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IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

IV. STUDIES OF ANIMAL PHYSIOLOGY

Warren R. Sanborn

MARCH 1968

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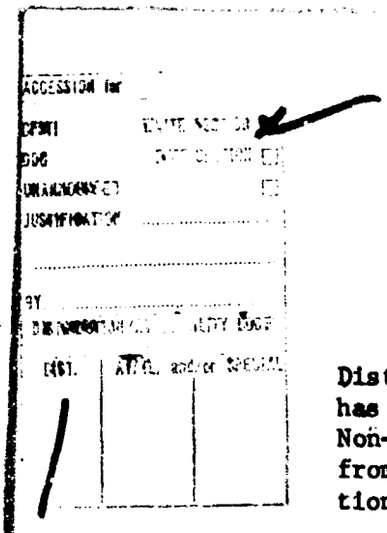
IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

IV. STUDIES OF ANIMAL PHYSIOLOGY

Warren R. Sanborn

March 1968

Technical Information Division
AEROBIOLOGY AND EVALUATION LABORATORY



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FOREWORD

The use of immunofluorescence, or fluorescent antibody, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The volume of literature related to immunofluorescence or fluorescent antibody and covering use of this technique has expanded explosively over the relatively few years since its inception. This expanding literature volume bears witness to the basic value of the technique. Through 1954, only about 40 articles had been published. In the next two years, 58 were added. During 1957 and 1958 there were 83 and 96, respectively. By 1961 the annual figure had reached more than 260 articles. For this supplementary second edition, the figures for 1963, 1964, and 1965 are 551, 764, and 678, respectively. These totals are testimony to Dr. Coons' genius.

Although it would be virtually impossible to cite every article that refers to the use of immunofluorescence, an attempt has been made to approach that limit. To that end, more than 445 journals were searched. In addition, six abstracting journals and the computer system of the National Library of Medicine, MEDLARS, were employed. Fifteen languages are represented. Translations were provided by colleagues of the compiler, government translating services, abstractors, and the compiler. The earliest entry in the original edition was 1905. In the present edition, entries covering the years 1963, 1964, and 1965 are the primary ones included, but there are also a few earlier entries not listed in the first edition. Further entries for 1966 and 1967 are now being compiled; these will be incorporated into further revisions of this bibliography.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. This entire second edition, Miscellaneous Publication 20, has the same overall title, "Immunofluorescence, an Annotated Bibliography," as the first edition (Miscellaneous Publication 3). The present edition also has the same six-volume structure: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending

undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author. A seventh volume, "Author and Subject Indexes," has been added to further aid the investigator in his search for articles relevant to his interest area.

Abstracts for citations in this edition have been prepared or modified in keeping with the central theme, the application of immunofluorescence to various problems. If the primary emphasis in the original article was immunofluorescence and the author's summary reflected this, the summary was generally left unchanged, except for minor changes and abbreviations simply to save space. In other instances, it was necessary to write a new abstract in order to indicate the proper place of immunofluorescent technique in the study. At the same time, the main point of such articles was maintained in abbreviated form in the abstract. Hopefully, this approach will be successful in bringing the application of immunofluorescence to the attention of the reader, while preserving each author's ideas at the same time. It is further hoped that this bibliography will aid investigators in avoiding duplication of effort and thus contribute to even greater and more imaginative applications of immunofluorescence.

Accession numbers have been assigned consecutively to citations throughout all six volumes of this edition. The plan for further future volumes allows this simple system. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in each volume.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

To avoid excess duplication and unwieldy size, the second parts of Volumes V and VI contain only basic citations for articles printed in the other four volumes. However, titles of articles are included to assist the reader in selection of those citations of possible interest. As in the other volumes, the references are placed in subject categories and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous forms. For unmistakable identification, they are listed below.

BSA	bovine serum albumin
DANS	a. 1-dimethylaminonaphthalene-5-sulfonic acid b. 5-dimethylamino-1-naphthalene sulfonic acid or chloride form.

FA	fluorescent antibody
FIC	fluorescein isocyanate
FITC	fluorescein isothiocyanate
FTA	fluorescent treponemal antibody
FTA abs	fluorescent treponemal antibody absorbed
FTA-200	a modification of the above based on serum dilution
PAP	primary atypical pneumonia
PAS	para-aminosalicylic acid
PBS	phosphate-buffered saline
RB 200	a. lissamine rhodamine RB 200
	b. lissamine rhodamine B 200
	c. lissamine rhodamine B
	d. sulphorhodamine B
	e. acid rhodamine B
TPFA	Treponema pallidum fluorescent antibody
TPI	Treponema pallidum immobilization

Generally, the citations follow the format prescribed by the second edition of Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations," Z39.5-1963, American Standards Association Incorporated, New York.

The compiler began to collect this immunofluorescence literature in 1957 while he was stationed at U.S. Navy Preventive Medicine Unit No. 2, Norfolk, Virginia The literature collection became more intense and organized after 1959 when he was transferred to Fort Detrick, Frederick, Maryland. Following his further transfer to the Microbiology Department of the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he continued this work with the encouragement and support of both of these latter installations. Work on the second edition began in 1964, and it has continued through support from both the U.S. Army and the Bureau of Medicine and Surgery of the U.S. Navy. This volume was completed while the compiler was assigned to U.S. Navy Medical Research Unit No. 3, FPO, New York, 09527, where he is currently serving as head of the Bacteriology Department.

The information in these volumes was originally recorded on coded marginal punch cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701, for improvement of the subsequent editions. Reader assistance in this area will be deeply appreciated.

ACKNOWLEDGMENTS

The essential team effort required for development of this immunofluorescence bibliography cannot be overstressed. As with many projects of this nature, the talents, advice, guidance, and assistance of many people led to the completion of this second edition. The compiler is deeply grateful to the many people who have contributed.

Financial support for this project at first was absorbed by the Pathology Division and the Walter Reed Army Medical Unit, Fort Detrick. However, completion of the first edition (through 1962) was made possible by special financial assistance from Physical Defense Division, Fort Detrick, under Dr. Charles R. Phillips. I am extremely grateful to him for his aid. Expenses for this second edition were primarily met through a generous grant from U.S. Navy Bureau of Medicine and Surgery, Preventive Medicine Division, under CAPT J. Millar, MC, USN. Many administration expenses also were borne by the Naval Medical Research Institute and by Fort Detrick.

A number of libraries kindly donated their services. In spite of the unusual requests required by this project, these libraries were very helpful and willingly assisted, often providing valuable suggestions. Libraries primarily involved were the Technical Library, Fort Detrick, under Mr. Charles N. Bebee and later Miss Joyce A. Wolfe, and the Technical Reference Library, Naval Medical Research Institute, Mrs. T.P. Robinson, librarian. Much valuable assistance was also rendered by the National Institutes of Health Library, Miss R. Connelly, reference librarian, the National Library of Medicine, and the Library of the Walter Reed Army Medical Unit, Fort Detrick. The staff members of these libraries were both helpful and patient. Without such fine assistance, the work could not have been completed.

It is a pleasure to acknowledge the highly competent secretarial help. Secretaries providing their capable and untiring talents were: Miss Sandra Rosenblatt, Miss Linda L. Zimmerman, Mrs. Marguerite M. Matovich, Mrs. Gene Heaven, Mrs. Linda Franklin, Mrs. Alberta Brown, Mrs. Margaret Raheb, and a number of others. Valuable assistance in double-checking problem references was provided by Mrs. Catherine F. Eaves and Mrs. Mary J. Gretzinger. Dr. George H. Nelson was a willing consultant for classification problems. Dr. Harold W. Batchelor provided an essential key to the development of this work by introducing the compiler to marginal punch card systems and guiding him in their application.

The Technical Information Division, under Mr. Gerald W. Beveridge, continually provided all types of assistance in addition to a home base from which to work. My gratitude for this cannot be fully expressed.

Last, but by no means least, the essential editorial work receives my highest praise. The tireless efforts, patience, and driving force supplied by these people were the prime factors in bringing this edition to completion. Mrs. Madeline Warnock Harp, in charge, Mrs. Mary D. Nelson, and Mrs. Ruth P. Zmudzinski all spent many hard weeks of work on this project. I shall always be indebted to them.

ABSTRACT

This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume IV contains 870 annotated literature citations, arranged according to major subject areas, and a complete author index.

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I. AUTOIMMUNE FACTORS IN DISEASE

A. DISEASE SYNDROMES

1. Hashimoto Syndrome and Thyroid Disease

6141

Anderson, J.R.; Gray, K.G.; Middleton, D.G.; Young, J.A. 1964. Autoimmunity and thyrotoxicosis. Brit. Med. J. 2:1630-1632.

The reported high incidences of thyroid and gastric antibodies in the serum of thyrotoxic patients were confirmed by FA, and it was shown that the two types of autoimmunity tend to occur especially in the same thyrotoxic individuals. Evidence that a genetically determined factor predisposes to various organ-specific autoimmunities is reviewed, and it is concluded, from the known occurrence of various autoimmunities in patients with thyrotoxicosis, that autoimmunization plays an important etiological part in thyrotoxicosis itself.

6142

Austoni, M.; Ziliotto, D. 1965. The clinical significance of autoimmune reactions in thyropathies. Minerva Med. 56:1821-1846. In Italian.

This study reports the clinical significance of autoimmune reactions in a broad range of thyropathies with particular emphasis on those postulated by autoaggressive genesis. With the indirect FA technique, antibodies have been localized in the colloid and in the cells. Antigens have also been located in the microsomic fraction of the parietal cells of the gastric mucosa. Note is made of the search for non-organ-specific antibodies.

6143

Beutner, E.H.; Gomez-Ustategui, J. 1964. Immunofluorescent staining with human antibodies to thyroid cells with a human complement staining system. J. Lab. Clin. Med. 64:747-795.

Immunofluorescent staining of thyroid cells was done with sections of rhesus monkey thyroids and selected human sera. Two techniques were applied, one utilizing antiserum prepared to amboceptor-sensitized sheep cells coated with human complement and another with antiserum to gamma globulin prepared as for conventional indirect FA. The FA staining of thyroid cells by these two methods seemed to be indistinguishable. Control experiments point to the specificity of these staining techniques for human complement and human gamma globulin. Indirect FA revealed colloid staining antibodies in some sera; the complement

staining method failed to do so. Among a group of 28 selected sera, 24 yielded comparable titers with cells of thyroid sections by the two staining methods; four yielded higher titers with the indirect staining method under the conditions utilized. The complement staining method appeared to be about ten times more sensitive than conventional complement fixation with thyroid microsomes.

6144

Beutner, E.H.; Witebsky, E. 1963. Studies on organ specificity: XV. Immunohistologic evaluation of reactions produced by thyroid autoantibodies. J. Immunol. 91:204-209.

Thyroglobulin antibodies occurring in the sera of patients with thyroiditis can best be demonstrated by using human thyroid extract to coat the tanned cells. Human autoantibodies directed against the cytoplasm of thyroid cells cross the species lines more freely than the thyroglobulin antibodies, as observed by immunofluorescent staining and complement fixation. Most thyroiditis sera react with the microsomes of the thyroid and not with those of any other organ when examined by means of the complement fixation test. In contrast to the results obtained by the complement fixation technique, immunofluorescent staining of the cytoplasm of thyroid cells with human antibodies seems to be specific for the thyroid and fails to cross-react with other organs as far as examined in this study.

6145

Cassano, C.; Andreani, D.; Ippolito, A.; Pagliari, M.; Mentzinger, G. 1963. Immunologic factors in thyroid pathology. Folia Endocrinol. Rome 16:263-284. In Italian.

Research has been carried out on the antithyroid antibodies in the serum of 202 patients suffering from various forms of thyroid pathology including simple hyperthyroidism, Basedow disease, toxic adenomatosis, simple goiter, myxedema, and thyroid neoplasia. Parallel analyses were made with different methods and indicated the presence of antibodies in a high percentage of the patients, varying from 70 to 80 per cent in Basedow patients and in those with Plummer's disease, and from 20 to 43 per cent in the groups suffering from neoplasia or simple hyperthyroidism. When titers of antibodies over 1:250 were considered, positivity was found only in 18 per cent of all the thyropathic patients and in 4 per cent of normal individuals. The authors discuss the importance of autoimmunization in the pathogenesis and evolution of thyroid pathology

6146

Croughs, W.; Visser, H.K.; De Boer, W.G.R.M. 1964. Two children with autoimmune thyroiditis. Ned. Tidjschr. Geneesk. 108:1252-1259. In Dutch.

Early symptoms of hyperthyroidism were seen in an 11-year-old girl suffering from goiter. Antibodies to thyroglobulin were found in the serum in a titer of 1:6400. The diagnosis of autoimmune thyroiditis was confirmed from pathological findings by the immunofluorescent technique. The serum of a 10-year-old girl, who had been treated for hypothyroidism (without goiter) for 3 years and for diabetes mellitus for 2 years, contained antibodies against thyroglobulin (1:1600) and against the parietal cells of gastric mucosa. Gastric biopsy demonstrated atrophic gastritis. There was achlorhydria after histamine and, in the early stage, macrocytic anemia. The presence of these different antibodies in the serum supports the diagnosis of autoimmune thyroiditis.

6147

Doniach, D.; Roitt, I.M.; Taylor, K.B. 1963. Autoimmune phenomena in pernicious anaemia: Serological overlap with thyroiditis, thyrotoxicosis, and systemic lupus erythematosus. Brit. Med. J. 1:1374-1379.

Because of the clinical association of thyroid diseases and pernicious anemia, the incidence of autoantibodies to thyroid gland and stomach has been determined in patients with pernicious anemia and healthy controls. Patients with Hashimoto's disease, thyrotoxicosis, and systemic lupus erythematosus (SLE) were examined. The incidence of thyroid antibodies was significantly higher in pernicious anemia patients without overt thyroid disease than in normal controls, although not as high as in the thyroid diseases. Gastric cytoplasmic antibodies, found in 83 per cent of patients with pernicious anemia, were detected in 27 per cent of patients with Hashimoto's disease and 33 per cent of patients with thyrotoxicosis. The incidence of antinuclear factors (ANF) in pernicious anemia was lower than in the controls In SLE the incidence of both thyroid and gastric cytoplasmic antibodies was lower than in the controls, whereas the incidence of ANF was 100 per cent.

6148

Federlin, K.; Oppermann, W ; Pfeiffer, E.F. 1965. Contribution to immune thyroiditis. Deut. Med. Wochensch. 90:247-255. In German.

Various serologic and fluorescence tests were made on two cases of Hashimoto's thyroiditis and one case of primary myxedema. With immune fluorescence, different localities of serum antibodies against thyroid

tissue could be determined. It was also possible to demonstrate a local uptake of antigen, thyroglobulin, through macrophages and the presence of gamma globulin in plasma cells. These findings speak for the fact that both cases with chronic thyroiditis represent true autoimmune diseases. The immunohistologically obtained result of thyroid antibodies in a primary myxedema supports the hypothesis that this disease forms the final stage of an immune thyroiditis.

6149

Fialkow, P.J.; Uchida, I.A.; Hecht, F.; Motulsky, A.G. 1965. Increased frequency of thyroid autoantibodies in mothers of patients with Down's syndrome. Lancet 2:868-870.

Parents of patients with Down's syndrome and a control group were tested for thyroid autoantibodies using tanned red cell agglutination and FA. The frequency of thyroid autoantibodies was similar among fathers of patients and controls. In contrast, 28 per cent of mothers were sero-positive as compared with 14 per cent of controls. This difference became more striking when only women of childbearing age were considered. Beyond 46 years of age, no difference between mothers and controls was found. The presence of thyroid autoimmune reactions in women appears to be associated with a higher risk of Down's syndrome in the offspring.

6150

Goudie, R.B.; McCallum, H.M. 1963. Loss of tissue-specific autoantigen in thyroid tumors. Lancet 2:1035-1038.

Twenty-two thyroids were studied by the fluorescent antibody technique, using cytotoxic Hashimoto's serum as a specific stain for the cytoplasmic autoantigen peculiar to thyroid epithelium. The cells of four thyroid carcinomata were found to be deficient in autoantigen, and less obvious autoantigen loss was readily demonstrated in some or all of the cells in three of six simple thyroid adenomata and in two of four adenomatous goiters. Autoantigen loss was trivial or absent in normal thyroid and in six of seven thyrotoxic thyroids. These immunological findings may have a bearing on invasive behavior of thyroid-carcinoma cells.

6151

Halberg, P. 1965. Thyro-cytotoxic factor. Acta Med. Scand. 177:509-518.

Many features suggest that chronic thyroiditis (Hashimoto's disease) may be an autoimmune disease. So far three different specific thyroid antibodies have been found. This cytotoxic factor might be identical with one of the thyroid antibodies demonstrable serologically, namely a complement fixing antibody. Evidence is presented that the cytotoxic

factor and the complement fixing antibody are identical. It is known that thyroid cells grown for more than 3 or 4 days are no longer sensitive to the cytotoxic factor. It is shown by an immune fluorescence technique that during 3 or 4 days of culture an antigenic deletion takes place that might explain this observation. The specific antigenicity of the cells, demonstrated by the immune fluorescence technique, suggests that they are thyroid follicular epithelial cells. Even though one of the circulating autoantibodies found in chronic thyroiditis, namely the complement fixing antibody, seems to have a specific cytotoxic effect on thyroid epithelial cells, the pathogenetic role of these antibodies is still very uncertain.

6152

Herbeuval, R.; Duheille, J.; Herbeuval, C.; Bellut, F. 1964. Studies of antithyroid autoantibodies in man. Compt. Rend. Soc. Biol. 158:141-145. In French.

Sera from five cases of hyperthyroidism, one of hyperfixing thyroid nodule, six of myxedema, two of simple colloid goiter, one of Hashimoto's disease, two of Biermer's disease, and three of miscellaneous diseases were examined for antithyroid autoantibodies by the indirect immunofluorescent method. The majority of the sera yielded negative results. Anticytoplasmic antibodies were detected in one case of hyperthyroidism. Anticolloidal antibodies were not detected in any sera.

6153

Hjort, T. 1963. The occurrence of antibody against second colloid antigen (CA-2 antibody) in patients with and without thyroid disease. Acta Med Scand. 174:147-154

The FA sandwich technique was used to study the occurrence of CA-2 antibody (antibody to the second colloid antigen) in 180 patients with medical diseases but without actual thyroid disease and in 370 patients with various thyroid disorders. Among the patients without thyroid disease CA-2 antibody was present in 3 per cent, but only in 1 per cent was it found alone. On the other hand, CA-2 antibody occurred fairly frequently among patients with thyroid disorders, often in combination with one or both of the other two thyroid antibodies. However, in 9 per cent of 196 patients with nontoxic goiter and in 9 per cent of 99 patients with thyrotoxicosis CA-2 antibody occurred alone, so that only FA could reveal the presence of autoimmunization. In patients with lymphadenoid goiter or myxoedema, FA was of less value, because most of these patients had thyroglobulin antibody in the serum in such high titers that CA-2 antibody, if any, for technical reasons could not be demonstrated. Three patients with acute thyroiditis had CA-2 antibody in the serum. In six patients with CA-2 antibody who underwent partial thyroidectomy, leakage of the second colloid antigen could not be demonstrated.

6154

Koffler, D.; Friedman, A. 1964. Immunocytochemical studies in chronic thyroiditis. J. Histochem. Cytochem. 12:18.

Surgical specimens of five cases of human chronic thyroiditis were studied by FA to determine the nature of the protein distribution in areas of active inflammation and exudation. Antisera were prepared to purified B-1C globulin (C'3 component of complement), gamma globulin, gamma macroglobulin, fibrinogen, and albumin in rabbits, and to rabbit gamma globulin in guinea pigs. They were conjugated with FITC and incubated with cryostat sections of thyroiditis, normal thyroid, and hyperplastic thyroid. Gamma globulin was deposited in colloid material contiguous to disrupted follicles and occasionally in the cytoplasm of epithelial cells of thyroid acini. B-1C globulin was demonstrated in similar areas utilizing the indirect technique. Fluorescence was decreased at pH 3.2. Foci of fibrinogen and albumin were found in some sections of thyroid, but their distribution did not correspond to gamma globulin. Macroglobulin was not found. Normal thyroid and hyperplastic thyroid did not exhibit protein localization in colloid. The presence of gamma globulin and B-1C globulin depositions in chronic thyroiditis suggests that antigen-antibody complexes may be present in the exudative lesions. Complete article.

6155

Koffler, D.; Paronetto, F. 1965. Complement-fixing antibody to colloid and epithelium in monkey thyroiditis. Nature 207:773-774.

Sera were assayed for antibody to thyroid antigens by indirect FA. Antinuclear antibodies were not observed. The presence of CF antibodies to colloid and epithelium in sera of monkeys with thyroiditis may indicate that both these antibodies have cytotoxic potential. Although serological similarities between primate and human thyroiditis are evident, the early histological lesions resembled those in guinea pigs, rats, rabbits, and dogs.

6156

Koffler, D.; Paronetto, F. 1965. Serologic and immunofluorescent studies of humoral antibody and gamma globulin localization in experimental autoimmune thyroiditis. J. Immunol. 94:329-336.

Immunofluorescent studies of thyroiditis in Hartley guinea pigs revealed humoral antibody to colloid antigen that correlated with the severity of thyroid lesions 3 weeks after the injection of homologous thyroid antigen. Hemagglutinating antibodies to thyroglobulin did not show a similar correlation. In vivo deposition of gamma globulin was demonstrated in guinea pigs with extensive thyroiditis. Humoral antibodies may be of significance in the pathogenesis of experimental thyroiditis.

6157

Massalski, W.; Mlodzki, M.; Brzosko, W.J.; Nowoslawski, A. 1964. Immunofluorescent tests in the course of I-131 administration. Pol. Tyg. Lek. 19:1596-1599. In Polish.

Indirect FA was used to detect thyroid autoantibodies in 66 patients before and after radio-iodine therapy. Before treatment only cytoplasmic fluorescence was observed. After treatment nuclear fluorescence was observed. In seven cases there was colloid fluorescence, in four cases diffuse fluorescence was seen, and there was one flocculation-type reaction. There was a distinct tendency for these reactions to disappear some time later. The problem of the appearance and disappearance of thyroid autoantibodies in the course of radio-iodine administration in diagnostic doses requires further study, which should also include groups of thyroid patients and healthy persons not treated. Immunofluorescence, due to its high sensitivity and the readiness with which the entire spectrum of thyroid autoantibodies can be visualized in one simple procedure on two tissue sections, should be employed in studies on the dynamics of thyroid autoimmunity.

6158

Nairn, R.C.; Ghose, T.; Porteous, I.B.; Urquhart, J.A. 1963. Detection of autoantibodies to cytoplasmic and nuclear antigens in freeze-dried thyroid. J. Clin. Pathol. 16:281-282.

The most important single factor for success with the present freeze-drying method is the suitability of the original thyroid tissue to provide antigenically active microscopical preparations. The thyroid should be hyperplastic to ensure an abundance of cytoplasmic microsomal antigen and a predominance of small acini to retain colloid. If satisfactory embedded tissue blocks were available commercially, the technique for cellular and colloid antigens described in this and the previous publication could be used for most routine laboratory investigations of clinically significant autoantibodies.

6159

Roitt, I.M ; Doniach, D. 1963. Lymphoid thyroiditis as a model for autoimmune disease. Acta Allergol. 18:474-478.

This is a discussion of the autoimmune nature of Hashimoto's disease. Several possible immunologic systems are outlined. Evidence for and against these is presented. The system is different from that of lupus erythematosus.

6160

Thomas, W.C., Jr.; Anderson, R.M.; Jurkiewicz, M.J.; Araujo, J.D.; Blizzard, R.M. 1965. Clinical studies in thyroiditis. Ann. Intern. Med. 63:808-818.

Thyroiditis is a clinically variable disorder that is being recognized with increasing frequency. Detailed studies of 25 patients with histologically proven thyroiditis have provided certain information that may be helpful in evaluating the manifestations of this malady. The salient features of these studies are summarized. Antithyroid antibodies are readily detected in the sera of all patients except those with granulomatous thyroiditis, but neither the intensity of the inflammatory reaction nor the functional status of the thyroid gland correlates with the incidence or titer of these circulating antibodies.

2. Lupus Erythematosus

6161

Aarons, I. 1964. Renal immunofluorescence in NZB/NZW mice. Nature 203:1080-1081.

The close similarities seen in the renal lesions of human lupus nephritis and the NZB/NZW mice have been indicated. Comparison of the fluorescent patterns seen in these cases, as well as in other forms of human nephritis, is possible. Such investigations would be of value in elucidating the nature of the lesions.

6162

Anonymous. 1964. Laboratory tests in systemic lupus erythematosus. Brit. Med. J. 2:1347-1348.

The relationship of LE cells to SLE is discussed. The nuclear immunofluorescence and LE cell tests are the most widely used routine diagnostic tests for SLE.

6163

Ansell, B.M.; Lawrence, J.S. 1963. A family study in lupus erythematosus. Arth. Rheum. 6:260.

The probands for this study comprised some 46 cases of classical sytemic lupus erythematosus. The relatives included were offspring and siblings,

aged 15 years and over, and parents who were living within the same prescribed area; spouses, when available, were included for control purposes. Full histories and results of physical examinations were recorded. For comparison, sera from population studies in England were matched for sex and age with those of the relatives, and were examined for the presence of antinuclear factor, latex test, and protein estimation. The sheep cell agglutination test results were compared with those of the population studies. There was some increase in the frequency of positive antinuclear factor tests in female relatives of probands with classical systemic lupus erythematosus.

6164

Azoury, F.J.; Jones, H.E.; Gum, O.B. 1965. Comparative study of antinuclear factors and intradermal tests in systemic lupus erythematosus and antinuclear factors in nephrotic syndrome and leprosy. Arth. Rheum. 8:428-429.

A comparative study of the significance of different antinuclear factors and different intradermal tests in the diagnosis of systemic lupus erythematosus (SLE) was carried out. Of 42 patients with SLE, 31 had positive LE tests and 40 had positive antinuclear factors. Of these 40, 23 had only homogeneous pattern, 10 had shaggy and homogeneous pattern, and seven had speckled pattern. The seven patients with a speckled pattern had nephrotic syndrome. Twelve patients with nephrotic syndrome secondary to other diseases had no antinuclear factors. The presence of the shaggy pattern correlated well with acute lupus or lupus nephritis. Of ten patients with leprosy, two had antinuclear factors and arthritic manifestations. Concurrently, intradermal testing was carried out on 22 patients with SLE, using homologous leukocytes, nucleoprotein, histone, and DNA. The ten patients positive to intradermal DNA had either acute lupus or lupus nephritis confirmed by renal biopsy, the shaggy pattern was present in all positives for antinuclear factors

6165

Barnes, C.W ; Sullivan, M.A.; Beutner, E.H.; Witebsky, E. 1965. In vitro and in vivo interaction of nuclear antibodies with corresponding antigens Experientia 21:485-488

Direct FA studies on specimens from SLE patients revealed that neither fresh buccal mucosa cells nor cells in liver biopsy specimens exhibited nuclear staining. However, films of leukocytes did. Possible explanations for variable staining reactions were explored. Antigen-antibody reactions appeared to be very rapid.

6166

Barnett, E.V.; Bakemeier, R.F.; Leddy, J.P.; Vaughan, J.H. 1965. Heterogeneity of antinuclear factors in lupus erythematosus and rheumatoid arthritis. Proc. Soc. Exp. Biol. Med. 118:803-806.

Twenty-six sera from adults and children with lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, or chronic active hepatitis were tested in a three-layer immunofluorescence test on human white cell nuclei. Rabbit antisera against human Type I and Type II Bence Jones protein were used to determine whether the antinuclear factors contained Type I or Type II L chains. Twenty-four of the sera were shown to contain ANF with both types of L chains. In the remaining two cases quantitative considerations limited the study. Both Type I and Type II ANF were also found in isolated fractions of gamma-2 and gamma-1M globulins in four lupus sera. Antinuclear factor was determined by the three-layer immunofluorescence test.

6167

Barnett, E.V.; Condemi, J.J.; Leddy, J.P.; Vaughan, J.H. 1963. Gamma-2, gamma-1A, and gamma-1M antinuclear factors in human sera. Arth. Rheum. 6:261-262.

An investigation of the classes of proteins containing antinuclear factors (ANF) in the sera of patients with lupus erythematosus or rheumatoid arthritis has been conducted with rabbit antisera specific for human gamma-2, gamma-1A, or gamma-1M immunoglobulins. The specificities of these rabbit antisera were confirmed by various tests, including specific inhibition of the ANF test. Certain sera from lupus erythematosus or rheumatoid arthritis patients had ANF in all three immunoglobulin types. The nuclear material reactive with all three types of ANF was destroyed by incubation with DNAase or DNAase and trypsin, but not with RNAase. Both gamma-1A and gamma-1M ANF were shown to be significantly reduced in titer following treatment with 0.1 M mercaptoethanol, but gamma-2 ANF was unaffected. The titers of gamma-1A and gamma-1M ANF were similar in both lupus erythematosus and rheumatoid arthritis but gamma-2 ANF was significantly lower in rheumatoid arthritis than in lupus patients. These differences may be interpreted in terms of autoimmunization by different nuclear antigens, differences in duration or degree of autoimmunization, or different genetic predispositions of the two populations for the production of the various types of ANF.

6168

Barnett, E.V.; Condemi, J.J.; Leddy, J.P.; Vaughan, J.H. 1964. Gamma-2, gamma-1A, and gamma-1M antinuclear factors in human sera. J. Clin. Invest. 43:1104-1115.

A modification of the indirect immunofluorescent technique was employed to detect antinuclear factors (ANF) of gamma-2, gamma-1A, or gamma-1M immunoglobulin classes. Gamma-1A ANF in two cases appeared to sediment as 7S globulins and be resistant to sulfhydryl treatment, but in other cases, they appeared to sediment faster than 7S globulins and to be inactivated by the sulfhydryl treatment. ANF and rheumatoid factors (RF), although frequently found together in the same serum, behaved as separate serological entities. Evidence was found that in some rheumatoid arthritis sera RF may interact with gamma-2 ANF on nuclei to give the appearance of gamma-1M ANF. ANF of all three immunoglobulin classes were detected in sera from both rheumatoid arthritis (RA) and lupus erythematosus (LE) patients. Gamma-2 ANF was found in higher titer in LE than in RA sera. Among the LE sera, gamma-2 and gamma-1A ANF were found in higher titer if the sera were from patients with disease for less than 1 year. LE patients with disease for more than 1 year, who generally were also in remission and on chloroquin or steroid therapy, had lower titers of gamma-2 and gamma-1A ANF. Results by FA tests of lesser sensitivity are presented.

6169

Barnett, E.V.; North, A.F., Jr.; Condemi, J.J.; Jacox, R.F.; Vaughan, J.H. 1965. Antinuclear factors in systemic lupus erythematosus and rheumatoid arthritis. Ann. Intern. Med. 63:100-108.

Antinuclear factors were heterogeneous. The origin and progression of these diseases were discussed and immunologic factors analyzed.

6170

Beck, J.S.; Rowell, N.R. 1963. Transplacental passage of antinuclear antibody Lancet 1:134-135.

Homogeneous antinuclear antibody has been followed in a child who was passively immunized with this antibody from his mother, who had systemic lupus erythematosus. Because the rate of destruction of the antibody was not accelerated (half-life 17.5 days), it is unlikely that the circulating antinuclear antibody was reacting with the infant's cell nuclei in vivo. This suggests that homogeneous antinuclear antibody is not the principal pathogenetic factor in the development of systemic lupus erythematosus.

6171

Beerman, H.; Mayock, R.L.; Lowney, E.D. 1965. Chronic panniculitis: (2) R/O lupus erythematosus or other collagen disease. Arch. Dermatol. 91:180-181.

In this case history the indirect FA test was borderline positive for thyroid autoantibody.

6172

Blumenfeld, H.B.; Kaplan, S.B.; Mills, D.M.; Clark, G.M. 1964. Disseminated lupus erythematosus in identical twins. J. Amer. Med. Ass. 185:667-669.

The fluorescent antinuclear test was one of the diagnostic tests reported in these case histories.

6173

Bonomo, L.; Tursi, A.; Dammacco, F. 1965. Characterization of the antibodies producing the homogeneous and the speckled fluorescence patterns of cell nuclei. J. Lab. Clin. Med. 66:42-52.

The antinuclear factors in sera from patients with systemic lupus erythematosus, rheumatoid arthritis, and other diseases that produce the homogeneous and speckled patterns of nuclear fluorescence were separated by diethylaminoethyl cellulose chromatography. The homogeneous patterns of nuclear fluorescence was constantly produced by a 7S gamma globulin factor, but the speckled pattern was produced by a factor associated with the macroglobulin class only.

6174

Bonomo, L.; Tursi, A.; Dammacco, F. 1965. Characterization of antinuclear factors showing immunofluorescence. Reumatismo 17:3:119-122. In Italian.

The antinuclear factors that produce the homogeneous and speckled pattern of nuclear fluorescence were separated by DEAE cellulose chromatography. Such fractionation procedure was carried out in sera from patients with systemic lupus erythematosus and rheumatoid arthritis. Although the homogeneous pattern of nuclear fluorescence appears constantly due to a 7S gamma globulin factor, the speckled pattern is produced by a factor associated with the macroglobulin class.

6175

Bonomo, L.; Tursi, A.; Del Zotti, G. 1963. Comparison between a total antinuclear reaction (antinuclear fluorescence) and a partial one (latexdeoxyribonucleoprotein) in systemic lupus erythematosus and other diseases. Reumatismo 15:426-433. In Italian.

A comparison was carried out between two tests for antinuclear factors: 176 sera were tested, 19 from patients with systemic lupus erythematosus (SLE) and 157 from patients with various diseases. Of these, 32 sera (18.1 per cent) demonstrated antinuclear factor (ANF); 19 of them were from patients with SLE and the remaining 13 from patients with various other diseases. Only 10 sera were positive in the LE test; 8 of such sera were from SLE patients. The higher sensitivity and the lower specificity of the ANF is attributed to its property of detecting all the ANF. In contrast, the LE test as well as the LE factor was able to detect only some ANF, namely the one active against the nucleoprotein.

6176

Bonomo, L.; Tursi, A.; Trimigliozzi, G.; Dammacco, F. 1965. LE cells and antinuclear factors in leprosy. Brit. Med. J. 2:689-690.

Serum antinuclear factors as detected by FA were found in 16 of 55 cases of leprosy. LE cells or 'rosettes' were present in the blood specimens of 4 of 10 leprosy patients with serum antinuclear factors.

6177

Brzosko, W.; Chorzelski, T.; Madalinski, K.; Nowoslawski, A. 1964. The use of immunofluorescence test in the diagnosis of disseminated lupus erythematosus. Pol. Tyg. Lek. 19:942-944. In Polish.

A report of the use of immunofluorescence test in the diagnosis of disseminated lupus erythematosus is presented. The sources of nuclear antigens were rabbit and human white blood cells as well as tissue sections from mouse liver and kidney. The indirect procedure was used for staining the smears and tissue sections with fluorescein isothiocyanate - labeled goat antihuman gamma globulin. The results of this preliminary study, compared with the results of simultaneously performed LE tests in a rather limited number of cases, indicate the usefulness of immunofluorescence in the routine diagnosis of lupus erythematosus.

6178

Burkholder, P.M. 1963. Complement fixation in diseased tissues: II. Fixation of guinea pig complement in renal lesions of systemic lupus erythematosus. Amer. J. Pathol. 42:201-223.

Immunohistologic tests for sites of human globulin and for fixation of guinea pig complement were used in an effort to identify complement-fixing antigen-antibody complexes in renal lesions of seven patients with systemic lupus erythematosus. Frozen sections of kidney were treated by FA for human globulin or with guinea pig complement and then FA for fixed guinea pig complement. The following were found to be sites of human globulin fixing guinea pig complement: glomerular capillary 'wire loops,' diffusely thickened glomerular capillary walls, walls of some arterioles, segments of a few glomerular capsular adhesions, and cytoplasmic granules of swollen endothelial cells in glomerular capillaries. Globulin in glomerular and arterial deposits behaved like antibody in complement-fixing antigen-antibody complexes. Lack of fixation of guinea pig complement at sites of human globulin in tubular casts and cytoplasmic droplets of tubular epithelium indicated a certain degree of selectivity of the immunohistologic complement fixation reaction used in this study.

6179

Burnham, T.K.; Fine, G.; Neblett, T.R. 1964. Imprints of tumors as nuclear substrate for the detection of antinuclear factors. Amer. J. Clin. Pathol. 42:517-518.

Sections and imprints of various tumors and other tissues were used as substrate in order to detect antinuclear factors. Imprints of 13 different tumors and five normal tissues were air-dried and stored at -15 C. Indirect FA was used with serum from various patients, including persons with scleroderma, lupus erythematosus, and several dermatoses. Imprints of tumors were superior to normal cells as nuclear substrate. The fluorescent nuclear speckles were brighter, more numerous, and usually larger than in imprints and sections of normal tissue. Nucleolar fluorescence was more impressive in imprints of tumors, with brighter, larger, and more numerous nucleoli being observed. Imprints of tumors, spleen, and lymph nodes manifested homogeneous and membranous nuclear fluorescence much better than smears of blood and histologic sections, as a result of the greater number, better delineation, and larger size (imprints of tumor only) of the nuclei. Superior nuclear definition is found in imprints. Slides may be prepared rapidly and stored at least 4.5 months prior to use. We suggest the use of imprints of tumors as a rapid, practical screening test for antinuclear factors. Complete article.

6180

Burnham, T.K.; Fine, G.; Neblett, T.R. 1964. Tumor imprints as a source of nuclear substrate for the detection of antinuclear factors. J. Invest. Dermatol. 42:7-9.

Sera of patients with positive lupus erythematosus cell tests, scleroderma, and various dermatoses were investigated for antibodies to skin components and antinuclear factors by the indirect fluorescent antibody technique. Speckled nuclear fluorescence in the epidermis was seen with several of the scleroderma sera. Epidermal nucleolar fluorescence occurred with one scleroderma serum; homogeneous epidermal nuclear fluorescence was seen with several of the lupus erythematosus cell positive sera. Tumor imprints were far superior for the detection of antinuclear factors to the conventional blood smears and tissue sections. Nuclear fluorescence was much more conspicuous because of greater number, size, and delineation of nuclei and the greater quantity of some of the nuclear antigens.

6181

Burnham, T.K.; Neblett, T.R.; Fine, G. 1963. The application of the fluorescent antibody technique to the investigation of lupus erythematosus and various dermatoses. J. Invest. Dermatol. 41:451-456.

The fluorescent antibody technique was applied to lesion biopsy specimens of 27 patients with various dermatoses and to biopsy specimens of normal skin from eight patients and one intradermal nevus. Lesions of patients with lupus erythematosus psoriasis and various dermatoses were stained by the direct immunofluorescent method. Localized bright yellow-green fluorescence was demonstrated at the dermal-epidermal junction, with diffuse patchy yellow-green fluorescence in the dermis in the various diseases studied. Lupus erythematosus lesions consistently showed a well-demarcated bright yellow-green fluorescent band at the basement membrane site. Although a brightly fluorescent but ill-defined band was also present at the dermal-epidermal junction in the other diseases, this was rather poorly demarcated and much less distinct than the well-demarcated band seen in the lupus erythematosus lesions. Normal skin and the intradermal nevus showed only the diffuse patchy yellow-green fluorescence in the dermis. Gamma globulin localization was thus demonstrated at the dermal-epidermal junction, suggesting this as the site of an antigen-antibody reaction in the various dermatoses.

6182

Caruntu, F. 1964. Immunofluorescent investigation of antinuclear factors in the serum of patients with disseminated lupus erythematosus and some of their first-degree relatives. Med. Interna 16:479-486. In Rumanian.

In two cases of disseminated LE, titers of 1:200 to 1:400 were found. However, these decreased after treatment with corticoid hormones. Low titers were found in three relatives. No antinuclear factor was found in eight controls. The significance of these findings is discussed, as well as their possible use in the diagnosis and study of the evolution of the disease.

6183

Casals, S.P.; Friou, G.J.; Myers, L.L. 1963. Significance of antibody to DNA in acute systemic lupus erythematosus. Arth. Rheum. 6:265.

The significance of antinuclear antibodies can be properly elucidated only by studies that distinguish among individual factors. Using a spot test for detection of antibody to nucleoprotein, and a characteristic nuclear reaction pattern produced by antibody to DNA, as well as DNA-complement fixation tests and another spot test specific for anti-DNA, we have investigated the relationship of serum anti-DNA to the course of the disease. SLE patients studied have all had antibody to nucleoprotein but, consistent with other reports, only about one-third have had demonstrable antibody to DNA. Those with negative DNA antibody tests were in a quiescent, chronic phase, in most instances due to corticosteroid therapy. Some with chronic disease and negative tests for anti-DNA did not require immediate treatment. Two of these untreated chronically ill patients subsequently developed acute exacerbations, and simultaneously anti-DNA antibody appeared. All patients with acute active disease had positive tests. Thus our studies have revealed a close correlation between this antibody and acute SLE, while antibody to nucleoprotein correlates best with diagnostic features rather than with the phase of disease.

6184

Casals, S.P.; Friou, G.J.; Myers, L.L. 1964. Significance of antibody to DNA in systemic lupus erythematosus. Arth. Rheum. 7:379-390.

A study was made of serum antibody to DNA and nucleoprotein in 35 patients with SLE, using immunofluorescent spot techniques. Anti-DNA was confirmed by complement fixation and observation of the shaggy nuclear fluorescent pattern. A striking correlation was observed between presence of anti-DNA and the acute phase of SLE. Anti-nucleoprotein, the most constant finding, showed only a general correlation of titer with phase of disease. Diagnostic and pathogenetic implications are discussed.

6185

Casals, S.P.; Friou, G.J.; Teague, P.O. 1963. Specific nuclear reaction pattern of antibody to DNA in lupus erythematosus sera. J. Lab. Clin. Med. 62:625-631.

By means of the indirect fluorescent antibody technique for detection of antinuclear antibodies, homogeneous and speckled nuclear patterns have previously been demonstrated. Of 34 sera yielding some kind of reaction with nuclei by the above technique, a new nuclear reaction pattern was seen with sera of five patients with systemic lupus erythematosus. The distinctive features of this new pattern are described, and the term shaggy is adopted for its designation. Because of positive complement fixation tests with DNA in only these five sera, an attempt was made to understand the significance of this new nuclear staining pattern. Evidence is given that DNA is exuding from nuclei when the cell preparations are incubated with phosphate buffer, pH 7, and that this shaggy pattern is the expression of DNA antigen and antibody precipitates.

6186

Cavellero, C.; Chiappino, G. 1963. Immunohistochemical investigations on collagen diseases. Ann. Sclavo 5:717-728. In Italian.

The results of immunohistochemical investigations made up on biopsy specimens and necroscopy material in cases of collagen diseases are reported. Bound gamma globulins are mainly in renal lesions of systemic lupus erythematosus. Indirect FA has also shown typical antinuclear and antisarcolemmal autoantibodies in the sera of patients with systemic lupus erythematosus and dermatomyositis. Rheumatoid factor was demonstrated in the wall of the small synovial vessels of joints in the course of rheumatoid arthritis as well as in some cells of subcutaneous nodules.

6187

Channing, A.A.; Kasuga, T.; Horowitz, R.E.; Dubois, E.L.; Demopoulos, H.B. 1965. An ultrastructural study of spontaneous lupus nephritis in the NZB/BL-NZW mouse. Amer. J. Pathol. 47:677-694.

An experimental model for the laboratory investigation of human systemic lupus erythematosus has been suggested by the spontaneous development of an SLE-like syndrome in the NZB/BL-NZW hybrid mouse. In this study, approximately 70 per cent of the animals developed lupus nephropathy as measured by the classical clinico-pathologic criteria. Ultrastructural features of the lupus-like glomerulopathy were compared with the glomerular changes in human SLE. In addition to endothelial, epithelial, and

mesangial alterations there was a variable but constant deposition of dense material, fibrinoid, within and on both sides of the true capillary basement membrane in the mouse observed. These features taken together are characteristic in human lupus glomerulopathy and indicate that this hybrid mouse provides a valid experimental model for SLE.

6188

Chorzelski, T.; Blaszczyk, M.; Langner, A. 1965. Effect of Resochin on the LE cells' formation in vitro. Przegl. Dermatol. 52:123-127. In Polish.

Modified Snapper and Nathan's method has been applied in investigating the effect of Resochin on LE cell formation in vitro. It has been found that Resochin in saturated solutions combines with leukocyte nuclei (autofluorescence) and causes inhibition of the LE cell formation. In the indirect FA technique blocking of the reaction between the LE factor and the leukocyte nuclei could be proved in a number of subacute lupus erythematosus cases. Absence of inhibition of the immunofluorescent reaction in the remaining cases could possibly be explained by the presence of some other antinuclear antibodies that remained unaffected by Resochin.

6189

Clark, R.F.; Burkhart, C.R.; Bates, H.R., Jr. 1963. Passive transfer of antinuclear activity in immunologically tolerant animals. Arth. Rheum. 6:573-580.

Rats made immunologically tolerant to human plasma protein were inoculated with human sera from patients with SLE containing antinuclear factors. This experiment demonstrated the transferability of antinuclear activity of human SLE sera in the rat by production of the LE cell phenomenon, the lack of difference in reactivity in the short-term experiments between the normal and immunologically tolerant animals, and the short duration of this transferred antinuclear activity. In addition, it demonstrated a possible in vivo reaction of the human antinuclear factors with tissues of the recipient because of the short duration of the transferred activity in contrast to persistently demonstrable human serum protein in the rat plasma.

6190

Colman, R.W.; Sturgill, B.C. 1965. Lupus-like syndrome induced by procaine amide: Association with anti-DNA antibody. Arch. Intern. Med. 115:214-216.

A second case in which a syndrome similar to disseminated lupus erythematosus occurred after procaine amide administration is presented. In addition to positive lupus erythematosus cell preparations, antibodies against deoxyribonucleic acid were demonstrated by FA and other means. These disappeared several months after drug withdrawal.

6191

Condemi, J.J.; Barnett, E.V.; Atwater, E.C.; Mongan, E.S.; Jacox, R.F.; Vaughan, J.H. 1963 Significance of antinuclear factor in rheumatoid arthritis Arth. Rheum. 6:266

In the past 2 years we have found antinuclear factor (ANF) in 36 of 132 patients with rheumatoid arthritis (RA), in 18 of 20 with systemic lupus erythematosus (SLE), and in 3 of 75 normal adults. To determine the significance of the presence of ANF in RA, 44 patients who fulfilled the A.R.A. criteria for definite RA were studied. Nodules occurred in 15 of 18 patients with ANF and 8 of 26 without ANF; P is less than 0.01. A battery of tests, case histories, signs, and symptoms revealed no other significant correlations. This study suggests that RA patients with ANF are not clinically distinguishable from those without ANF. RA patients with nodules may produce a greater variety of abnormal serum factors, i.e., rheumatoid factor and ANF. Complicating infarctive vasculitis is not more frequent in patients with rheumatoid arthritis with ANF than in those without ANF.

6192

Cormane, R.H. 1964 'Bound' globulin in the skin of patients suffering from chronic discoid lupus erythematosus and from systemic lupus erythematosus Dermatologica 129.304-305

Both forms of LE share corresponding globulin factor bound to the basal membrane of the epidermis with fluorescein-labeled antihuman globulin. This phenomenon, although to a lesser degree, can sometimes also be encountered in the basal membrane of the clinically normal skin in patients with systemic LE, but not in the clinically normal skin of patients with chronic discoid LE. Pretreatment of sections from the normal skin with LE positive serum resulted only in a positive staining of the nuclei; however, the basal membrane was not stained by this procedure. The globulin bound to the basal membrane appears, therefore, not to be identical with the antinuclear factor or with the LE factor.

6193

Cormane, R.H. 1964 Bound globulin in the skin of patients with chronic discoid lupus erythematosus and systemic lupus erythematosus Lancet 1 534-535

In both discoid and systemic lupus erythematosus, the region of the basal membrane can be specifically stained with fluorescein-conjugated antihuman globulin serum. A similar bound globulin factor seems to be present in both diseases. Moreover, in systemic lupus erythematosus, it seems sometimes to be present, although to a much lesser

extent, in the basal membrane of the clinical normal skin. This bound globulin is not apparently related to the antinuclear factor or to the so-called LE factor. This method may be of use in cases in which the clinical and histological diagnoses are questionable. Perhaps it also contributes to a better understanding of the skin lesions in both discoid and systemic lupus erythematosus.

6194

Doniach, D.; Roitt, I.M.; Taylor, K.B. 1963. Autoimmune phenomena in pernicious anaemia: Serological overlap with thyroiditis, thyrotoxicosis, and systemic lupus erythematosus Brit. Med. J. 1:1374-1379

Because of the clinical association of thyroid diseases and pernicious anemia, the incidence of autoantibodies to thyroid gland and stomach has been determined in patients with pernicious anemia and healthy controls Patients with Hashimoto's disease, thyrotoxicosis, and systemic lupus erythematosus (SLE) were examined. The incidence of thyroid antibodies was significantly higher in pernicious anemia patients without overt thyroid disease than in normal controls, although not as high as in the thyroid diseases. Gastric cytoplasmic antibodies, found in 83 per cent of patients with pernicious anemia, were detected in 27 per cent of patients with Hashimoto's disease and 33 per cent of patients with thyrotoxicosis. The incidence of antinuclear factors (ANF) in pernicious anemia was lower than in the controls. In SLE the incidence of both thyroid and gastric cytoplasmic antibodies was lower than in the controls, whereas the incidence of ANF was 100 per cent.

6195

Dubois, E.L.; Horowitz, R.E.; Demopoulos, H.B.; Teplitz, R 1965. NZB/NZW mice as a model of systemic lupus erythematosus Arth Rheum 8.441.

A strain of highly inbred New Zealand mice spontaneously develop a disease resembling human SLE. Fifty-seven hybrids were observed for 8 months or longer Antinuclear antibody was positive in 23 of 50 mice Fluorescent antibody technique revealed heavy deposits of mouse gamma globulin in glomerular basement membrane Two new lesions suggestive of human SLE were observed The first was an acute hepatitis reminiscent of early human lupoid hepatitis in 8 of 33 animals (24 per cent) The second lesion was a peculiar splenic fibrosis without evidence of onionskin arrangement about the arterioles observed in 8 of 31 animals This disease represents the first naturally occurring model of human SLE.

6196

Faber, V.; Elling, P. 1964. Antinuclear factor: A new serological reaction. Ugeskr. Laeger 126:1003-1009. In Danish.

A material comprising 1129 sera from normal individuals and from patients with various diseases has been investigated for antinuclear factor (ANF) using the fluorescent antibody technique in order to fix the positive frequency of ANF in the different groups. In healthy individuals 4.6 per cent of the sera showed positive reaction for ANF in low titers. A similar or lower positivity was found in most of the groups of patients with various medical and malignant diseases. ANF was present in 97 per cent of patients with disseminated lupus erythematosus and also frequently in rheumatoid arthritis, hepatic cirrhosis, and in other autoimmune diseases. The diagnostic value of the ANF test is discussed.

6197

Faber, V., Elling, P. 1965. Antinuclear factors (ANF) as determined by the immunofluorescent antibody technique. Acta Med. Scand. 177:309-319.

A material comprising 1,129 sera from normal individuals and from patients with various diseases has been investigated for antinuclear factors (ANF) using the fluorescent antibody technique in order to determine the occurrence of ANF in the different groups. In healthy individuals, 4.6 per cent of the sera showed a positive reaction for ANF in low titers. A similar or lower positivity was found in most of the groups of patients with various medical and malignant diseases. ANF was present in 97 per cent of the sera of patients with disseminated lupus erythematosus and also frequently in rheumatoid arthritis, hepatic cirrhosis, and other autoimmune diseases. The diagnostic value of the ANF test is discussed.

6198

Gargour, G., MacGaffey, K., Locke, S., Stein, M.D. 1964. Anterior radiculopathy and lupus erythematosus cells: Report of a case. Brit. Med. J. 1964:799-801.

The case of a 32 year-old woman with a chronic polyneuropathy manifest as an anterior radicular syndrome is reported. Weakness evolved during a 3.5-year period and was associated with high spinal fluid protein, positive lupus erythematosus cells, and positive serum fluorescent antibody reaction. There was no evidence of systemic lupus. The possibility that lupus presents as an indolent disorder of nerve roots is suggested. The fluorescent antinuclear factor test was negative.

6199

Gonzalez, E.N.; Rothfield, N.F. 1965. The relation of immunoglobulin class of antinuclear factor, pattern of nuclear fluorescence and clinical manifestations in patients with systemic lupus erythematosus. Arth. Rheum. 8:444.

The results of indirect fluorescent antibody tests in 100 patients with SLE were analyzed to determine the effect of disease activity, duration of disease, age, and clinical manifestations on the class of immunoglobulin antinuclear factor (ANF) and on the pattern of nuclear fluorescence. ANF was present in sera from all patients, although only 53 patients had a positive LE cell preparation on the same sample of blood. The presence of IgG or IgM ANF could not be correlated with disease duration, clinical disease activity, or age. IgA ANF was present in 60 per cent of patients with active disease and in 40 per cent of patients in remission. The diffuse pattern of nuclear fluorescence was found most commonly and was produced by all three classes of ANF. The incidence of diffuse pattern was significantly higher in patients in remission than in those with clinical disease activity. The incidence of peripheral pattern was significantly higher in patients with active disease. The peripheral pattern was not found in 65 patients with other diseases in whom ANF was present. The peripheral pattern of nuclear fluorescence may be specific for patients with SLE and is most frequently found in those patients with clinical evidence of active disease.

6200

Good, R.A. 1963. Experimental models in systemic lupus erythematosus. Arth. Rheum. 6:490-512.

Experimental allergic encephalomyelitis is discussed as one possible disease model. FA is mentioned as a tool to find antibody on erythrocytes in HA tests and in the antinuclear factor test. Antibody demonstrated on erythrocytes in hemagglutination tests.

6201

Halberg, P; Bertram, U.; Soborg, M.; Nerup, J. 1965 Organ antibodies in disseminated lupus erythematosus Acta Med Scand 178:291-299

Sera from 29 cases of disseminated lupus erythematosus were tested by FA for the presence of specific thyroid, adrenal, parietal cell, and salivary antibodies. They were also tested for anti-nuclear factor by FA. Other serologic tests were also employed. Organ-specific antibodies were rare, but non-organ-specific antibodies were frequently found. Results were compared with findings from sera of patients with diseases such as chronic thyroiditis, Addison disease, and pernicious anemia. Findings for these latter diseases were generally the reverse of the lupus sera findings.

6202

Hamard, M.; Cannat, A.; Seligmann, M. 1964. Detection of antinuclear antibodies by immunofluorescence: Study of 1,430 sera. Rev. Franc. Etud. Clin. Biol. 9:716-728. In French.

Antinuclear factors were detected by indirect FA in the sera of patients with various diseases, including lupus erythematosus and myasthenia gravis. The significance of the per cent of positives versus type and severity of disease is discussed.

6203

Hamashima, Y. 1964. Systemic lupus erythematosus and the fluorescent antibody technique. Jap. J. Allergy 13:315-319. In Japanese.

Present experimental studies demonstrate significant relationship between the anti-nuclear factor in the serum of LE patients and DNA. An experimental LE condition was also created with anti-DNA rabbit serum. With FA technique, gamma globulin was detected in the nuclei of collagen fibers and renal tissues of rabbits immunized by DNA. This result indicates that gamma globulin can enter the nucleus.

6204

Hamashima, Y.; Odaka, T. 1964. Autoimmune globulins in systemic lupus erythematosus. Jap. J. Med. Sci. Biol. 17:267-277.

Interaction of antinuclear and anticytoplasmic antibodies in the serum of SLE with DNA and experimental production of antinuclear antibodies in immunized rabbits with denaturated DNA-protein combination or denaturated DNA-progesteron combination are discussed. These autoimmune globulins are contained in gamma-2 globulin. Macroglobulins were found in three patients' sera out of 18 cases of SLE, but the macroglobulin did also react with the cell nuclei when tested by the immunofluorescent technique. Two mothers of SLE female patients and one younger brother of an SLE male patient showed antinuclear antibodies in their sera. There might be a hereditary basis in the system of dysresponse in the body.

6205

Hamashima, Y.; Odaka, T. 1964. Antinuclear phenomenon and nucleic acid. Autoimmune globulins in systemic lupus erythematosus. Jap. J. Med. Sci. Biol. 17:282

An abnormal gamma globulin has been found in the sera of patients with acute systemic lupus erythematosus in which LE cells are present. This abnormal protein was investigated by FA, enzymatic digestions, and inhibition procedures. It possesses an apparently specific reactive

affinity for intranuclear DNA in autologous, homologous, and heterologous tissues. Sera from 18 SLE patients and their families were used for the agar-diffusion, immunoelectrophoresis, FA, and ultracentrifugation. Nuclear staining was seen in serum of three relatives. Some patients had both the antinuclear factor and anticytoplasmic factor, and these factors are contained in gamma-2 globulin. These factors react with DNA or DNA-protein, and anticytoplasmic factor reacts with the human hepatic cell mitochondrial fraction and also with the rat hepatic cell mitochondrial fraction non-specifically. Fresh organ specimens were obtained from six autopsy cases of SLE. Nuclear staining on the paraffin sections was shown in mesenchymal cells and secreting cells such as the liver parenchyma, intestinal mucous membrane, glandular cells of pancreas or salivary glands, and renal tubular epithelium. Remarkable nuclear staining was observed in the leukocyte nuclei. In the brain, fluorescent fine granules were observed in the lumen of the cerebral blood vessels. The nucleus and cytoplasm of nerve cells reacted with the factors of SLE serum.

6206

Heide, G. 1963. Production of antinuclear antibodies in animal experiments: Studies on the immunology of visceral lupus erythematosus. Deut. Arch. Klin. Med. 209:144-165. In German.

Rabbits were immunized with calf thymus nuclei and calf thymus deoxyribonucleoprotein. The antisera obtained reacted in complement fixation tests against nuclei of different species including rabbit nuclei. Antibodies were detected against a species-specific antigen in a buffer extract of calf thymus nuclei, histone, and deoxyribonucleic acid. It was thus shown that cell nuclei have antigenic properties. The antibodies were shown to be of low molecular weight. Antibodies could be absorbed from the experimental sera with calf thymus nuclei and were eluted from them in a purified state. Serological investigations indicated that the buffer-extract antibody differed from the corresponding antibody found in human LE sera, although antibodies against histone and DNA are found similarly in human LE sera. The antisera also contained a factor capable of inducing nucleophagocytosis; there was a distinct similarity to human LE cells. It was not finally established whether the nucleophagocytosis-promoting factor of the experimental sera was completely identical with the LE factor present in human LE sera.

6207

Holborow, J.; Johnson, G.D. 1964. Antinuclear factor in systemic lupus erythematosus: A consideration of the immunofluorescent method of detecting antinuclear antibodies, with results obtained in a family study. Arth. Rheum. 7:119-127.

An immunofluorescent technique used for demonstrating antinuclear factor is discussed and a possible influence of differences in method on ANF findings in family studies already reported is considered. The incidence of serum antinuclear factor in first degree relatives of patients suffering from systemic lupus erythematosus was higher than in matched population controls; the difference was a small but a significant one

6208

Homma, M.; Stevens, M.B.; Townes, A.S.; Shulman, L.E. 1965. Anti-DNA antibodies in systemic lupus erythematosus and other disorders. Arth. Rheum. 8:447.

Employing the DNA spot test, we have studied sera from patients with SLE, drug-induced lupus, rheumatoid arthritis, a variety of other disorders, and normal controls. Of more than 100 sera from the 40 patients with SLE studied thus far, all except one have been found to have anti-DNA antibody by this technique, and 80 per cent of these sera have had titers of 1.4 or higher. In general, patients with active disease and those with nephritis had high titers. However, several patients with neither clinical evidence of active disease nor renal involvement had similarly high titers. Low titers of anti-DNA antibody were also demonstrable in some patients with rheumatoid arthritis as well as various other diseases. Of the 25 normal controls studied thus far, only two have shown a minimal reaction. These data indicate that anti-DNA antibodies, as determined by this technique are found in almost all patients with SLE, with or without nephritis and whether the disease is active or not. Moreover, small amounts of anti-DNA antibody may be found in individuals who do not have clinical evidence of SLE.

6209

Horowitz, R.E.; Dubois, E.L.; Channing, A.A. 1965. Pathology of systemic lupus erythematosus in the mouse. Federation Proc. 24:3060:683.

Helyer and Howie in 1961 described a hybrid mouse that, in addition to a hemolytic anemia, showed typical LE cells in the peripheral blood. Tissues from fifty NZB-NZW mice with positive LE preparations, anemia, proteinuria, and ascites were examined histologically, immunocytochemically, and by electron microscopy. The kidneys showed characteristic wire-loop lesions, focal fibrinoid necrosis, and occasional

hematoxylin bodies. The thickened basement membranes fluoresced brightly when stained with fluoresceinated anti-mouse gamma globulin. The glomeruli showed endothelial cell swelling, subendothelial fibrinoid, basement membrane thickening and fusion of the podocytes on electron microscopy. The livers of one-fourth of the mice showed active single-cell necrosis and regeneration and inflammation reminiscent of lupoid hepatitis. The spleens of another one-fourth of the animals showed dense perivascular fibrosis. The clinical and morphologic features these animals present suggest that they may serve as useful models for the study of human systemic lupus erythematosus. Complete article.

6210

Johnson, G.D.; Bencze, G. 1965. The effect of heparin on nuclear immunofluorescence. Proc. Congr. Int. Soc. Blood Transfus. 10:40-42.

Following the observation that production of lupus erythematosus cells in the blood of patients suffering from systemic lupus erythematosus may be inhibited by adding excessive amounts of heparin to the specimen, the effect of heparin in the fluorescent test for antinuclear factor has been studied. Treatment of cryostat sections with heparin prevented staining of nuclei in the antinuclear factor test and also abolished the Feulgen reaction. Similar results were obtained with fresh human leukocyte preparations.

6211

Joseph, R.R.; Zarafonetis, C.J.D. 1965. Fatal systemic lupus erythematosus in identical twins: Case reports and review of the literature. Amer. J. Med. Sci. 249:190-199.

Two cases of fatal systemic lupus erythematosus in identical twins together with evidence of rheumatic disease in their father have been added to the growing literature on familial occurrence of this disease. The pre-existing case reports have been reviewed, as has the literature on the existence of laboratory stigmata of rheumatic disease in asymptomatic relatives of patients with systemic lupus. A genetically determined defect may underlie this disease.

6212

Kalsbeek, G L ; Cormane, R H ; Utrecht, M B ; Leyden, M D 1964 'Bound' complement in the skin of patients with chronic discoid lupus erythematosus and systemic lupus erythematosus. Lancet 2:178-180.

These results show that, in the skin lesions of discoid and systemic lupus erythematosus, anti-human complement (factors C'3a and C'4) and anti-human globulin are concomitantly bound to the region of the basal membrane In both forms of lupus erythematosus, therefore, the localized gamma globulin is probably immunological in character The phenomenon does not occur in psoriasis, atopic dermatitis, eczematous dermatitis, or seborrheic dermatitis The use of fluorescein-labeled gamma globulin in combination with fluorescein-labeled anti-human complement seems appropriate for diagnostic purposes.

6213

Lange, K , Ores, R ; Strauss, W ; Wachstein, M 1965 Steroid therapy of systemic lupus erythematosus based on immunologic considerations Arth Rheum 8.244-259

Patients with systemic lupus erythematosus were treated continuously with large doses of glucocorticoids until the immunologic abnormalities had returned to normal, irrespective of prior relief from subjective complaints Thereafter, prolonged intermittent steroid therapy with large doses was used, guided by immunologic data and continued for at least 2 years With this therapy immunosuppression was maintained and the disease was controlled Side effects were minimal Loss of renal function markedly improved under this therapy even in advanced cases Immunofluorescent histology was performed on specimens from renal biopsies

6214

Lange, K ; Ireser, G , Wachstein, M ; Wasserman, E 1964 Routine immunofluorescent histology as an aid in the diagnosis and prognosis of renal diseases Amer J Pathol 44:14a-15a

Kidney biopsy specimens from normal individuals and 53 patients with renal diseases were studied by FA for human gamma globulin, undivided complement, complement component C'1, complement component C 3, and human albumin Antigens were deposited by immune processes and not by transudation into inflamed tissues In acute glomerulonephritis there was intense staining for gamma globulin and all complement components The staining was diffuse on or near the basement membrane and in the area of the mesangium With clinical healing, the staining became segmental and disappeared with complete clinical healing Even slight urinary abnormalities were accompanied by segmental hyperfluorescence In chronic glomerulonephritis there was

diffuse intensive staining of the basement membrane. Segmental or no staining appeared in the fibrotic and hyalinized regions. Intense staining was associated with active SLE. It receded or disappeared after prolonged glucocorticoid treatment. In active pure nephrosis, staining for gamma globulin and complement components was in the basement membranes. Staining of the mesangium was lacking. A patient with severe Bence Jones proteinuria exhibited no staining. Complete article.

6215

Larsson, O. 1963. Thymoma and systemic lupus erythematosus in the same patient. Lancet 2:665-666.

A case of systemic lupus erythematosus and simultaneous thymoma is described. The two diseases developed at the same time and a connection between them seems probable. The thymoma was extirpated and investigated. With the fluorescent antibody technique no trace of antibody production was found in it.

6216

Lukanina, K.V.; Dolzhanskii, V.M. 1965. Use of the fluorescent antibody method for the laboratory diagnosis of systemic lupus erythematosus. Terap. Arkh. 37:10:70-73. In Russian.

An FA method has been suggested for laboratory diagnosis of systemic lupus erythematosus. Nuclei of chick erythrocytes are used as an antigen. A total of 140 serum samples were studied; 23 from patients with systemic lupus erythematosus in the active stage and 14 from those in the remission stage, 23 from patients with other collagenoses, 42 from patients with other pathology, and 30 from healthy donors. A markedly positive reaction was produced only with the sera of patients having systemic lupus erythematosus in the remission stage. Some serum samples from patients with other collagenoses exhibited a slightly positive reaction.

6217

Mazzei, D.; Luporini, G.; Del Giacco, G.S. 1964. Antinuclear factors in collagen diseases. Rass. Clin. 40:305-313. In Italian.

The techniques and antigens used by researchers in the investigation of antinuclear factors in collagen diseases are tabulated and discussed. A total of 57 sera taken from patients with mesenchymal diseases was examined and compared with controls. The following methods were used: test for lupus erythematosus (LE) cells, conditioned agglutination, precipitation with DNA in agar, and immunofluorescence. The fluorescent sera used were human anti-gamma globulin serum, anti-7S gamma globulin serum, and anti-beta-2M globulin serum, each conjugated with FITC.

Several different types of nuclear fluorescence were distinguished. In addition to finding LE cells in all cases of disseminated lupus erythematosis, a positive result for antinuclear antibodies in all 24 cases tested with immunofluorescence was obtained. The best results in diagnosis can still be had by relying on the LE cell test. Although the immunofluorescence test yields the same specificity, it is more elaborate to perform

6218

McCormick, J.N.; Day, J. 1963. The association of rheumatoid factor with antinuclear factor activity. Lancet 2:554-556.

The removal of rheumatoid factor by exhaustive absorption with sensitized sheep cells and/or aggregated human gamma globulin was accompanied by complete or substantial loss of 7S and 19S antinuclear factor, demonstrated by FA, from serum of eight patients with rheumatoid arthritis, one with systemic lupus erythematosus (SLE), and one with dermatomyositis. When rheumatoid factor was originally absent, as in four other SLE sera and in the serum from a patient with a drug reaction, similar absorption procedures caused no appreciable loss of antinuclear factor activity. From these findings it is suggested that 7S antinuclear factor may be associated with rheumatoid factor in an ambivalent 19S/7S complex.

6219

Meneghini, C.L.; Cozza, G. 1963. The demonstration of antinuclear factors in the blood of patients with lupus erythematosus by the fluorescent antibody technique; Immunoelectrophoretic observations. G. Ital. Dermatol. 104.323-326. In Italian.

Antinuclear factors were shown in acute LE sera by a fluorescent antibody technique. Antinuclear factors were also found in four sera of 10 examined of subacute LE with rather fixed lesions. The authors admit a unitary pathogenesis of LE in all its clinical forms. The fluorescent techniques have a higher sensitivity, in comparison with the other methods, to show antinuclear factors. Nucleated chicken red blood cells are suggested as substratum, because they allow clear-cut results in a more simple way. The immunoelectrophoresis of acute LE sera demonstrated an increase of macro alpha-2, lipo, beta-2M, and gamma globulins; in some cases there was a decrease of the beta-1A fraction. The same sera, after absorption on nuclear material, when tested with antihuman globulin rabbit sera, did not show a specific disappearance of any band of precipitation.

6220

Meneghini, C.L.; Cozza, G. 1964. Experimental pathogenetic studies with the fluorescent antibody technique in lupus erythematosus: Apropos of the unitary pathogenesis of acute and chronic lupus erythematosus. G. Ital. Dermatol. 105:45-52. In Italian.

This report of 1,151 cases of lupus erythematosus emphasizes the unitary pathogenesis of all the different clinical forms of LE and describes two cases of subacute LE with nonfixed and superficial lesions that evolved to the serious acute systemic forms. The serologic tests performed with the fluorescent antibody technique showed antinuclear antibodies in all the cases of acute LE, in five cases of 20 subacute LE and in three cases of 63 chronic fixed LE. On the bases of this data, together with the changes of plasma proteins, the unitary autoimmune pathogenesis of LE is proposed.

6221

Paronetto, F.; Deppisch, L.; Tuchman, L.R. 1964. Lupus erythematosus with fatal hemorrhage into the liver and lesions resembling those of periarteritis nodosa and malignant hypertension. Amer. J. Med. 36:948-955.

Immunocytochemical studies were made in a patient with lupus erythematosus also exhibiting lesions resembling those seen in periarteritis nodosa and malignant nephrosclerosis, including healed and healing vascular lesions in medium-sized arteries in various abdominal organs, rupture of an aneurysm of a branch of the hepatic artery, and necrotizing lesions in the renal arterioles. Gamma globulin, complement, albumin, and fibrinogen were found in the vascular lesions; in the renal glomeruli, gamma globulin was associated only with complement. It is suggested that these lesions have a common pathogenesis. Harmful antigen-antibody complexes and complement are localized in glomeruli and vessels, the latter injured by hypertension, to produce increased permeability and secondary inhibition with plasma proteins.

6222

Paronetto, F.; Koffler, D. 1965. Immunohistochemical observations in systemic lupus erythematosus and glomerulonephritis. Federation Proc. 24:3059:683.

To assess the relationship of protein deposition to tissue damage, kidneys of 16 patients with SLE, and of 15 patients with acute, subacute, and chronic glomerulonephritis were studied with the fluorescent antibody technique as to: type of immunoglobulin; complement, beta-1C globulin; and deposition of other plasma proteins, fibrinogen, alpha-2 macroglobulin

and albumin Patients with SLE with lupus nephritis exhibited gamma-2 and gamma-1M globulin, complement, and fibrinogen in the renal glomeruli and arterioles with fibrinoid necrosis. Gamma-1A globulin, alpha-2 macroglobulin, and albumin were rarely seen Three cases showed immunoglobulin staining of nuclei of renal epithelial cells. In acute glomerulonephritis fibrinogen, gamma globulin and complement were localized in renal glomeruli. In subacute and chronic glomerulonephritis glomeruli exhibited plasma proteins similar to that seen in SLE but in decreased amounts. Gamma-2 and gamma-1M globulin are the immunoglobulins present in the lesions of SLE and glomerulonephritis. The localization of complement suggests the presence of immune complexes in the lesions. Renal glomerular deposits of fibrinogen indicate that alterations in blood coagulation may play a role in the pathogenesis of these diseases. Complete article.

6223

Peterson, W. C., Jr.; Fusaro, R. M. 1963. Antinuclear factor in light sensitivity and lupus erythematosus. Arch. Dermatol., 87:563-565.

Antinuclear antibodies were studied by FA in the sera of 17 patients with chronic discoid lupus erythematosus and 17 cases of light sensitivity eruptions. In the cases of chronic discoid lupus erythematosus ten sera showed positive fluorescence in the nuclei, while seven were negative. All the sera from patients with light sensitivity eruptions were negative for nuclear fluorescence with one exception in which weakly positive fluorescence was observed. Use of this technique is suggested as an aid in differential diagnosis.

6224

Pollak, V. E. 1963. Hereditary factors in systemic lupus erythematosus. J. Lab. Clin. Med. 62:1001.

Clinical reports have suggested the possible importance of hereditary factors in the genesis of systemic lupus erythematosus (SLE). Sera from 189 relatives of 43 patients with SLE were studied for antinuclear antibodies by FA. Serial dilutions of serum were made. FA staining ≥ 4 was called positive. In 25 of 43 families, antinuclear antibodies were observed in one or more relatives. Positive tests were found in 47 of the 142 first degree relatives and in 10 of the 47 second degree relatives studied. All tests were negative in sera from 71 healthy subjects, from 24 relatives of 10 matched healthy probands, and from spouses of seven patients. In addition, 271 patients with diseases unrelated to SLE were investigated; 11 positive tests were found. Titers of antinuclear antibodies in relatives were lower than those in 334 sera from 112 patients with SLE but were comparable to those in patients with SLE in remission.

A second case of clinical SLE occurred in four families and of acute scleroderma in one. The mothers in two families and the grandmother in a third were healthy initially and developed clinical diseases later. An increase in antinuclear antibody titer accompanied the appearance of clinical SLE. The incidence of antinuclear antibodies was significantly higher, P is less than 0.01, in these five families than in 38 families in which no second case of SLE has developed. Hereditary factors are important in the production of both antinuclear antibodies and clinical disease SLE.

6225

Pollak, V.E. 1964. Antinuclear antibodies in families of patients with systemic lupus erythematosus. New Engl. J. Med. 271:165-171.

Antinuclear antibodies were found in 91.5 per cent of serum samples from patients with systemic lupus erythematosus and in 25 per cent of patients with rheumatoid arthritis and other collagen diseases. They occurred in only 4 per cent of 271 patients with diseases other than systemic lupus erythematosus and the collagen diseases, and in none of the 71 healthy subjects. Antinuclear antibodies were found in relatives of patients with systemic lupus erythematosus in 25 of 43 families studied. In all, positive tests were found in 33 per cent of 142 first-degree and 21 per cent of 47 second-degree relatives. They were not detected in serum specimens of 24 relatives of matched healthy subjects. These observations strongly suggest that hereditary factors are important in the production of antinuclear antibodies.

6226

Pollak, V.E.; Pirani, C.L.; Schwartz, F.D. 1964. The natural history of the renal manifestations of systemic lupus erythematosus. J. Lab. Clin. Med. 63:537-550.

Serial clinical observations and histologic studies were made on the kidneys of 87 patients with systemic lupus erythematosus over periods ranging from 7 months to 8 years. A total of 176 renal biopsy and necropsy specimens were analyzed by a semiquantitative method, and the histologic findings were classified: normal kidney, lupus glomerulitis, active lupus glomerulonephritis, and membranous lupus glomerulonephritis. Progression from the milder forms of renal involvement to severe active lupus glomerulonephritis was uncommon. Most patients with active lupus glomerulonephritis died in renal failure, but the clinical and histologic progression of the disease was slowed or halted in many by prolonged treatment with large doses of prednisone. Antinuclear antibodies were found in all patients.

6227

Ritchie, R.F.; Bayles, T.B.; Harter, J.G. 1964. A fluorescent antibody inhibition method for classification of serum antinuclear factors. Arth. Rheum. 7:339.

FITC conjugates were prepared from gamma globulins of patients with systemic lupus erythematosus or rheumatoid arthritis. It was possible to inhibit fluorescence of these conjugates with normal and diseased sera. Fluorescence of conjugates from rheumatoid arthritis globulins were nearly always inhibited by diseased or normal sera. Fluorescence of conjugates of lupus globulins was nearly always inhibited by serum of lupus patients only. Globulin conjugates from patients with other than classic lupus reacted similarly to those of rheumatoid patients. This inhibition technique permits the multiple factors present in sera to be distinguished. It offers a potential method of assessing clinical significance.

6228

Rothfield, N.F.; Frangione, B.; Franklin, E.C. 1965. Slowly sedimenting mercaptoethanol-resistant antinuclear factors related antigenically to M immunoglobulins (gamma-1M globulin) in patients with systemic lupus erythematosus. J. Clin. Invest. 44:62-72.

An unusual antinuclear antibody in sera from patients with systemic lupus erythematosus has been reported. The antibody is a small molecule that is antigenically related to the M immunoglobulins but sediments slowly in a sucrose density gradient and is eluted after the first peak from a Sephadex G-200 column. The antibody retains its activity after treatment with mercaptoethanol. The unusual antibody is present in a high proportion of male patients and is most frequently found in sera from patients with active disease of short duration. The possible significance of the unusual antibody has been discussed, and alternative explanations for its presence in the sera from patients with systemic lupus erythematosus have been proposed.

6229

Schwartz, R.S.; Lewis, R.M.; Henry, W.B.; Gilmore, C.E. 1963. Systemic lupus erythematosus in the dog. J. Clin. Invest. 42:976-977.

Seven young adult dogs that spontaneously developed typical features of systemic lupus erythematosus have been studied. They had the following features: severe autoimmune hemolytic anemia in each case, idiopathic thrombocytopenic purpura in four cases, and severe glomerulonephritis in four cases. There was no relationship to breed or sex. One dog had salicylate-responsive lameness, and eruption on the butterfly

area of the face, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, and glomerulonephritis. Biopsy of this dog's skin rash showed lesions identical to discoid lupus. The LE clot test was positive in six dogs. Two of four animals tested had fluorescent antinuclear antibody, as did the asymptomatic daughter of one of the propositi. The antiglobulin test was positive during the phase of the disease in which hemolytic anemia was present. Eluates prepared for antiglobulin-positive erythrocytes sensitized randomly selected, normal, dog red cells. The Hinton test was negative in all. Pathological findings included wire loop lesions of glomeruli, myocarditis, and hepatitis. Systemic lupus erythematosus may occur in at least three vertebrate species, mouse, dog, and man. The sequential development of multiple immunologic abnormalities in these dogs bears a strong resemblance to systemic lupus erythematosus in man.

6230

Seligmann, M.; Hurez, D.; Hamard, M. 1964. Study of different varieties of antinuclear antibodies detected in the serum of patients with disseminated lupus erythematosus. Bibl. Haematol. 19:322-328. In French.

A description is given of the different varieties of antinuclear factors found in the sera of patients with lupus erythematosus, as well as of the nuclear constituents toward which they react, and of the techniques used to detect them in the sera. The practical usefulness of each one of the methods is discussed; attention is drawn to their respective sensitivity and specificity. The possible pathogenic action of these factors in vivo and the nature of the mechanism leading to their synthesis are briefly discussed.

6231

Shrank, A.B.; Doniach, D. 1963. Discoid lupus erythematosus: Correlation of clinical features with serum auto-antibody pattern. Arch. Dermatol. 87:677-685.

Eighty patients with discoid lupus erythematosus (LE) were surveyed clinically and serologically for evidence of systemic involvement. The incidence of autoantibodies to three thyroid constituents, gastric parietal cells, and cell nuclei (ANF) were compared in the patients and controls matched for age and sex. There was a threefold increase in positive ANF in the patients of both sexes, but the incidence of thyroid antibodies was increased only in female patients. Fourteen of the patients were considered to have mild systemic involvement, but there was no evidence of progression from chronic discoid LE or from this intermediate state to systemic LE in this series followed up for 2 to 30 years.

6232

Shulman, L.E.; Gumpel, J.M.; D'Angelo, W.A.; Souhami, R.L.; Stevens, M.B.; Townes, A.S.; Masi, A.T. 1964 Antinuclear factor in inbred strains of mice: The possible role of environmental influence. Arth. Rheum. 7:753.

We have attempted to induce systemic lupus erythematosus in the A/J mouse strain by the chronic administration of hydralazine. Other strains, both genetically related, as the A/He, and unrelated, as the C-57-B1/6J, were selected as controls. Twenty-five per cent of A/J retired breeders have antinuclear factor. Unexpectedly, antinuclear factor was also found in the control strains: 39 per cent of A/He and 20 per cent of C-57-B1/6J mice. Their finding has confirmed that antinuclear factor is not present in younger mice of the A/J strain, but we were able to find it in 20 per cent of the younger A/He mice. LE cell testing of positive sera revealed extracellular LE bodies, but not LE cells. Because antinuclear factor had been demonstrated in several different inbred strains of mice, we sought to test the influence of the environment on the formation of antinuclear factor by obtaining the same inbred strains from another producer. The proportions of antinuclear factor in retired breeders of the same age from the second producer were strikingly lower; 5 per cent of the A/He strain and 3 per cent of the C-57-B1/6J strain. We confirmed by skin grafting that the strains had maintained their genetic identity. These data suggest that there may be important environmental determinants in the development of antinuclear factor in mice

6233

Solheim, B.G. 1963. Antinuclear factors: Demonstration by means of fluorescent antibodies. Nord. Med. 70:1343-1346. In Swedish.

The demonstration of antinuclear factors by immunofluorescence is simple in a modern equipped special laboratory. The result can be available after 2 to 3 hours and is easy to interpret. The test for antinuclear factors will nearly always indicate if there is evidence of lupus erythematosus disseminatus In addition, their presence, especially in high concentrations, generally indicates a change in the immunological apparatus Regular examination of patients with positive reactions can be important in evaluation of the danger of transition to lupus erythematosus disseminatus. A regular checking of the titer of the patient with this disease will be helpful when such a patient returns to the hospital with a relapse. It will then be easier to determine whether the relapse is due to an intercurrent infection or an exacerbation, as, in the latter case, the titer will often be significantly higher than in the controls.

6234

Souhami, R.L.; Honma, M.; Gumpel, J.M.; Stevens, M.B.; Shulman, L.E. 1965. The absence of antinuclear factors (by immunofluorescence) in certain patients with abnormal LE cell tests. Arth. Rheum. 8:470.

Over the past 2 years, 22 patients have been identified with an abnormal lupus erythematosus cell test and a negative antinuclear factor test, as determined by immunofluorescence. These sera were tested using human white blood cells as a substrate in the immunofluorescent test, because previous studies had suggested that the sensitivity was thereby enhanced. With this method, five of the seven patients with LE cells and 11 of the 15 with ECM were negative. The remaining six were positive in very low titer, and in only one of these was this reproducible. The clinical analysis of these patients was interesting in that none had definite systemic lupus erythematosus. The difference between these cases and SLE was further emphasized by finding that anti-DNA antibodies were present in titers of 1:4 or greater in only three of these 22 patients, compared with a frequency of 80 per cent in SLE sera. The presence of an abnormal LE cell test in the absence of antinuclear factor is a departure from the findings in SLE. It may reflect either a different mechanism of LE cell formation, possibly due to differences in antibody, or the presence of serum factors inhibiting the immunofluorescent test.

6235

Sturgill, B.C.; Carpenter, P.R. 1964. Antibody to ribosomes in systemic lupus erythematosus. Arth. Rheum. 7:347-348.

Antibodies against a number of cytoplasmic constituents, including a trypsin-sensitive extract of microsomes, have been demonstrated in patients with systemic lupus erythematosus. We have used rat and rabbit liver ribosomes adsorbed onto bentonite particles to test sera from patients with SLE. Absorption of positive sera with ribosomes or calf thymus nucleoprotein abolished the antiribosomal reactivity, but absorption with native or thermally denatured DNA, thymine, uracil, ADP, or yeast RNA was without effect. A positive serum was fractionated on DEAE cellulose, and anti-ribosomal activity was found in gamma globulin by immunoelectrophoresis. The analogous fraction from normal serum was nonreactive. Most sera with antiribosomal activity have shown cytoplasmic fluorescence in indirect immunofluorescence staining of tissue, which could be abolished by prior absorption with ribonucleoprotein or ribosomes. In one patient, the level of antiribosomal antibody has closely paralleled the anti-DNA antibody titer and the clinical course of the illness.

6236

Sturgill, B.C.; Carpenter, R.R. 1965. Antibody to riborcmes in systemic lupus erythematosus. Arth. Rheum. 8:213-218.

Antibody to ribosomes has been demonstrated in the sera of several patients with SLE. The specificity of the reaction appears to be related, at least in part, to soluble RNA. This reactivity appears to occur almost exclusively in the serum of patients with SLE. Antinuclear antibody was detected by FA.

6237

Sturgill, B.C.; Carpenter, R.R ; Strauss, A.J.L.; Goodman, H.C. 1964 Antibodies in systemic lupus erythematosus and myasthenia gravis which react with thermally denatured DNA-coated bentonite. Proc. Soc. Exp. Biol. Med. 115:246-251.

Sera from 66 per cent of 35 patients with SLE flocculated bentonite particles coated with thermally denatured DNA; only 29 per cent of these same sera flocculated bentonite particles coated with native DNA All of these sera contained antinuclear antibody. Twenty per cent of sera from 44 patients with myasthenia gravis flocculated the bentonite particles coated with denatured DNA, but none reacted with the native DNA bentonite. Thirty-seven per cent of the sera from patients with myasthenia gravis contained antinuclear antibody. Rheumatoid factor and antibodies to thyroglobulin were occasionally seen in low titer in the serum from patients with myasthenia gravis.

6238

Svec, K.H.; Kaplan, M.H. 1963. A variant LE cell factor reactive only with altered nuclear material. Arth. Rheum. 6:11-22.

An antinuclear factor from the serum of a patient with systemic lupus erythematosus has been described that exhibits LE cell factor activity although it is nonreactive with nuclei in tissue sections or with beef thymus nucleoprotein. This factor demonstrated reactivity limited to nuclear material of leukocytes only after the cells were subjected to mechanical trauma. As determined by absorption tests with traumatized leukocytes, it is suggested that this factor is related to the LE cell activity present in the same serum Evidence is presented that this factor is fundamentally different from the previously described LE cell factor and represents a second type of lupus factor with LE cell activity. This second LE cell factor presumably occurs rarely in sera of lupus patients.

6239

Takahashi, T.; Lee, C.L.; Biava, C.; Davidsohn, I. 1965. Localization of gamma-globulin within inclusion bodies of L.E. (lupus erythematosus) cells. Nature 207:1071-1073.

Three methods were used: autoradiography, immunoelectron microscopy, and FA. The methods were complementary. The presence of gamma globulin in the inclusion bodies of LE cells was demonstrated. This gamma globulin may consist, in part, of the LE factor.

6240

Tan, E.M.; Kunkel, H.G. 1964. Immunofluorescent study of skin lesions of systemic lupus erythematosus. Arth. Rheum. 7:348-349.

Skin lesions of patients with systemic lupus erythematosus were biopsied and studied for localization of gamma globulin by the immunofluorescent method. Three principal findings were observed. Gamma globulin was frequently observed at the junctional region between epidermis and dermis as a continuous layer of dense speckles. In this region, gamma globulin was also frequently present in large, discrete clumps that resembled hematoxylin bodies. The staining for gamma globulin of these structures could be abolished by treatment with DNAase, but not RNAase. A third finding was the demonstration of gamma globulin in the nuclei of epidermal cells, in the nuclei of sweat glands, and in nuclei of cells surrounding small blood vessels in the dermis. Skin biopsies were taken from the same patient in areas with and without rash. The areas without rash occasionally showed scattered small bands of speckled staining at the junctional layer but none of the other findings. Biopsies of normal skin from patients with other diseases were uniformly negative. These studies have demonstrated extensive in vivo localization of antibodies in skin lesions of SLE, both in nuclei of cells and in other skin structures.

6241

Thivolet, J.; Verjus, F.; Kratchko, A. 1964. Hereditary factors in disseminated lupus erythematosus: Familial, clinical, and serologic investigations. Ann. Dermatol. Syphiligr. 91:361-378. In French.

The results of systemic, clinical, and serological investigations on consanguinous subjects with disseminated lupus erythematosus (LE) are reported. Clinically, 149 individuals of 13 different families were examined. Among these one case of chronic lupus erythematosus, one of hemolytic anemia, and one of Raynaud's disease were detected, but no disseminated LE. Serologically, 4 per cent of 50 sera showed antinuclear antibodies by the immunofluorescence method and 11.4 per cent revealed a clear-cut hypergammaglobulinemia. These investigations reveal the existence of a hereditary predisposition to disseminated LE.

6242

Thompson, G.R ; Simpson, J.F.; Westerberg, M.R. 1965. Antinuclear, antimuscle, and rheumatoid factors in systemic lupus erythematosus and myasthenia gravis. Arth. Rheum. 8:475.

A number of gamma globulin fractions with the properties of antibodies against cells and components of cells have been identified in serum from patients with systemic lupus erythematosus. Similar antibodies have been reported in myasthenia gravis (MG) sera In addition, skeletal and cardiac muscle-binding globulins have been found in MG sera. To assist in evaluating the incidence and specificity of these factors, 39 MG and 18 SLE sera were studied Antimuscle antibodies were studied by the fluorescent antibody technique. Skeletal muscle-binding globulins were present in 30.8 per cent of MG sera; specific fluorescence of cardiac muscle was observed in 31.2 per cent. All sera were concordant for skeletal and cardiac muscle reactivity except one, which was positive only with cardiac muscle. No specific binding was observed with smooth muscle. None of the SLE sera reacted with skeletal, cardiac, or smooth muscle. Three of 33 control sera reacted with muscle, but the pattern of binding was distinctive and different. It is of interest that none of the SLE sera contained muscle-binding globulins

6243

Wierzchowiecki, M; Jeromski, J. 1965. Antiglobulin consumption test and studies of immunofluorescence in the diagnosis of systemic lupus erythematosus Pol. Arch Med. Wewnetranej 35:1019-1024. In Polish.

Results of two methods are presented for detecting nuclear antibodies in the serum of patients with SLE. The antiglobulin consumption test was positive in all patients with SLE studied, while the test using FA was positive in 11 of 12 subjects. It is claimed that the tests are of great diagnostic value in patients suspected of SLE.

6244

Willkens, R F.; Decker, J.L. 1963 Rheumatoid arthritis with serologic evidence suggesting systemic lupus erythematosus: Clinical, serologic, and chromatographic studies. Arth. Rheum. 6:720-735.

Fourteen patients with long-standing rheumatoid arthritis characterized by severe articular deformities, subcutaneous nodules, and visceral manifestations are described Eight had chronic ulcerative skin lesions of the lower extremities. Despite the presence of a variety of antinuclear factors in their sera, they were not considered to have systemic lupus erythematosus. Serum protein chromatography suggested that the antinuclear factors in rheumatoid arthritis

were more frequently composed of larger protein molecules than in systemic lupus erythematosus. The antinuclear factors are considered to be a manifestation of rheumatoid arthritis and clinical evidence suggested that chronic infection may have played a role in their development.

6245

Wilske, K.R.; Shalit, I.E.; Willkens, R.F.; Decker, J.L. 1965. Findings suggestive of systemic lupus erythematosus in subjects on chronic anticonvulsant therapy. Arth. Rheum. 8:260-266.

An institutionalized population was examined under standardized conditions for physical and laboratory abnormalities seen with systemic lupus erythematosus or other connective tissue diseases. Multiple abnormalities were found in about 8 per cent of those on chronic anticonvulsant therapy and in none of those not receiving such treatment; phenobarbital was as closely related as diphenylhydantoin but the combination may be most important. The abnormalities were interpreted as reactive results of the drugs; the ability to react was considered to be restricted to a small proportion of the population. FA detected antinuclear antibodies in subjects.

6246

Wolf, P.L.; Pearson, B.; Clifford, G.O. 1963. The production of glomerular lesions in canine kidney by injection of plasma from patients with systemic lupus erythematosus Lab. Invest. 12:853-854.

When plasma from patients with systemic lupus erythematosus is infused intravenously into dogs, a renal lesion develops in the majority of the recipient animals. The renal changes consist of thickening of glomerular capillaries, hypercellularity, hyperemia, and occasional focal lesions resembling wire loops. PAS stains indicate thickening of basement membrane or marked endothelial cell swelling. Infusion of plasma from normal subjects and patients with other collagen diseases did not produce similar changes. FA studies have indicated that a factor in systemic lupus erythematosus plasma adheres to the glomeruli of normal dogs when overlaid directly. The glomeruli of dogs previously infused with systemic lupus erythematosus plasma show some fluorescence when overlaid with fluorescein-labeled anti-human globulin. A factor in systemic lupus erythematosus plasma may be capable of producing a lesion in the kidneys of the dog resembling that of the disease in the human. An immunologic mechanism may be involved.

6247

Zingale, S.B.; Minzer, L.; Rosenberg, B.; Lee, S.L. 1963. Drug-induced lupus-like syndrome: Clinical and laboratory syndrome similar to systemic lupus erythematosus following antituberculous therapy, report of a case. Arch. Intern. Med. 112:63-66.

A case of a 61-year-old man, who developed clinical and laboratory manifestations of systemic lupus erythematosus while undergoing treatment for tuberculosis, is presented. Antinuclear antibody was measured by FA. Isoniazid induced the systemic lupus erythematosus - like syndrome in this patient because of the relationship between the dose administered and the length of time the patient was treated with this drug.

6248

Zitnan, D 1964. Antinuclear factors and their relationship to the activity and aggressiveness of systemic lupus erythematosus. Cas. Lek. Ceskych 103:515-519. In Czech.

Antinuclear factors examined by the indirect method of fluorescent antibodies can be detected in practically all cases of systemic lupus erythematosus. When estimated semi-quantitatively, they are usually found in higher titers, particularly in active cases with symptoms of affection of internal organs, specially the kidneys. Their level is markedly reduced by glucocorticoids administered in suppressive doses. In view of a certain agreement between levels of antinuclear factors and the intensity and progression of the disease, they can be considered in the quantitative sense as indicators of the activity and aggressiveness of the fundamental pathological process in lupus erythematosus disseminatus.

3. Myasthenia Gravis

6249

Abernathy, R.S. 1965. Auto-immunologic reactions to muscle in myasthenia gravis. Ann. Intern. Med. 62:1087.

Indirect FA on four patients showed binding of their serum gamma globulins to slices of either normal or MG muscle obtained by biopsy. Specific globulin binding was localized to the muscle striations. It was blocked by prior absorption of the serum with muscle but remained after absorption with human liver powder. Diffuse sarcolemmal staining was also seen but was not specific. In three MG patients such antibodies were

shown to be truly autoimmune, as they combined with the patient's own muscle. Presence of muscle autoantibodies could not be correlated with the duration, activity, or severity of the disease. In four MG patients muscle obtained by biopsy was examined by direct FA. No muscle-bound globulin was found in any patient, even though serum from three patients bound with muscle in vitro. Some patients with MG possess circulating autoantibodies to muscle. The role of such antibodies in the pathogenesis of MG remains conjectural.

6250

Adner, M.M.; Sherman, J.D.; Ise, C.; Schwab, R.S.; Dameshek, W. 1964. An immunologic survey of forty-eight patients with myasthenia gravis. New Engl. J. Med. 271:1327-1333.

An immunologic survey of 48 patients with myasthenia gravis revealed a significant incidence of antibodies directed against normal tissues. Ability to express delayed (cellular) hypersensitivity reactions, as measured by the development of contact sensitivity to dinitrochlorobenzene, significantly decreased in only 45 per cent of patients with myasthenia gravis as compared with 86 per cent of a control group. There was no evidence of impairment of humoral-antibody production. There were no immunologic abnormalities that could be attributed to the effect of thymectomy per se. Myasthenia gravis may be an autoimmune disorder in which antibodies produced, stimulated or otherwise controlled by the thymus in some way, interfere with motor end-plate function. It is also possible that a cellular hypersensitivity mechanism is involved in the pathogenesis of this disease and that the abnormal contact-sensitivity reactions noted in the myasthenic patients reflect alterations in this mechanism.

6251

Anonymous. 1965. Myasthenia gravis: A third tissue-specific antibody? J. Amer. Med. Ass. 191:31.

Controversy surrounds tentative evidence of thymus-specific antibody. Clinical correlation of cross-reactive antibody to muscle and thymus, or muscle only, is reported. FA titration studies of 30 patients showed 15 had good correlations between positive levels of antibody and changes in clinical state.

6252

Barnett, E.V.; Bakemeier, R.F.; Leddy, J.P.; Vaughan, J.H. 1965. Heterogeneity of antinuclear factors in lupus erythematosus and rheumatoid arthritis. Proc. Soc. Exp. Biol. Med. 118:803-806.

Twenty-six sera from adults and children with lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, or chronic active hepatitis were tested in a three-layer immunofluorescence test on human white cell nuclei. Rabbit antisera against human Type I and Type II Bence Jones protein were used to determine whether the antinuclear factors contained Type I or Type II L chains. Twenty-four of the sera were shown to contain ANF with both types of L chains. In the remaining two cases quantitative considerations limited the study. Both Type I and Type II ANF were also found in isolated fractions of gamma-2 and gamma-1M globulins in four lupus sera. Antinuclear factor was determined by the three-layer immunofluorescence test.

6253

Beutner, E.H.; Leff, I.L.; Fazekas, G.; Witebsky, E. 1965. Immunologic studies of two cases of fatal myasthenia gravis. J. Amer. Med. Ass. 191:461-465.

Tissues and blood sera obtained from two cases of fatal myasthenia gravis were examined serologically by indirect immunofluorescent staining and the tanned-cell hemagglutination test. The sera of both patients contained antibodies specific for skeletal and heart muscle, but no complement fixing antibodies to skeletal muscle. In both cases immunofluorescent staining was observed with sections of the muscle of the patient. The tanned-cell test performed with globulins from the skeletal muscle of the patients yielded positive results in one case. The heart antigen, but not the antigens prepared from various skeletal muscles of the other patient, contained the active antigen; the absence of muscle antigen appeared to be due to its neutralization in the extraction process by the antibodies of the patient.

6254

Beutner, E.H.; Witebsky, E.; Leff, I. 1963. Autoimmune responses in myasthenia gravis: Observations on S and SH antigens, on species specificity, and on in vivo reactions. Federation Proc. 22:341:217.

Autoantibodies to skeletal, S, and skeletal and heart, SH, antigens of muscle may be demonstrated in myasthenic sera by complement fixation and by direct and indirect immunofluorescent staining respectively. Absorption of sera with the saline-insoluble fraction of heart muscle

abolished the indirect staining activity on heart muscle sections but the indirect or complement staining activity on skeletal muscle section remains. This activity is by definition S antibody. Thus indirect immunofluorescent staining reveals both S and SH antibody activity. Species-specificity studies by complement fixation, immunofluorescent staining for complement, and conventional method reveal cross-reactions with skeletal muscle antigens of dog, rat, duck, turtle, and frog to the same titer as with human and monkey antigen. Extensive cross-reactions also occur in the indirect immunofluorescent staining. Direct immunofluorescent staining for gamma globulin reveals small foci of staining in sections of muscle biopsies of myasthenic patients. These have been observed in some biopsies from clinically affected muscle but not in biopsies from unaffected myasthenic muscle or in non-myasthenic muscle. Complete article.

6255

Djanian, A.Y.; Beutner, E.H.; Witebsky, E. 1964. Tanned-cell hemagglutination test for detection of antibodies in sera of patients with myasthenia gravis. J. Lab. Clin. Med. 63:60-70.

Previous studies revealed the presence of two types of autoantibodies in the sera of some patients suffering from myasthenia gravis, one reactive with both skeletal muscle and heart and the other with skeletal muscle only. This article deals with the demonstration of muscle antibodies by the tanned-cell hemagglutination technique. Comparative tests were performed with sera from 45 patients with myasthenia gravis by tanned-cell hemagglutination, immunofluorescent staining, and complement fixation; 14 were considered positive. Of the 45 sera, ten yielded positive tanned-cell tests with human muscle fractions, eight yielded positive immunofluorescent staining of muscle striations, and six yielded positive complement fixation with muscle extracts. None of the 58 control sera yielded unequivocally positive reactions. The hemagglutination reaction is at least as sensitive and specific as immunofluorescent staining in the detection of SH antibodies in sera of patients with myasthenia gravis.

6256

Feltkamp, T.E.W.; Geld, H., van der; Oosterhuis, H.J.G.H. 1963. Studies on sera from cases of myasthenia gravis, using the fluorescent antibody technique. Vox Sang. 8:317-327.

The serum of 111 patients with myasthenia gravis was examined by indirect FA for the presence of antibodies against muscle tissue (rat diaphragm). Many sera (42 per cent) showed a positive picture, three different types of fluorescence being clearly recognizable. Nuclear fluorescence was observed in 13.5 per cent. The same technique performed on human thyroid tissue revealed the presence of antibodies against thyroid tissue in 32 per cent.

6257

Feltkamp, T.E.W.; Geld, H., van der; Kruyff, K.; Oosterhuis, H.J.G.H. 1963. Antinuclear factor in myasthenia gravis. Lancet 1:667.

The high incidence of antinuclear factors indicates that myasthenia gravis should be classed among the autoimmune diseases characterized by the presence of antinuclear factors.

6258

Geld, H., van der; Feltkamp, T.E.W.; Van Loghem, J.J.; Oosterhuis, H. J.G.H.; Biemond, A. 1963. Multiple antibody production in myasthenia gravis. Lancet 2:373-375.

The demonstration of multiple antibodies in myasthenia gravis, its clinical course with typical remissions and exacerbations, and the female sex preponderance, are all reminiscent of other autoimmune diseases such as systemic lupus erythematosus and Hashimoto's disease, and have led us to conclude that myasthenia gravis is an autoimmune disease, probably resulting from disturbed tolerance. The fluorescent ANF test was one of those employed.

6259

Geld, H., van der; Feltkamp, T.E.W.; Oosterhuis, H.J.G.H. 1964. Reactivity of myasthenia gravis serum gamma globulin with skeletal muscle and thymus demonstrated by immunofluorescence. Proc. Soc. Exp. Biol. Med. 115:782-785.

Sera from patients with myasthenia gravis, 36 out of a total of 90, showed reactivity with epithelial cells in the thymus by means of the immunofluorescence technique. This antibody was cross-reactive with skeletal muscle. All nine sera of patients whose myasthenia was associated with a thymoma showed strong reactivity in serum dilutions up to 1:60. Antigenic change in epithelial cells is postulated as a possible mechanism initiating an auto-immune response in the thymus.

6260

Geld, H., van der; Strauss, A.; Kemp, P., Jr.; Exum, E. 1964. Reactivity of myasthenia gravis serum gamma globulin with thymic epithelial cells: Incidence, specificity, and cross-reactivity with skeletal muscle A bands. Federation Proc. 23:1445:1442.

The present study was performed to determine the incidence and specificity of this phenomenon. Sera from 28[illegible] myasthenia gravis patients, 100 individual normal controls, and 750 individual disease controls from the Laboratory of Immunology, NIAID, were studied. Thirty per cent of the myasthenia gravis sera in these series were reactive in titers from 1:60 through 1:1920. No control sera were reactive with thymic epithelial cells. Reactivity was uniformly associated with reactivity against skeletal muscle A bands. Reciprocal absorption studies with muscle and thymus point toward common antigenic determinants in thymic epithelial cells and A bands of skeletal muscle. Complete article.

6261

Goldin, H.; Robbins, W.C. 1963. A patient with myasthenia gravis, thymoma, and lupus nephritis. Arth. Rheum. 6:272-273.

Auto-antibodies, especially to DNA, occur frequently in systemic lupus erythematosus, and there is growing evidence that autoimmune processes may be of importance in myasthenia gravis. A clinical history and test results are presented. Complement fixation with calf thymus nuclei was positive 1:32, and with calf thymus DNA 1:256. By immunofluorescence, gamma globulin from this serum combined diffusely with cell nuclei with concentration at the nuclear membrane. Nucleolar staining was not noted. This report is additional evidence that there may be a significant association of myasthenia gravis with autoimmune diseases such as systemic lupus. It supports the hypothesis that both diseases may be related to abnormal behavior of the thymus.

6262

Gordon, S.V.; Hess, J.W.; Frederick, R.J. 1965. Myasthenia gravis: Studies on in vivo binding of gamma globulin to muscle. Arch. Intern. Med. 115:405-410.

The presence of a muscle-binding 7S gamma globulin (MBGG) in the serum of certain patients with myasthenia gravis has been repeatedly demonstrated. This study was an attempt to investigate the etiologic implications of this serologic phenomenon by looking for in vivo binding of MBGG in myasthenic muscle. Using the indirect immunofluorescent technique, sera from 29 myasthenia gravis patients were screened for MBGG. Three

patients whose sera gave positive reactions and one with a negative reaction were chosen for muscle biopsy. In addition, a frozen specimen of myasthenic muscle obtained at autopsy was available. One of the four biopsy specimens showed occasional areas of faint cross-striational fluorescence when fluorescein-tagged rabbit antihuman 7S gamma globulin was applied directly to the muscle. The other three were negative. The autopsy specimen showed rather diffuse cross-striational fluorescence, but the circumstances make interpretation of this observation difficult. From this study it appears that significant in vivo binding of MBGG to muscle does not occur regularly.

6263

Grob, D.; Namba, T.; Sato, T.; Kline, D.R. 1965. Effect on skeletal muscle of serum of myasthenic patients and of rabbits immunized with muscle constituents. Federation Proc. 24:3119:693.

The serum of patients with myasthenia gravis, or of rabbits immunized with rat muscle ribonucleoprotein prepared by precipitation with d-tubocurarine, contains a globulin that reacts in vitro with normal human or animal skeletal muscle, as demonstrated by immunofluorescent staining. Similar binding in vivo was demonstrated by intramuscular or intra-arterial injection of serum of myasthenic patients or immunized rabbits into the rat tibialis anterior, followed by immunofluorescent staining of excised muscle. The staining of cross striations was similar to that observed in vitro, but was more marked, especially in red muscle, and longitudinal strands were also stained. Binding of globulin was also demonstrated, without addition of serum, in muscle removed from myasthenic patients. Antiserum to saline extract or homogenate of rat muscle produced less binding in vivo and in vitro than did antiserum to d-tubocurarine binding ribonucleoprotein; antiserum to actomyosin produced none. Despite evidence of binding to muscle, the intra-arterial injection into rats of serum of myasthenic patients or immunized rabbits had no effect on action potentials or tension evoked by nerve stimulation. Complete article.

6264

Hale, W.L.; Beutner, E.H. 1965. Studies of the serological relation of myosin and actomyosin to antigens reactive with muscle antibodies in the sera of myasthenia gravis patients. Federation Proc. 24:3117:693.

Sera of patients with myasthenia gravis that contained muscle antibodies were absorbed with structural proteins extracted from monkey skeletal muscle. The absorbed sera were tested for the presence of muscle and heart antibodies by indirect and complement-fixation immunofluorescent staining techniques and by the tanned-cell hemagglutination test for muscle antibodies. Actomyosin, prepared as a by-product of myosin extraction, and once-purified myosin were equally

efficient absorbents of antibodies reactive with skeletal muscle and heart, SH antibodies, but neither protein absorbed antibodies reactive with skeletal muscle only, S antibodies. Quantitative analyses of these results as well as further purification of the myosin by high-speed centrifugation indicated that soluble myosin, itself, was not the active antigen. Thus, the actomyosin or myosin extraction procedure may be used as primary steps in the purification of the antigen that combines with SH antibodies in the sera of myasthenia gravis patients. Complete article.

6265

Hamard, M.; Cannat, A.; Seligmann, M. 1964. Detection of antinuclear antibodies by immunofluorescence: Study of 1,430 sera. Rev. Franc. Etud. Clin. Biol. 9:716-728. In French.

Antinuclear factors were detected by indirect FA in the sera of patients with various diseases, including lupus erythematosus and myasthenia gravis. The significance of the per cent of positives versus type and severity of disease is discussed.

6266

Hess, E.; Eliasson, S.; Ziff, M. 1963. A study of serum factors in myasthenia gravis. Arth. Rheum. 6:275-276.

It has been postulated that myasthenia gravis may be an autoimmune disorder, and the work of a number of investigators has indicated the important role of the thymus in the formation of immunologically competent cells. Four cases are presented. Three of the patients had antinuclear factors, demonstrated by the fluorescent antibody technique, two had positive LE tests, two thyroglobulin antibodies, and one a positive latex fixation test and increased serum gamma globulin level. Thymus abnormalities existed. Two thymomas, one hyperplastic thymus gland, and three normal glands were studied by the immunofluorescent technique for the presence of cells containing gamma globulin, and a few cells with positive cytoplasmic staining were noted only in the hyperplastic gland. Gamma globulin was not seen in the thymomas. Sera from four patients were tested against autologous and homologous myasthenic muscle as well as normal muscle, and factors reactive with the sarcolemmal and cross-striatal components were observed in three. Eleven family members of the patient with a hyperplastic thymus and positive LE test were studied. Six had thyroid and one had muscle antibodies. Only one had antinuclear factor and none had rheumatoid factor.

6267

Kornfield, P.; Siegal, S.; Weiner, L.B.; Osserman, K.E. 1965. Studies in myasthenia gravis: Immunologic response in thymectomized and nonthymectomized patients. Ann. Intern. Med. 63:416-428.

Thirty-four adult patients with myasthenia gravis, of whom 21 were thymectomized, and 29 normal controls were studied for H agglutinin response to triple typhoid vaccine. Immunofluorescent muscle autoantibodies were demonstrated in nine of 21 thymectomized myasthenics and in three of 13 nonthymectomized patients. There is a definite relationship between presence of such antibodies, clinical severity of the disease, and type of thymic pathology, especially thymoma.

6268

Myers, S.J.; Grob, D. 1965. Muscle-binding serum globulin in myasthenia gravis and connective tissue diseases. Federation Proc. 24:3118:693.

The serum of patients with myasthenia gravis contains 7S globulin that binds, as demonstrated by immunofluorescent staining, with cross striations of skeletal and cardiac muscle of normal man, dog, rabbit, guinea pig, hamster, rat, mouse, and toad, but not fish or lobster. Binding was inhibited by pretreatment of muscle with ribonuclease, or with d-tubocurarine, the effect of which was reversed by incubation with acetylcholine, choline, or neostigimine. Binding of 7S globulin was shown to occur predominantly with a muscle ribonucleoprotein that could be precipitated by d-tubocurarine. There was weaker binding to saline extract of muscle, and none to actomyosin. Staining of normal muscle was produced by high concentrations of serum of most normal subjects, but only serum of patients with myasthenia gravis or connective tissue diseases, especially the former, produced staining in low concentrations. Staining occurred with serum of 23 per cent of normal subjects at dilution of 1:30, and in none at 1:90; in 93 per cent of myasthenic patients at dilution of 1:30, 62 per cent at 1:90, and 37 per cent at 1:270; in 40 per cent of patients with lupus and 10 to 20 per cent of patients with scleroderma, myositis, and rheumatoid arthritis at dilution of 1:30. Complete article.

6269

Namba, T.; Himei, H.; Grob, D. 1964. Autoimmune phenomena in myasthenia gravis. Federation Proc. 23:1447:342.

The serum of myasthenic patients contains, in the globulin fraction, substances that combine with A bands, nuclei, and possibly endplates of normal or myasthenic muscle, and with nuclei of other cells, such as thymus, liver, and mucous membrane as demonstrated by direct and indirect immunofluorescent techniques. Similar localization of staining was produced by serum globulin of rabbits immunized with a ribonucleoprotein obtained from extract of normal or myasthenic skeletal muscle by precipitation with d-tubocurarine. The staining of A bands of muscle corresponded to the site of overlapping of myosin and actin filaments. The staining of nuclei was more stippled than the diffuse fluorescence produced by the serum of patients with lupus erythematosus. The binding sites of globulin in A bands and nuclei corresponded to the localization of ribonucleic acid, as indicated by pyronine staining. The binding globulin could be absorbed from the serum of myasthenic patients or immunized rabbits preferentially by the ribonucleoprotein obtained from muscle by d-tubocurarine precipitation. This ribonucleoprotein may be the antigen responsible for the presence of muscle, nuclear, and endplate binding antibody in the serum of myasthenic patients. Complete article.

6270

Namba, T.; Sato, T.; Myers, S.J.; Nakamura, T.; Grob, D. 1965. Muscle- and nuclear-binding serum globulin in patients with myasthenia gravis, muscular dystrophy, and connective tissue diseases. J. Clin. Invest. 44:1079-1080.

Serum of myasthenic patients, or rabbits immunized with muscle ribonucleoprotein prepared by precipitation with d-tubocurarine, contains 7S globulin that combines with A bands of normal human or animal skeletal or cardiac muscle, with cytoplasm of thymic epithelial cells, and with nuclei of muscle thymic, and buccal mucosal cells, as demonstrated by FA. Staining of A bands of skeletal muscle occurred in 62 per cent of myasthenic patients, 17 per cent of patients with other muscle diseases, including muscular and myotonic dystrophy, 4 per cent of patients with connective tissue diseases, and in no normal subjects. Serum also stained films of d-tubocurarine binding ribonucleoprotein strongly and films of muscle extract weakly. Blocking controls were satisfactory. Binding of globulin to muscle A bands in vivo was demonstrated, with and without adding myasthenic serum. Despite evidence of binding to muscle, intra-arterial injection into rats of serum of myasthenic patients or immunized rabbits had no effect on action potentials or tension evoked by nerve stimulation. Serum of most myasthenic patients, and of some patients with muscular or myotonic dystrophy or connective tissue disease, contains 7S globulin that binds to cytoplasmic and nuclear ribonucleoprotein in muscle and other tissues. There is no effect of such binding on muscle function.

6271

Osserman, K.E.; Burnett, L. 1965. Studies in myasthenia gravis: Changes in demonstration of antibody fluorescence correlated with clinical course. Federation Proc. 24:3115:693.

Four hundred thirty-seven sera of 118 pedigreed myasthenic patients were studied over various periods of time, 1 day to 9 years, for the demonstration of the presence of fluorescent antibody on striated muscle, using an indirect immunofluorescent technique, employing a rabbit anti-human 7S gamma globulin. Twenty-seven patients, 23 per cent, displayed reversibility in antibody fluorescence in that reactions of their sera changed from positive to negative or negative to positive. In 21 of these 27 patients there was good correlation with changes in the demonstration of antibody fluorescence with the clinical course of myasthenia gravis. Changes in antibody fluorescence show an apparent inverse relationship between both clinical classification of the illness and thymic pathology. Hypothesis of autoimmune mechanism in myasthenia gravis may be further supported by the presence of fluorescent antibody against striated muscle that varies with the course of the disease. Complete article.

6272

Osserman, K.E.; Weiner, L.B. 1965. Studies in myasthenia gravis: Immunofluorescent tagging of muscle striation with antibody from serums of 256 myasthenic patients. Ann. N.Y. Acad. Sci. 124:730-743.

This is a general review of the knowledge surrounding the disease myasthenia gravis. On an indirect FA test, serum of myasthenia patients causes fluorescent staining of muscle striations. Of a seven-section classification of the disease, the lightest per cent of positive immunofluorescent reactions occurs in adult forms of localized or general disease. Other relationships of immunofluorescent reaction to clinical picture are discussed.

6273

Osserman, K.E.; Weiner, L.B. 1965. Studies in myasthenia gravis: Correlation of antibody immunofluorescence with clinical course. New Engl. J Med. 273:615-620.

Four hundred and thirty-five sera of 117 myasthenic patients were studied over various periods (1 day to 9 years) for the demonstration of the presence of immunofluorescent antibody on striated muscle, with the use of an indirect immunofluorescent technique employing a rabbit antihuman 7S gamma globulin. Twenty-two per cent displayed reversibility in antibody immunofluorescence, in that reactions of

their sera changed from positive to negative or negative to positive. In 81 per cent of these 26 patients there was good correlation between changes in the demonstration of antibody immunofluorescence and the clinical course of myasthenia gravis. Changes in antibody immunofluorescence showed an apparent inverse relation between clinical classification of the illness and the thymic abnormality.

6274

Rowland, L.P.; Griffiths, C.O.; Kabat, E.A. 1965. Myasthenia gravis, thymoma and cryptococcal meningitis. New Engl. J. Med. 273:620-627.

A patient with myasthenia gravis was found to have a locally invasive thymoma, which could not be removed surgically. Subsequently, cryptococcal meningitis, which seemed to be successfully treated with amphotericin, developed. She ultimately died of acute bacterial pneumonitis, however, and at autopsy cryptococci were present in the meninges. The immunologic findings resemble those of patients with Hodgkin's disease, in which there is a predilection for cryptococcal infection, but the immunologic state differs from that of patients with agammaglobulinemia, in which there may be a predilection for rheumatoid arthritis and related diseases. The relationship of the observed immunologic state to myasthenia is not known.

6275

Strauss, A.; Geld, H., van der; Kemp, P., Jr.; Exum, E.; Goodman, H.; Osserman, K. 1964. Reactivity of myasthenia gravis serum gamma globulin with skeletal muscle A bands: Incidence, specificity, certain clinical and serologic correlations. Federation Proc. 23:1444:341.

Sera from 285 myasthenia gravis, MG, patients were studied for reactivity against skeletal muscle. In this series, indirect immunofluorescence technique for specific detection of gamma globulin binding to tissue was employed. Incidence of reactivity was 30 per cent; titers ranged from 1:60 through 1:1920. Positive reactions were universally seen with sera from MG patients having thymomas. Reactivity was also observed in sera from patients with apparent MG of 1 to 3 months duration, not treated with anticholinesterases. Reactivity, within test limits, was not observed in 100 normal control sera, nor in sera from more than 750 individuals with inflammatory and endocrinopathic myopathies, muscular dystrophies, hereditary neuromuscular diseases, autoimmune diseases, dysproteinemias, tuberculosis, non-myasthenics treated with anticholinesterases, etc. Reactions of MG sera with muscle were consistently associated with binding to cytoplasm of thymic epithelial cells and vice versa. Complete article.

6276

Strauss, A.J.L.; Geld, H.W.R., van der; Smith, C.W.; McFarlin, D.E.; Barlow, M. 1965. Autoimmune reactivities in serums of patients with thymomas unassociated with myasthenia gravis. Federation Proc. 24:3116:693.

Serum gamma globulins of 30 per cent of myasthenia gravis patients bind in vitro, in high titers, to both skeletal muscle A bands and thymic epithelial cell cytoplasm, as shown by indirect immunofluorescence technique. Sera of virtually all patients with myasthenia gravis and associated thymomas react in this way. In a coded study of sera from 803 individuals without myasthenia gravis, the same reactivity occurred only in one healthy donor and in one patient with a thymoma and an aregenerative anemia. Recent extension of this survey revealed that sera from 10 of 33 patients with thymomas, or histories thereof, without myasthenia gravis also bound to skeletal muscle and thymus. Titers of reactivity were in the same range as in myasthenia gravis patients. A curare provocation test on one such patient was negative. Antinuclear reactivity was present in sera of five of the same patients, as well as in sera of five of the remaining 23. Sera from 200 cases of other neoplasia did not react with skeletal muscle or thymus. Complete article.

6277

Sturgill, B.C.; Carpenter, R.R.; Strauss, A.J.L.; Goodman, H.C. 1964 Antibodies in systemic lupus erythematosus and myasthenia gravis which react with thermally denatured DNA-coated bentonite. Proc. Soc. Exp. Biol. Med. 115:246-251.

Sera from 66 per cent of 35 patients with SLE flocculated bentonite particles coated with thermally denatured DNA; only 29 per cent of these same sera flocculated bentonite particles coated with native DNA. All of these sera contained antinuclear antibody. Twenty per cent of sera from 44 patients with myasthenia gravis flocculated the bentonite particles coated with denatured DNA, but none reacted with the native DNA bentonite. Thirty-seven per cent of the sera from patients with myasthenia gravis contained antinuclear antibody. Rheumatoid factor and antibodies to thyroglobulin were occasionally seen in low titer in the serum from patients with myasthenia gravis.

6278

Thivolet, J.; Kratchko, A. 1964. Antimuscle and antinuclear antibodies during myasthenia: Detection by the immunofluorescence technique, preliminary note. Rev. Franc. Etud. Clin. Biol. 9:212-215. In French.

Using an indirect immunofluorescence technique, antimuscular antibodies were found in three of nine patients with myasthenia gravis, but these were absent in 16 patients with various other diseases, including muscular pathology. Some correlation of the presence of the autoantibodies with a thymus tumor seemed probable. No antinuclear antibodies were found in the nine myasthenic patients.

6279

Thompson, G.R.; Simpson, J.F.; Westerberg, M.R. 1965. Antinuclear, antimuscle, and rheumatoid factors in systemic lupus erythematosus and myasthenia gravis. Arth. Rheum. 8:475.

A number of gamma globulin fractions with the properties of antibodies against cells and components of cells have been identified in serum from patients with sytemic lupus erythematosus. Similar antibodies have been reported in myasthenia gravis (MG) sera. In addition, skeletal and cardiac muscle-binding globulins have been found in MG sera. To assist in evaluating the incidence and specificity of these factors, 39 MG and 18 SLE sera were studied. Antimuscle antibodies were studied by the fluorescent antibody technique. Skeletal muscle-binding globulins were present in 30.8 per cent of MG sera; specific fluorescence of cardiac muscle was observed in 31.2 per cent. All sera were concordant for skeletal and cardiac muscle reactivity except one, which was positive only with cardiac muscle. No specific binding was observed with smooth muscle. None of the SLE sera reacted with skeletal, cardiac, or smooth muscle. Three of 33 control sera reacted with muscle, but the pattern of binding was distinctive and different. It is of interest that none of the SLE sera contained muscle-binding globulins.

6280

Vetters, J.M. 1965. Immunofluorescence staining patterns in skeletal muscle using serum of myasthenic patients and normal controls. Immunology 9:93-95.

By indirect immunofluorescent tests upon the sera of patients with myasthenia gravis and of normal subjects, two types of staining pattern were found. The first, A-band staining, was seen only with myasthenic sera. The second, or I-band pattern, has not previously been described and was seen in an equal proportion of normal controls and of myasthenic patients. I-band staining is a possible source of confusion in immunofluorescent tests for muscle antibody.

6281

Wolf, P.L.; Pearson, B.; Hess, J.W. 1963. Evidence for the binding of fluorescein-tagged gamma globulin to muscle in myasthenia gravis. Lab. Invest. 12:870-871.

Relatively pure gamma globulin was obtained from normal and myasthenic patients by DEAE Sephadex fractionation of sera. The gamma globulin was FITC labeled. Slides were prepared from muscle biopsy frozen sections with and without specific inhibition by untagged gamma globulin. There was binding of the labeled myasthenic gamma globulin to the sarcolemmal region of the isologous muscle. There was no cross-reaction between normal gamma globulin and myasthenic muscle. In one patient in whom studies were performed at 1-year intervals, the staining was much more intense at the time of exacerbation of the disease. Similar studies on thymoma tissue removed from a patient with myasthenia gravis revealed specific binding of the tagged gamma globulin in some areas of the tumor. Antibody may be present in the gamma globulin fraction of patients with myasthenia gravis that binds to the sarcolemmal region of myasthenic muscle. Observations in one case also indicate that there are gamma globulin-binding properties common to thymoma tissue and myasthenic muscle.

4. Pernicious Anemia

6282

Dagg, J.H.; Goldberg, A.; Anderson, J.R.; Beck, J.S.; Gray, K.G. 1964 Autoimmunity in iron-deficiency anemia. Brit. Med. J. 1964:1349-1350.

The sera of 64 patients with iron-deficiency anemia have been tested by an immunofluorescent technique for the presence of autoantibodies to the parietal cells of the gastric mucosa. Approximately one-half of these patients had a histamine-fast achlorhydria, the other half were able to secrete acid gastric juice. The antibody was detected in 13 of 64 iron-deficient patients and in 4 of 64 normal control subjects. The difference in incidence between these two groups is statistically significant. The antibody was present in 11 of 33 anemic patients with a histamine-fast achlorhydria and in 2 of 31 anemic patients with acid gastric juice. The difference between the two groups is statistically significant. The relevance of these results to the pathogenesis of iron-deficiency anemia and to the relationship of iron-deficiency anemia to pernicious anemia is discussed.

6283

De Boer, W.G.R.M.; Nairn, R.C.; Maxwell, A. 1965. Pernicious anemia autoantibody to gastric parietal cells. J. Clin. Pathol. 18:456-459.

Rat stomach provided an excellent substrate for immunofluorescence testing of gastric parietal cell autoantibodies in 65 human sera. The results of similar tests using human stomach corresponded closely in the 42 cases examined. The rat stomach had some advantage over human stomach in its ready availability in the fresh state, its occasional brighter staining reactions, and its avoidance of nonspecific staining by the sandwich immunofluorescence technique.

6284

Fisher, J.M.; Taylor, K.B. 1965. A comparison of autoimmune phenomena in pernicious anemia and chronic atrophic gastritis. New Engl. J. Med. 272:499-503.

Sera of 86 patients with pernicious anemia and 16 patients with biopsy-proved chronic atrophic gastritis were tested for antibodies to gastric parietal cytoplasm, gastric mucosa, intrinsic factor, thyroglobulin, and thyroid acinar-cell cytoplasm. Of the group with pernicious anemia, 84.5 per cent had parietal-cell antibodies by immunofluorescent techniques, 53 per cent had gastric-mucosa antibodies by complement-fixation tests, and 56 per cent had antibodies to intrinsic factor. Of the group with chronic gastritis, 62.5 per cent had parietal-cell, and 38.5 per cent gastric-mucosa antibodies. Both groups showed a significant incidence of antibodies to thyroglobulin, 25.5 per cent in pernicious anemia and 50 per cent in gastritis. Common etiologic factors and autoimmune phenomena may occur in both diseases. The specific lesion in many cases of pernicious anemia may be due to the formation of antibodies to intrinsic factor.

6285

Irvine, W.J.; Davies, S.H.; Haynes, R.C.; Scarth, L. 1965. Secretion of intrinsic factor in response to histamine and to gastrin in the diagnosis of Addisonian pernicious anaemia. Lancet 2:397-401.

The content of intrinsic factor in the samples of gastric juice obtained during a standard gastric analysis when either histamine or gastrin was used as stimulant was correlated with the microscopic appearances in the stomach and the patient's ability to secrete acid and to absorb oral vitamin B-12. Screening tests for gastric antibodies should become a routine investigation in persons at special risk. Those with positive antibody tests should undergo gastric analysis. Antibody to parietal cells was detected by FA and CF.

6286

Jeffries, G.H.; Sleisenger, M.H. 1965. Immunofluorescent studies in pernicious anemia. Gastroenterology 48:823.

Patients with Addisonian pernicious anemia exhibit circulating autoantibodies that react with human intrinsic factor and with gastric parietal cell cytoplasm. Immunofluorescent studies were carried out to define the reactivity of immunoglobulins with parietal cell cytoplasmic antigen and to determine the role of the mononuclear cell infiltrate of both normal and atrophic gastric mucosae in the production of immunoglobulins. Parietal cell antibodies were detected by serial incubation of fresh frozen, acetone-fixed sections of rat gastric mucosa with diluted pernicious anemia sera, and with fluoresceinated antihuman immunoglobulins. Parietal cell antibody activity was predominantly in the gamma-2 globulin fraction of serum, was present to a lesser degree in the gamma-1A globulin fraction, but was not detected in the gamma-1M globulin fraction. Fresh frozen, acetone-fixed sections of human gastric mucosa from normal subjects and from patients with pernicious anemia were incubated with fluoresceinated antihuman immunoglobulins, and the distribution of immunoglobulin in the mucosa was determined by fluorescence microscopy. Immunoglobulins were thus detected in the cells infiltrating the lamina propria. Gamma-1A globulin was detected in the majority of infiltrating plasma cells, whereas gamma-2 and gamma-1M globulins were present in fewer cells. Complete article.

6287

Rubin, W.; Fauci, A.S.; Sleisenger, M.H.; Jeffries, G.H. 1965. Immunofluorescent studies in adult celiac disease. J. Clin. Invest. 44:475-485.

To investigate the possible role of immunologic processes in the pathogenesis of adult celiac disease, duodenal and/or jejunal biopsies from nine patients with adult celiac disease and from five control patients were studied by immunofluorescent techniques. Immunoglobulins were demonstrated in mononuclear cells in the lamina propria of all tissue sections. Gamma-1A was the major immunoglobulin present. No in vivo or specific in vitro fixation of beta-1C globulin component of complement was observed in these tissues. In specimens from all subjects, the beta-1C globulin component of complement was fixed in vitro at the superficial margin of the epithelial cells and in goblet cells, a distribution corresponding to mucin. This nonspecific complement fixation may be secondary to a nonspecific in vitro fixation of immunoglobulin, particularly gamma-1M globulin, which was fixed in the same areas. Autoantibodies reactive against epithelial cell cytoplasm could not be demonstrated. The cytoplasm of the intestinal epithelium from celiac patients bound gliadin, but not casein, bovine serum albumin, or ovalbumin.

6288

te Velde, K.; Abels, J.; Anders, G.J.P.A.; Arends, A.; Hoedemaeker, Ph.J.; Nieweg, H.O. 1964. A family study of pernicious anemia by an immunologic method. J. Lab. Clin. Med. 64:177-187.

The incidence of an antibody against gastric parietal cells was determined with an immunofluorescence method in relatives of 21 patients who suffered from pernicious anemia and had the antibody. It was found in 42 of 220 of these relatives, or 20 per cent. In a group of 120 patients with pernicious anemia a positive result was obtained in 87 per cent. Three of 78 allied relatives who were studied as a control group had the parietal cell antibody. In a second control study, 6 of 100 normal sera contained the parietal cell antibody. Development of this antibody is controlled by a dominant autosomal gene. The expression of the gene is pleiotropic. The full pattern of effect implies the development of pernicious anemia. Additional data were obtained in 20 asymptomatic relatives who had the parietal cell antibody. The detection of the parietal cell antibody, which appears to be an early sign of pernicious anemia, is a convenient method for selecting those relatives of patients with pernicious anemia who run the risk of also developing the disease. If on further examination these people are found to lack the intrinsic factor, they should receive treatment with vitamin B-12 to prevent the development of manifest pernicious anemia.

6289

te Velde, K.; Abels, J.; Anders, G.J.P.A.; Arends, A.; Nieweg, H.O. 1964. An investigation into the genetic background of pernicious anemia by an immunological method. Ned. Tijdschr. Geneesk. 108:2109-2110. In Dutch.

In 120 pernicious anemia patients, antiparietal cell antibodies were found in 87 per cent by immunofluorescence. A study of these antibodies which appear to be an essential manifestation of the disease, in members of families of patients produced 20 per cent positives. Family history data indicated that the occurrence of antibody is determined by a dominant gene with incomplete penetrance in a heterozygote carrier.

6290

Woolf, N.; Pilkington, T.R.E. 1965. The immunohistochemical demonstration of lipoproteins in vessel walls. J. Pathol. Bacteriol. 90:459-463.

The application of FA to a study of the distribution of lipoproteins within the arterial intima shows that beta-lipoproteins are present in a variety of patterns of localization in atherosclerosis. In uncomplicated lesions, the lipoprotein may be derived from circulating plasma as a result of a filtration process.

5. Rheumatic Fever

6291

Angelino, P.F.; Vacca, G. 1964 Immunofluorescence on human myocardium of rheumatic subjects. Minerva Med. 55:49-54. In Italian

Conspicuous deposits of gamma globulin were detected by FA in the cardiac auricular tissue of patients with a history of rheumatic fever.

6292

Angelino, P.F.; Vacca, G. 1965 Immunofluorescence with streptococcic antigens in chronic rheumatic myocarditis Minerva Med. 56 2349-2352 In Italian

In 14 of 26 patients studied, gamma globulin on amputated auricles reacted with fluorochrome-labeled streptococcic antigens (O-streptolysin, streptokinase, and streptohyaluronidase). The immunofluorescent reactions were localized in the interstitial tissue and vessel walls and, in two cases, in the myofibers These findings seem to support the assumption of an evolutionary rheumatic pathology of the organ.

6293

Hess, E.V.; Fink, C.W.; Taranta, A; Ziff, M. 1964 Heart muscle antibodies in rheumatic fever and other diseases J. Clin Invest 43 886-893

Serum factors reactive with human heart muscle were investigated by the indirect immunofluorescent technique in 624 subjects Two main staining patterns were observed. sarcolemmal-subsarcolemmal and diffuse An intermyofibrillar pattern was also seen In acute rheumatic fever patients, 41 5 per cent showed the sarcolemmal-subsarcolemmal pattern, the latter at times accompanied by intermyofibrillar staining In the presence of carditis, this increased to 63 4 per cent Twenty-seven per cent of patients with myocardial infarction showed the diffuse type of immunofluorescent reaction The diffuse staining pattern was also occasionally seen in patients with acute glomerulonephritis and rheumatoid arthritis Other groups showed a low incidence of positive reactions There was a striking increase in incidence of positive reactions after cardiac surgery for rheumatic heart disease There appeared to be no relationship between the incidence of positive reactions and anti-streptolysin-O titer The serum factors are antibody in nature.

6294

Kantor, F.S. 1965. Fibrinogen precipitation by streptococcal M protein: II. Renal lesions induced by intravenous injection of M protein into mice and rats. J. Exp. Med. 121:861-872.

Intravenous injection of Type 1 streptococcal M protein into mice and rats produced lesions confined to renal glomeruli. Thrombi of eosinophilic amorphous material, seen to occlude glomerular capillaries, were shown to contain M protein and fibrinogen. Gradual regression of the morphological lesions was observed during the 3 weeks following injection. Initial abnormal proteinuria and azotemia returned to control levels by the end of the 1st week; a second rise in urinary protein excretion and urea retention was demonstrated in some rats coincident with appearance of anti-M antibodies. The mechanism of renal localization of streptococcal M protein by means of a complex with fibrinogen was suggested, which may comprise an initial phase in the pathogenesis of acute poststreptococcal glomerulonephritis.

6295

Kaplan, M.H. 1963. Immunologic relation of streptococcal and tissue antigens: I. Properties of an antigen in certain strains of Group A streptococci exhibiting an immunologic cross-reaction with human heart tissue. J. Immunol. 90:595-606.

Antisera prepared in rabbits against cell walls or M protein preparations of a Group A Type 5 strain of Streptococcus have been found reactive by immunofluorescence and complement fixation with human heart tissue. The reactant in heart tissue was localized to cardiac myofibers of all heart specimens tested and in smooth-muscle elements of vessel walls and endocardium of a proportion of heart specimens tested. Rheumatic and nonrheumatic hearts showed comparable reactivity. The reactant was also identified in human skeletal muscle and in heart and skeletal muscle of the rabbit. In the Streptococcus, the cross-reactive antigen was demonstrated in cell walls and in acid extracts of cell walls by immunofluorescent absorption tests. Following absorption of antisera with cell walls, antibody bound to cell walls could be eluted at pH 3.0 and shown to possess serologic reaction with heart. The immunologic relationship between streptococcal cell wall antigen and myofibers and smooth muscle of vessel walls is consistent with the hypothesis that bound gamma globulin observed in rheumatic hearts in these sites is derived from immune bodies

6296

Kaplan, M.H. 1965. Induction of autoimmunity to heart in rheumatic fever by streptococcal antigens cross-reactive with heart. Federation Proc. 24:109-112.

Within the limits of current information, it is not apparent how the immunologic relationship of streptococcal cells and human tissue described can be related to the many varied clinical features of rheumatic fever. Induced autosensitization to myofibers and vessel walls may have significance for such manifestations as myocarditis, arteritis, erythema marginatum, and possibly even Aschoff lesions in the context of the above proposition, but a relationship to other rheumatic manifestations, including valvular lesions, subcutaneous nodules, chorea, pneumonitis, or encephalitis is not obvious. Possibly other cross-reactive antigens of the Streptococcus, that have recently been uncovered, and to which autoimmune mechanisms might be induced, may offer insight into these questions.

6297

Kaplan, M.H. 1965. Multiple nature of cross-reactive relationship between Group A streptococci and heart tissue. Federation Proc. 24:275:176.

The immunologic relationship of Group A streptococcal cells and mammalian heart tissue has been demonstrated by immunofluorescence of anti-streptococcal serum, precipitin reaction of anti-heart serum, and precipitin-absorption of human post-streptococcal serum with heart tissue. At least three different antigenic determinants in streptococcal preparations have been implicated in this cross-reactive relationship. Antigen 1 is present in cell walls and to a lesser extent in cell membranes of certain strains only; it is trypsin-sensitive, and has cathodal mobility. Antigen 3 is present in cell walls or membranes of all Group A strains, is trypsin-sensitive, and has anodal mobility. In the case of antigen 2, it has been possible to demonstrate that the precipitin line given with rabbit or chicken anti-streptococcal sera shows fusion with the precipitin line given with anti-heart serum. Complete article.

6298

Kaplan, M.H.; Bolande, R.; Rakita, L.; Blair, J. 196 Presence of bound immunoglobulins and complement in the myocardium in acute rheumatic fever: Association with cardiac failure. New Engl. J. Med. 271:637-645.

Clinical, electrocardiographic, pathological, and immunopathological findings in five children who died of acute rheumatic fever in cardiac failure are presented. In all five cases intensive deposits of gamma globulin and the beta-1A component of human complement were observed

throughout the myocardial segments available for study. Variable staining for gamma-1A (beta-2A) and gamma-1M (beta-2M) globulins was also observed. These deposits were localized primarily in sarcolemma of cardiac myofibers and also in smooth muscle of vessel walls and endocardium, and in interstitial connective tissue. The possible relation of these findings to the concept of streptococcal-induced autoimmunity to heart is considered. An autoimmune mechanism of immune injury to myocardium may participate in the pathogenesis of rheumatic myocarditis.

6299

Kaplan, M.H.; Craig, J.M. 1963. Immunologic studies of heart tissue: VI. Cardiac lesions in rabbits associated with autoantibodies to heart induced by immunization with heterologous heart. J. Immunol. 90:725-733.

Rabbits given repeated injections of beef or rat heart homogenates in aluminum hydroxide gel adjuvant developed focal cardiac lesions associated with antibodies to heart. Myocardial lesions of varying severity were observed in seven of nine animals injected with beef heart and six of twelve animals injected with rat heart. In 13 control rabbits injected with human gamma globulin in adjuvant, these scattered focal lesions were not observed. The incidence and severity of cardiac lesions could not be related to the titer of antibodies to heart as measured by complement fixation or immunofluorescence or necessarily, to presence of flocculating antibodies. Bound gamma globulin was detected within cardiac myofibers in focal sites of myocardium in several animals of the experimental groups, but not in the control animals. The sparseness of such deposits of bound gamma globulin in myocardium of most of the experimental animals, despite the presence of circulating antibodies to heart, suggested that impermeability of myofibers to antibody may represent a limiting factor in the pathogenesis of these lesions.

6300

Kaplan, M.H.; Svec, K H. 1964. Immunologic relation of streptococcal and tissue antigens: III. Presence in human sera of streptococcal antibody cross-reactive with heart tissue; association with streptococcal infection, rheumatic fever, and glomerulonephritis. J. Exp Med 119:651-666.

Sera from some patients with rec streptococcal infection or non-suppurative sequelae exhibit a precipitin reaction with a partially purified streptococcal antigen that is immunologically related to human heart tissue. This precipitin could be absorbed from sera with normal human heart tissue homogenates but not with homogenates of other organs. Cross-reaction by heart absorption was dependent both upon the serologic properties of individual sera and the nature or purity of the streptococcal product employed as test antigen Antigen was localized to cell walls and to a lesser extent to cell membranes of these strains. Reactive sera showed diminution or

loss of serological activity following heat inactivation at 56 C or after prolonged storage at 4 C. Sera containing cross-reactive antibody exhibited FA reaction with sarcolemma of cardiac myofibers, which was inhibited by streptococcal cross-reactive antigen. Antibody to streptococcal cross-reactive antigen was observed in 24 per cent of patients with recent streptococcal infection and in the majority of patients with acute rheumatic fever, rheumatic heart disease, or acute glomerulonephritis. Induction of cross-reactive autoantibody to heart in certain individuals is associated with streptococcal infection.

6301

Kaplan, M.H.; Svec, K.H.; Kushner, I.; Arana-Sialer, J.; Suchy, M.L. 1963. Evidence in Group A streptococcal cells of cross-reactive antigens related to mammalian heart. Federation Proc. 22:340:217.

A cross-reaction between rabbit antiserum to Group A cell walls and mammalian heart has been demonstrated by complement fixation and immunofluorescence. The reverse cross-reaction between antisera to human or rabbit heart and streptococcal cells and cell walls may be shown by agar diffusion and immunoelectrophoresis. A single line of precipitation is observed with solubilized cell wall and three distinct lines with acid extracts or sonicates of whole cells. One of these latter antigens is related to cell wall, a second was present also in culture filtrates, and the third has been detected only in cell extracts. Absorption tests have confirmed the separate specificities of these antigens. Analysis of sera from patients with acute rheumatic fever frequently shows presence of antibody, with specificity directed to cross-reactive antigen of cell walls and heart. These data give direct support to the concept of the induction by streptococcal infection of autoimmunity to heart in rheumatic fever. Complete article

6302

Lindberg, L.H.; Raffel, S.; Vosti, K.L. 1964. Streptococcal glomerulonephritis in rats. Federation Proc. 23.2446.509

Experimental streptococcal glomerulonephritis was induced in rats by intraperitoneally implanting diffusion chambers containing broth cultures of hemolytic streptococci. The rats were placed in individual metabolism cages and urine was collected for protein determinations. Anti-streptolysin O, anti-kidney, and anti-M protein antibody titers were determined on individual sera obtained prior to insertion of the capsule and at the termination of the experiment. Sixty days after implantation of the capsules the rats were sacrificed, capsules were removed, and the chamber contents cultured. The kidneys were perfused with isotonic saline solution until grossly cleared of blood prior to removal, and portions were quick-frozen at -70 C. Sections were stained with fluorescein-labeled antisera

against gamma globulin, appropriate streptococcal antigens, and complement. This model readily demonstrated the presence of tissue-bound gamma globulin, streptococcal antibody and antigen, and the fixation of complement on the glomerular basement membranes of kidneys from rats with proteinuria and elevated anti-M protein antibody titers. These observations support the concept that the pathogenesis of human post-streptococcal glomerulonephritis has an immunologic origin. Complete article.

6303

Mardiney, M.R.; Shuler, S.E.; Feldman, J.D. 1965. Immunology and morphology of human post-streptococcal glomerulonephritis. Federation Proc. 24:3056:682.

Kidneys of a 10-year-old boy were biopsied 7 days and 7 months after the onset of post-streptococcal glomerulonephritis, PSGn. The acute lesion was characterized by large glomeruli in which capillaries were obstructed by swollen endothelial and mesangial cells, and by unobtrusive focal alterations of the basement membranes, BM. By fluorescence microscopy, gamma-2 globulin was distributed in or on the BM; beta-1C was similarly distributed, but in a segmental fashion; a few small foci of antigen, Group A, beta hemolytic streptococci, acid extract of walls, were deposited apparently randomly. After complete clinical recovery, the second biopsy showed patency of glomerular capillaries, an increased number of mesangial cells, some residual swollen endothelium, and focal alterations of the BM. By fluorescence microscopy, gamma-2 globulin was still distributed in or on the BM but with diminished intensity; beta-1C was found mostly in mesangial zones; antigen was not detected. In many details human PSGn was similar to serum sickness nephritis in the rabbit. However, there was still no firm evidence that complexes of antigen antibody and complement were responsible for the human lesion. Complete article.

6304

Markowitz, A.S.; Lange, C.F., Jr. 1965. Streptococcal-related glomerulonephritis: I. Isolation, immunochemistry, and comparative chemistry of soluble fractions from Type 12 nephritogenic streptococci and human glomeruli. J. Immunol. 92:565-575.

Soluble fractions obtained from pooled human glomeruli and the cell membrane of nephritogenic streptococci were shown to be immunologically cross-reactive. One of the components present in the glomerular extract was shown to be unrelated to the streptococcal cross-reactive material but related to a component present on all human red cells tested. Chemical and physical analysis of soluble fractions obtained from isolated human glomeruli and streptococcal cell membranes indicated that they are low-molecular-weight glycoproteins. The glomerular extract has approximately 78.9 per cent protein and 13 to 15 per cent carbohydrate; the streptococcal extract has 80 per cent protein and 7 per cent carbohydrate

The glomerular fraction contains 1.5 to 2 per cent sialic acid but no appreciable phosphorus; the streptococcal fraction has no sialic acid but 3.7 per cent phosphorus. FA was used to localize glomerular antigen at the basement membrane.

6305

Renoux, M. 1964. Specific cardiac antibodies and their significance. Coeur Med. Intern. 3:391-399. In French.

Various immunological techniques, including immunofluorescence, have aided in finding specific antibodies of the cardiac tissue in a large proportion of the sera of subjects with rheumatic disease, postcommissurotomy syndrome, or myocardial infarction. Immune-appearing gamma globulins are fixed on the myocardium of these patients. The studies described here prove a specific antigenicity of the myocardium. In Bouillard's disease, the demonstration of a cross reaction between a myocardiac antigen and a proteinaceous wall antigen of certain group A hemolytic streptococci seems to explain the course of the streptococcal infection in rheumatic disease. The role of autoimmunity in postcommissurotomy syndrome, Dressler's syndrome, and some myocarditis is suggested but not proved.

6306

Seegal, B.C.; Andres, G.A.; Hsu, K.C.; Zabriskie, J.B. 1965. Studies on the pathogenesis of acute and progressive glomerulonephritis in man by immunofluorescein and immunoferritin techniques. Federation Proc. 24:100-108.

Findings are currently compatible with a hypothesis that in acute glomerulonephritis, antigen-antibody complexes may circulate in the blood, aggregate in glomerular capillaries, penetrate among proliferating cells, and form deposits beneath the basement membrane. Complement seems involved. Type 12 streptococcal products appear to be a source of antigen. In subacute nephritis, presence of gamma globulin and complement indicates a role in progression of this disease. FA has been most helpful in these studies, but we are only at the beginning of the problem.

6307

Shiokawa, Y.; Yamada, S ; Shibayama, H. 1963. Studies on rheumatic fever with the immunofluorescent method. Jap. Heart J. 4:407-416.

The proposition that autoimmunity in the pathogenesis of rheumatic fever and rheumatic heart disease has been discussed, but the presence and nature of autoantibodies still remain uncertain. We examined sera from cases with rheumatic fever, rheumatic heart disease, and other diseases by the indirect immunofluorescent method and found positive reactions in sera of rheumatic fever and other diseases. Rabbit heart tissue was used instead of human heart. Sera reacted in three reactive patterns to rabbit and human heart tissues. The results suggest the multiplicity of antibodies to heart tissues.

6308

Wells, H. 1964. The use of immunofluorescent techniques as a diagnostic aid in rheumatic heart diseases. Virginia Med. Mon. 91:516-518.

Fifteen sera of patients suspected of having rheumatic heart disease and 15 sera of patients with unrelated diseases were layered on 3-micron-thick sections of fetal heart and incubated with fluorescein-tagged goat anti-human globulin. All of the sera from patients with clinically diagnosed rheumatic heart disease contained anti-heart immune antibodies detectable by the fluorescent antibody technique. None of 15 control sera produced fluorescent activity. In the first trial run, insufficient washing resulted in flecks of fluorescence remaining on the tissue and upon foreign particles. The procedure is quickly performed and reproducible. Present evidence suggests that clinical activity of the disease correlates with intensity of fluorescence in carefully controlled tests.

6309

Zabriskie, J.B.; Freimer, E.H.; Seegal, B. 1964. An immunological relationship between streptococcal membranes and human heart tissue. Federation Proc. 23:1454:343.

Our immunological studies of streptococcal protoplast membranes reveal that they cross-react with human cardiac tissue. This property is present in all Group A strains tested as well as some Group C strains. In contrast, other streptococci and gram-positive bacteria are non-reactive. By means of the fluorescent antibody technique, this reactivity can be localized to the myofibers and vascular smooth muscle of both normal and rheumatic hearts. Fractionation experiments clearly demonstrate that the immunologically active material in the streptococcal cell resides in the cell membrane. Membranes, free of other cell fractions and antisera to these membranes, have been used. The activity in these antisera can be removed by prior absorption with purified membranes. In addition, those cell wall preparations that partially block the reaction are contaminated with membrane material. Extracts obtained by HCl or pepsin digestion of membranes also extinguish fluorescence, and the active substance appears to be a dialyzable polypeptide. Complete article.

6. Rheumatoid Arthritis

6310

Barnett, E.V.; Bakemeier, R.F.; Leddy, J.P.; Vaughan, J.H. 1965. Heterogeneity of antinuclear factors in lupus erythematosus and rheumatoid arthritis. Proc. Soc. Exp. Biol. Med. 118:803-806.

Twenty-six sera from adults and children with lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, or chronic active hepatitis were tested in a three-layer immunofluorescence test on human white cell nuclei. Rabbit antisera against human Type I and Type II Bence Jones protein were used to determine whether the antinuclear factors contained Type I or Type II L chains. Twenty-four of the sera were shown to contain ANF with both types of L chains. In the remaining two cases quantitative considerations limited the study. Both Type I and Type II ANF were also found in isolated fractions of gamma-2 and gamma-1M globulins in four lupus sera. Antinuclear factor was determined by the three-layer immunofluorescence test.

6311

Barnett, E.V.; Condemi, J.J.; Leddy, J.P.; Vaughan, J.H. 1963. Gamma-2, gamma-1A, and gamma-1M antinuclear factors in human sera. Arth. Rheum. 6:261-262.

An investigation of the classes of proteins containing antinuclear factors (ANF) in the sera of patients with lupus erythematosus or rheumatoid arthritis has been conducted with rabbit antisera specific for human gamma-2, gamma-1A, or gamma-1M immunoglobulins. The specificities of these rabbit antisera were confirmed by various tests, including specific inhibition of the ANF test. Certain sera from lupus erythematosus or rheumatoid arthritis patients had ANF in all three immunoglobulin types. The nuclear material reactive with all three types of ANF was destroyed by incubation with DNAase or DNAase and trypsin, but not with RNAase. Both gamma-1A and gamma-1M ANF were shown to be significantly reduced in titer following treatment with 0.1 M mercaptoethanol, but gamma-2 ANF was unaffected. The titers of gamma-1A and gamma-1M ANF were similar in both lupus erythematosus and rheumatoid arthritis but gamma-2 ANF was significantly lower in rheumatoid arthritis than in lupus patients. These differences may be interpreted in terms of autoimmunization by different nuclear antigens, differences in duration or degree of autoimmunization, or different genetic predispositions of the two populations for the production of the various types of ANF.

6312

Barnett, E.V.; Condemi, J.J.; Leddy, J.P.; Vaughan, J.H. 1964. Gamma-2, gamma-1A, and gamma-1M antinuclear factors in human sera. J. Clin. Invest. 43:1104-1115.

A modification of the indirect immunofluorescent technique was employed to detect antinuclear factors (ANF) of gamma-2, gamma-1A, or gamma-1M immunoglobulin classes. Gamma-1A ANF in two cases appeared to sediment as 7S globulins and be resistant to sulfhydryl treatment, but in other cases, they appeared to sediment faster than 7S globulins and to be inactivated by the sulfhydryl treatment. ANF and rheumatoid factors (RF), although frequently found together in the same serum, behaved as separate serological entities. Evidence was found that in some rheumatoid arthritis sera RF may interact with gamma-2 ANF on nuclei to give the appearance of gamma-1M ANF. ANF of all three immunoglobulin classes were detected in sera from both rheumatoid arthritis (RA) and lupus erythematosus (LE) patients. Gamma-2 ANF was found in higher titer in LE than in RA sera. Among the LE sera, gamma-2 and gamma-1A ANF were found in higher titer if the sera were from patients with disease for less than 1 year. LE patients with disease for more than 1 year, who generally were also in remission and on chloroquin or steroid therapy, had lower titers of gamma-2 and gamma-1A ANF. Results by FA tests of lesser sensitivity are presented.

6313

Barnett, E.V.; North, A.F., Jr.; Vaughan, J.H. 1963. Antinuclear fractions in juvenile rheumatoid arthritis. Arth. Rheum. 6:761.

Antinuclear factors have been found in the sera of adults and children with lupus erythematosus and of a proportion of adults and children with rheumatoid arthritis. This study describes the incidence of antinuclear factor in gamma-2, gamma-1A, and gamma-1M immunoglobulin classes in the sera of children with rheumatoid arthritis. The sera of 13 patients with definite juvenile rheumatoid arthritis, 7 patients with possible or probable juvenile rheumatoid arthritis, and 20 age-matched, non-arthritic hospitalized pediatric patients were examined for antinuclear factor by the three-layer immunofluorescent technique. Of 13 patients with definite rheumatoid arthritis, 8 had antinuclear factor in one or more immunoglobulin classes. None of these patients was latex-positive. None of the seven children with possible or probable rheumatoid arthritis and none of the 20 controls showed antinuclear factor. It appeared that the presence of antinuclear factor was correlated with longer duration and greater severity of disease. The distribution of antinuclear factor among different immunoglobulin classes in this group of patients more closely resembles that found in lupus erythematosus than that found in adult rheumatoid arthritis.

6314

Barnett, E.V.; North, A.F., Jr.; Condemi, J.J.; Jacox, R.F.; Vaughan, J.H. 1965. Antinuclear factors in systemic lupus erythematosus and rheumatoid arthritis. Ann. Intern. Med. 63:100-108.

Antinuclear factors were heterogeneous. The origin and progression of these diseases were discussed and immunologic factors analyzed.

6315

Bonomo, L.; Tursi, A.; Dammacco, F. 1965. Characterization of the antibodies producing the homogeneous and the speckled fluorescence patterns of cell nuclei. J. Lab. Clin. Med. 66:42-52.

The antinuclear factors in sera from patients with systemic lupus erythematosus, rheumatoid arthritis, and other diseases that produce the homogeneous and speckled patterns of nuclear fluorescence were separated by diethylaminoethyl cellulose chromatography. The homogeneous pattern of nuclear fluorescence was constantly produced by a 7S gamma globulin factor, but the speckled pattern was produced by a factor associated with the macroglobulin class only.

6316

Bonomo, L.; Tursi, A.; Dammacco, F. 1965. Characterization of antinuclear factors showing immunofluorescence. Reumatismo 17:3:119-122. In Italian.

The antinuclear factors that produce the homogeneous and speckled pattern of nuclear fluorescence were separated by DEAE cellulose chromatography. Such fractionation procedure was carried out in sera from patients with systemic lupus erythematosus and rheumatoid arthritis. Although the homogeneous pattern of nuclear fluorescence appears constantly due to a 7S gamma globulin factor, the speckled pattern is produced by a factor associated with the macroglobulin class.

6317

Bonomo, L.; Tursi, A.; Minerva, V. 1963. Rheumatoid factor in liver shown by immunofluorescence method. Boll. Soc. Ital. Biol. Sperim. 39.1372-1375. In Italian.

The results of a study of a small group of patients show the presence of gamma globulins fixed to the tissue of liver biopsies in cases of rheumatoid arthritis, chronic liver disease, and chronic bronchitis. The cellular location of the fluorescence is noted.

6318

Cavellero, C.; Chiappino, G. 1963. Immunohistochemical investigations on collagen diseases Ann. Sclavo 5:717-728. In Italian.

The results of immunohistochemical investigations made up on biopsy specimens and necroscopy material in cases of collagen diseases are reported. Bound gamma globulins are mainly in renal lesions of systemic lupus erythematosus. Indirect FA has also shown typical antinuclear and antisarcolemmal auto-antibodies in the sera of patients with systemic lupus erythematosus and dermatomyositis. Rheumatoid factor was demonstrated in the wall of the small synovial vessels of joints in the course of rheumatoid arthritis as well as in some cells of subcutaneous nodules.

6319

Condemi, J.J.; Barnett, E.V.; Atwater, E.C.; Mongan, E.S.; Jacox, R.F.; Vaughan, J.H. 1963. Significance of antinuclear factor in rheumatoid arthritis. Arth. Rheum. 6:266.

In the past 2 years we have found antinuclear factor (ANF) in 36 of 132 patients with rheumatoid arthritis (RA), in 18 of 20 with systemic lupus erythematosus (SLE), and in 3 of 75 normal adults. To determine the significance of the presence of ANF in RA, 44 patients who fulfilled the A.R.A. criteria for definite RA were studied. Nodules occurred in 15 of 18 patients with ANF and 8 of 26 without ANF; P is less than 0.01. A battery of tests, case histories, signs, and symptoms revealed no other significant correlations. This study suggests that RA patients with ANF are not clinically distinguishable from those without ANF. RA patients with nodules may produce a greater variety of abnormal serum factors, i.e., rheumatoid factor and ANF. Complicating infarctive vasculitis is not more frequent in patients with rheumatoid arthritis with ANF than in those without ANF.

6320

Condemi, J.J.; Barnett, E.V.; Atwater, E.C.; Jacox, R.F.; Mongan, E.S.; Vaughan, J.H. 1965. The significance of antinuclear factors in rheumatoid arthritis. Arth. Rheum 8:1080-1093.

Sixty-eight patients with definite or classic rheumatoid arthritis were studied to determine the significance of the presence of antinuclear factors. The patients with positive antinuclear factor determinations were those with more severe disease. These patients almost always had subcutaneous nodules, which were not always associated with a high latex titer for rheumatoid factor. Necrotizing vasculitis occurred in both antinuclear factor positive and negative patients Our data do not support the notion that a positive antinuclear factor test or a positive LE cell test should be used to exclude the diagnosis of rheumatoid arthritis.

6321

Del Giacco, G.S.; Mazzei, D. 1965. Demonstration by immunofluorescence of a serum antiperinuclear factor in rheumatoid arthritis. Reumatismo 17:60-64. In Italian.

In 55 sera of patients (20 with rheumatoid arthritis, others with other diseases), an attempt was made to demonstrate the antiperinuclear factor by means of FA. Immunofluorescence was shown by using smears of human buccal mucosa placed in contact with the serum to be tested and then with human gamma globulin antiserum labeled with fluorescein. Tests were positive in 11 of 20 cases of rheumatoid arthritis and not in the other cases. The factor appears to be a 7S gamma globulin directed against the keratohyaline granules present in the cytoplasm of the cells of the mucosa and appears to be specific for rheumatoid arthritis. The significance of this factor is not yet known. One can consider it as an antibody associated with complex immunological response common to all collagen diseases.

6322

Douglas, W. 1965. The digital artery lesion of rheumatoid arthritis. Ann. Rheum. Dis. 24:40-45.

Digital vessels removed by biopsy from seven patients with rheumatoid arthritis were examined for the presence of rheumatoid factor by immunofluorescent techniques. Rheumatoid factor was not found within the thickened intima of these vessels, although it was readily demonstrated in lymph nodes from sero-positive rheumatoid patients, where it was seen in plasma cells, in germinal centers, and upon the surface of vascular endothelium and blood elements.

6323

Faber, V.; Elling, P. 1964. Antinuclear factor: A new serological reaction. Ugeskr. Laeger 126:1003-1009. In Danish.

A material comprising 1129 sera from normal individuals and from patients with various diseases has been investigated for antinuclear factor (ANF) using the fluorescent antibody technique in order to fix the positive frequency of ANF in the different groups. In healthy individuals 4.6 per cent of the sera showed positive reaction for ANF in low titers. A similar or lower positivity was found in most of the groups of patients with various medical and malignant diseases. ANF was present in 97 per cent of patients with disseminated lupus erythematosus and also frequently in rheumatoid arthritis, hepatic cirrhosis, and in other autoimmune diseases. The diagnostic value of the ANF test is discussed.

6324

Faber, V.; Elling, P. 1965. Antinuclear factors (ANF) as determined by the immunofluorescent antibody technique. Acta Med. Scand. 177:309-319.

A material comprising 1,129 sera from normal individuals and from patients with various diseases has been investigated for antinuclear factors (ANF) using the fluorescent antibody technique in order to determine the occurrence of ANF in the different groups. In healthy individuals, 4.6 per cent of the sera showed a positive reaction for ANF in low titers. A similar or lower positivity was found in most of the groups of patients with various medical and malignant diseases. ANF was present in 97 per cent of the sera of patients with disseminated lupus erythematosus and also frequently in rheumatoid arthritis, hepatic cirrhosis, and other autoimmune diseases. The diagnostic value of the ANF test is discussed.

6325

Fish, A.J.; Michael, A.F. 1965. Immunopathologic changes in rheumatoid arthritis synovia. Federation Proc. 24:3062:683.

Within recent years, studies by several groups of investigators have shown that some patients with rheumatoid arthritis display a moderate to marked depression of complement levels in the synovial fluid in the presence of normal or elevated serum complement levels. To investigate the possibility that lower synovial fluid complement levels in rheumatoid arthritis may represent local utilization of complement components in an immune process, synovial tissue from ten cases of rheumatoid arthritis was examined using immunofluorescent methods to detect specific deposition of gamma-G globulin and beta-1C component of C3. Nine of ten patients displayed moderate to marked accumulation of gamma-G globulin and beta-1C globulin that were deposited in the connective tissue stroma of the synovium. Two patients with recently active disease showed, in addition, well-defined localization of gamma-G globulin and beta-1C globulin in the cytoplasm of large lymphocytes and plasma cells. Synovia from ten patients with diseases other than rheumatoid arthritis, osteoarthritis, traumatic arthritis, or chronic synovitis, were similarly examined and failed to exhibit any gamma-G globulin or beta-1C globulin. Complete article.

6326

Francois, R.J. 1965. Beta-haemolytic streptococci and antistreptolysin-O titres in patients with rheumatoid arthritis and a matched control group. Ann. Rheum. Dis. 24:369-377.

Antistreptolysin-O titer was determined and a throat and a nose swab were taken monthly for 6 months in a group of patients with rheumatoid arthritis and a control group. At any moment of the study the prevalence

of beta-hemolytic streptococci of Groups A, C, and G was significantly higher in the patients with rheumatoid arthritis than in the controls. This was due in part to a slightly higher acquisition rate, but mainly to reduced elimination rate. Equal numbers of raised antistreptolysin-O titers were found in both groups, but significantly more higher titers in the rheumatoid patients. This seems to agree with the lower elimination rate, as prolonged infection leads to higher antibody response.

6327

Frangione, B.; Cooper, N.S.; McEwen, C. 1963. Rheumatoid factor in rheumatoid variants. Arth. Rheum. 6:772.

Rheumatoid factors (RF) have been found in the serum of most rheumatoid arthritis patients. RF is virtually always absent from sera of patients with so-called variants of rheumatoid arthritis, including ankylosing spondylitis, the arthritis of ulcerative colitis, psoriatic arthropathy, and Reiters syndrome. FA was employed to examine synovium obtained at autopsy or surgically from patients with variants of rheumatoid arthritis as well as those with rheumatoid arthritis. RF was found in most of the specimens from rheumatoid patients; it was seen in plasma cells, lymphocytes, unidentifiable mononuclear cells, synovial lining cells, and apparently extracellularly in vessel walls and tissue spaces. RF was also found in synovium from three of six patients with ankylosing spondylitis and two patients with Reiters syndrome. The distribution of RF in the synovium from the variants was similar to that in the rheumatoids, but it tended to be quantitatively less. RF was not found in the serum of the variants. It seems probable that these findings are another example of the nonspecificity of some rheumatoid factors

6328

Hedberg, H. 1964. The depressed synovial complement activity in adult and juvenile rheumatoid arthritis. Acta Rheumatol. Scand. 10:109-127.

These are case histories in which the ANF test was performed by FA.

6329

Huijmans, W.; Valkenburg, H.A.; Muller, A.S.; Gratama, S. 1964. Rheumatoid arthritis in Liberia with an assessment of serological findings. Ann. Rheum. Dis. 23:45-49

Because of the important information to be gained from demographic studies, the details are presented of the first fully established case of rheumatoid arthritis in a native girl in Liberia, a country formerly said to be devoid of this disease. Two additional adult patients are also

discussed. The serological findings, which included tests for rheumatoid and antinuclear factors, were compared with those in a large number of local control subjects.

6330

Homma, M.; Stevens, M.B.; Townes, A.S.; Shulman, L.E. 1965. Anti-DNA antibodies in systemic lupus erythematosus and other disorders. Arth. Rheum. 8:447.

Employing the DNA spot test, we have studied sera from patients with SLE, drug-induced lupus, rheumatoid arthritis, a variety of other disorders, and normal controls. Of more than 100 sera from the 40 patients with SLE studied thus far, all except one have been found to have anti-DNA antibody by this technique, and 80 per cent of these sera have had titers of 1:4 or higher. In general, patients with active disease and those with nephritis had high titers. However, several patients with neither clinical evidence of active disease nor renal involvement had similarly high titers. Low titers of anti-DNA antibody were also demonstrable in some patients with rheumatoid arthritis as well as various other diseases. Of the 25 normal controls studied thus far, only two have shown a minimal reaction. These data indicate that anti-DNA antibodies, as determined by this technique, are found in almost all patients with SLE, with or without nephritis and whether the disease is active or not. Moreover, small amounts of anti-DNA antibody may be found in individuals who do not have clinical evidence of SLE.

6331

Kass, H.; Hanson, V. 1965. Antinuclear antibodies in childhood. Arth. Rheum. 8:450-451.

This study was designed to test the following hypotheses: Antinuclear antibodies occur in childhood in the same general disease distribution as in adult life; the occurrence of antinuclear antibodies will be influenced by the sex and age of the child, and antinuclear antibodies may be found more frequently in the presence of other abnormal immune factors. In the study group of 68 children with definite rheumatoid arthritis, 10 of 44 girls and 2 of 24 boys demonstrated antinuclear factor by immunofluorescent methods. The peak incidence of antinuclear factor in rheumatoid children occurred in the 1- to 5-year and 11- to 15-year age range. There were no children under 6 years of age with positive rheumatoid factor. The incidence of antinuclear factor in the childhood rheumatic diseases follows a pattern similar to that in the adult. The data suggest that there is an increased production of abnormal immune factors in female children. The bimodal peak incidence of antinuclear factor in both the very young and the adolescent rheumatoid child is contrasted to the directly increasing seropositivity for rheumatoid factor as the child approaches adolescence.

6332

Nienhuis, R.L.F.; Mandema, E. 1964. A new serum factor in patients with rheumatoid arthritis: The antiperinuclear factor. Ann. Rheum. Dis. 23:302-305.

Using indirect FA with epithelial cells from healthy human buccal mucosa as substrate, a new factor was found in the sera of about 50 per cent of patients with rheumatoid arthritis. This factor is called antiperinuclear factor because it gives a perinuclear fluorescence. It is probably an antibody of the 7S gamma globulin type against keratohyaline granules in buccal mucosa cells. The incidence of the factor in healthy people seems to be very low, and its presence in the other diseases so far studied also seems to be very rare. It should be noted that the factor was not present in the serum of 42 patients with ankylosing spondylitis.

6333

Restifo, R.A.; Lussier, A.J.; Rawson, A.J.; Rockey, J.H.; Hollander, J.L. 1965. Studies on the pathogenesis of rheumatoid joint inflammation: III. The experimental production of arthritis by the intra-articular injection of purified 7S gamma globulin. Ann. Intern. Med. 62:285-291.

In one of the preceding papers, a hypothesis was presented for the pathogenesis of rheumatoid joint inflammation. It proposes that such inflammation may be the result of the leukocytic response to the local deposition of complexes of 7S gamma globulin with rheumatoid factor in the joint. This study was designed to test this hypothesis by the intra-articular injection of autologous purified 7S gamma globulin into the uninvolved knees of rheumatoid arthritic patients known to harbor the rheumatoid factor. Five of six trials of autologous, rheumatoid 7S gamma globulin elicited an acute reaction. The one patient who failed to react was later found to have a negative serum latex fixation test. When purified rheumatoid 7S gamma globulin was injected into osteoarthritic knees, there was no inflammatory response in five such trials. Other classes of 7S gamma globulin evoked no response. Although preliminary, this study tends to support the above hypothesis.

6334

Riddle, J.M.; Bluhm, G.B.; Barnhart, M.I. 1965. Ultrastructure and immunocytology of exudative neutrophilic phagosomes in rheumatoid arthritis. Federation Proc. 24:2686:616.

Neutrophils from synovial fluids of four classical rheumatoid arthritis patients were investigated by light, fluorescence, and electron microscopy. Discrete cytoplasmic inclusions were present. In ultrathin sections these were identified as membrane-bound phagosomes.

Phagosomal contents were diverse and consisted of cellular constituents, homogeneous background material with a substructure, and granular masses. With immunofluorescence, the phagosomal contents were identified as fibrin, fibrin associated with rheumatoid factor, and rheumatoid factor - 7S gamma globulin complexes. Our interpretation of these data is presented. Fibrin formation in the joint stimulates and sustains neutrophil migration. In turn, these leukocytes are cellular agents for fibrin removal. The combination of rheumatoid factor and fibrin may function to recycle the acute inflammatory response. In fact, this molecular aggregate may confront the exudative neutrophil with a structure that cannot be digested by the neutrophilic enzymes. Complete article.

6335

Ritchie, R.F.; Bayles, T.B.; Harter, J.G. 1964. A fluorescent antibody inhibition method for classification of serum antinuclear factors. Arth. Rheum. 7:339.

FITC conjugates were prepared from gamma globulins of patients with systemic lupus erythematosus or rheumatoid arthritis. It was possible to inhibit fluorescence of these conjugates with normal and diseased sera. Fluorescence of conjugates from rheumatoid arthritis globulins was nearly always inhibited by diseased or normal sera. Fluorescence of conjugates of lupus globulins was nearly always inhibited by serum of lupus patients only. Globulin conjugates from patients with other than classic lupus reacted similarly to thc e of rheumatoid patients. This inhibition technique permits the multiple factors present in sera to be distinguished. It offers a potential method of assessing clinical significance.

6336

Ritchie, R.F.; Bayles, T.B ; Harter, J.G. 1965. The significance of a negative and a false negative test for antinuclear factors in the diagnosis of rheumatic disease. Arth. Rheum. 8:463-464

Concern over the nonspecificity of the antinuclear factor (ANF) test has arisen. Of 140 patients with definite rheumatoid arthritis, 28 per cent had a positive ANF test but there was no definite correlation of the titer with the degree of clinical activity, sedimentation rate, gamma globulin levels, or the extent of disease, and only a poor correlation with the titer of rheumatoid factor and the presence of LE cells. Seventy-two per cent of patients with active rheumatoid arthritis had a negative test in the whole serum; however, on dilution 8 per cent of these sera produced bright nuclear fluorescence. In several instances titers of 1:128 were found and in one patient a titer of 1:1024. Although none of the 45 patients with SLE or active scleroderma was found to be negative in the whole serum, a similar increase in fluorescence was noted with moderated dilution. This phenomenon is freely and reversibly removed by dialysis

and probably does not represent a prozone effect. If the test is to be used as a diagnostic aid, both the whole serum and a dilution of 1:8 should be examined. All of 200 normals and patients without rheumatic disease had a negative test, and none of these showed the false negative reaction. A negative ANF test has considerable diagnostic value in the exclusion of SLE or scleroderma.

6337

Rodman, W.W.; Williams, R.C., Jr.; Bilka, P.J.; Muller-Eberhard, H.J. 1964. Immunofluorescent localization of beta-1C and beta-1E globulin complement components in synovial tissues from rheumatoid arthritis patients. Arth. Rheum. 7:749.

The purpose of this study was to examine rheumatoid synovial tissues directly with fluorescein-conjugated anticomplement antisera to determine whether any specific localization of complement components was present. Fresh synovial tissues obtained by needle biopsy from patients with rheumatoid arthritis and normal synovia from various orthopedic procedures were examined using FA conjugates to: 7S gamma globulin, 19S gamma globulin, beta-1C globulin, and beta-1E globulin. Tissue-bound rheumatoid factors were localized with conjugated human aggregated gamma globulin. All synovia from patients with rheumatoid arthritis showed synovial membrane staining for 7S gamma globulin. More localized areas of 19S gamma globulin and rheumatoid factors were noted within synovial membranes and around blood vessel walls. Beta-1C and -1E globulin components were found both in synovial membrane filaments and bound to nuclei of fibrocytes as well as blood vessel walls. The synovial tissue localization of both beta-1C and beta-1E globulin corresponded most closely with concurrent 7S gamma globulin staining. Parallel deposition of complement and 7S gamma globulin in rheumatoid synovial tissues suggests that if immunologic injury be present thereby, it is related to 7S gamma globulin and not to rheumatoid factor localization

6338

Turner-Warwick, M.; Doniach, D. 1965. Auto-antibody studies in interstitial pulmonary fibrosis. Brit. Med. J. 1:886-891.

Auto-antibody studies were performed in 48 patients with interstitial pulmonary fibrosis, of whom 14 also had rheumatoid arthritis. Positive results in one or more tests were obtained in 32, most of the antibodies being of non-organ-specific nature. Rheumatoid factors were present in 49 per cent of the cases, including all the patients with arthritis, but also in 11 of 34 cases without joint manifestations, and in these the differential agglutination titer was more often positive than the latex F II test. Antinuclear factors were detected in 28 per cent

compared with 4 per cent of matched controls, and two-thirds of positive reactors had negative differential agglutination titers. Non-organ-specific complement fixing antibodies were found in 19 per cent of patients (controls 2 per cent), all of whom also had antinuclear factors or rheumatoid factors. Auto-antibodies specific to lung could not be detected by immunofluorescent techniques in the patients' sera, nor could fixed antigen-antibody complexes be demonstrated in biopsies on two cases of interstitial pulmonary fibrosis.

6339

Vacca, G. 1964. Quantitative research of antinuclear factors in rheumatoid arthritis by the immunofluorescent technique. Minerva Med. 55:2131-2133. In Italian.

The sera of 42 rheumatoid arthritis patients were tested for the presence of antinuclear antibodies by the FA technique. In eight cases titers of 1:1 to 1:64 were obtained. The results agreed with those obtained by agglutination.

6340

Ward, D.J.; Johnson, G.D.; Holborow, E.J. 1964. Antinuclear factor in rheumatoid arthritis: Its incidence and clinical significance. Ann. Rheum. Dis. 23:306-310.

The results of repeated ANF tests in 273 patients with classical or definite rheumatoid arthritis have been studied in retrospect. Considering initial tests only, 81 per cent were negative and 19 per cent positive. Taking all tests into account, 77 per cent of the initial 273 cases remained negative, 13 per cent remained positive, and 10 per cent varied, having both positive and negative results. This should be compared with an incidence of less than 1 per cent of positive tests found in a normal population by the method described, and an incidence of 100 per cent in 62 cases of clinically active SLE. Implications and variance of findings are discussed.

6341

Williamson, N.; Ling, N.R. 1965. Cellular reaction to complexes formed between rheumatoid factor and aggregated human gamma globulin. Ann. Rheum. Dis. 24:513-521.

Intracellular and extracellular bodies were produced when aggregated gamma globulin was added to cultures of leukocyte-rich plasma from rheumatoid patients. Similar cells and bodies occurred in synovial fluid smears from rheumatoid patients. Histochemical and FA studies were undertaken

to establish the nature of these structures. The specificity and significance of this phenomenon in rheumatoid arthritis is discussed, and the possible application to diagnosis is considered.

7. Sjogren Syndrome

6342

Beck, J.S.; Anderson, J.R.; Bloch, K.J.; Buchanan, W.W.; Bunim, J.J. 1965. Antinuclear and precipitating autoantibodies in Sjogren's syndrome. Ann. Rheum. Dis. 24:16-22.

Tests for antinuclear antibodies were performed by the indirect fluorescent technique in 42 patients with Sjogren's syndrome. Eighteen had rheumatoid arthritis (Group A), two had probable rheumatoid arthritis (Group B), three had progressive systemic sclerosis (Group C), three had polymyositis (Group D), and 16 had the sicca syndrome alone (Group E). Of the 42 patients who had positive antinuclear factor tests, the incidence and titer of the antibodies was greater in patients of Group E than in Group A. There was a direct relationship between the presence of antinuclear factor and parotid enlargement indicated by history or examination, and a direct relationship between the titers of antinuclear factor and the gamma globulin levels. Study of the morphological pattern of nuclear fluorescence showed that the prevalence of homogeneous antibody was the same in Groups A and E, but that speckled antinuclear antibody was more common in Group E. Antinuclear antibody was found exclusively in patients of Group E. Three precipitating autoantibodies were found. Incidence and titer of the precipitating autoantibodies were higher in Group E than in Group A.

6343

Bertram, U.; Halberg, P. 1964. Chronic sialodenitis. Acta Allergol. 19.399-405.

A 68 year-old woman whose daughter had died of disseminated lupus erythematosus was admitted because of a tumor in the palate. A biopsy of the tumor revealed chronic sialodenitis. No swelling of other salivary glands could be found, but the patient had xerostomy, and sialography showed changes also in the parotid glands. The patient had diffuse hypergammaglobulinemia, and complement fixing antibody directed against extracts of salivary glands was found in her serum. The antibody was, however, not specific because the serum could also fix complement with extracts of various other organs. The serum contained antinuclear factor as demonstrated by the immunofluorescence technique, but no cytoplasmic

fluorescence could be obtained in sections of salivary glands. It is emphasized that, even though chronic sialodenitis (Sjogren syndrome) has features in common with autoimmune diseases, all attempts to demonstrate organ-specific antibodies against salivary antigens in sera from patients with this disease have so far been unsuccessful.

6344

Bertram, U.; Halberg, P. 1964. A specific antibody against the epithelium of the salivary ducts in sera from patients with Sjogren syndrome Acta Allergol. 19:458-466.

Sjogren syndrome has many features in common with autoimmune diseases clinically, pathologically, and serologically. However, all previous attempts to demonstrate a specific antibody against salivary tissue in sera from patients with Sjogren syndrome have been unsuccessful. In this paper a serological phenomenon is presented that might represent a specific antibody against an antigen localized in the cytoplasm of the epithelial cells of the salivary ducts of parotid, submaxillary, and sublingual glands, demonstrated by an immunofluorescence technique. No antigenicity was found in the acini. The antibody was found in sera from 11 of 19 patients with Sjogren syndrome, in one of 24 control sera, in one of 19 sera from patients with Hashimoto disease, and in 4 of 16 sera from patients with disseminated lupus erythematosus.

6345

Bunim, J J.; Buchanan, W.W.; Wertlake, P.T.; Sokoloff, L.; Bloch, K.J ; Beck, J S.; Alepa, F P. 1964. Clinical, pathologic, and serologic studies in Sjogren's syndrome. (Combined Clinical Staff Conference at the National Institutes of Health.) Ann. Intern. Med. 61:509-530.

As a result of the several studies and observations described at this conference, former concepts of the clinical, pathological, and immunological characteristics of Sjogren's syndrome have been revised and broadened. The clinical classification of cases into five subgroups was substantiated by distinctive patterns of diverse tissue antibodies. The presence of rheumatoid factors in almost every case of Sjogren's syndrome, even in the absence of rheumatoid arthritis, was an unexpected finding, as were the occurrence of multiple antibodies to tissue components. Other new clinical features are described.

6346

Harrison, W.J. 1965. Thyroid, gastric (parietal-cell), and nuclear antibodies in ulcerative colitis. Lancet 1:1350-1353.

The frequency of thyroid and parietal-cell antibodies in ulcerative colitis sera was found to be normal. The frequency of factors reacting with liver and thyroid cell nuclei was normal, but 3 of 100 colitis sera contained, at a titer of less than 1:10, an antinuclear factor specific for polymorphs.

6347

Pittman, F.E.; Holub, D.A. 1965. Sjogren's syndrome and adult celiac disease. Gastroenterology 48:869-876.

A patient with Sjogren's syndrome, nutritional deficiencies, and diarrhea was shown to have primary intestinal malabsorption caused by adult celiac disease. Abnormalities of small intestinal mucosal function and morphology improved after a gluten-free diet. Instillation of gliadin-purified wheat protein into the jejunum after regeneration of the mucosa had occurred caused a severe histological reaction and a generalized urticarial skin eruption. Studies with fluorescein-labeled antibody demonstrated increased gamma globulin deposition in the lamina propria of the jejunal mucosa.

6348

Shearn, M.A.; Tu, W.-H. 1965. Nephrogenic diabetes insipidus and other defects of renal tubular function in Sjogren's syndrome. Amer. J. Med. 39:312-318.

Polyuria and polydipsia were the presenting symptoms in a young woman with Sjogren's syndrome. Various clinical test results are listed. FA was used to demonstrate anti-thyroid antibody.

6349

Talal, N.; Bunim, J.J. 1963. The development of malignant lymphoma in patients with Sjogren's syndrome. Arth. Rheum. 6:302.

Three of 58 patients with Sjogren's syndrome have developed reticulum cell sarcoma, and lesions resembling those of Waldenstrom macroglobulinemia have appeared in a fourth case. In one case, beta-2M globulins were increased in serum and lymph nodes, as determined by immunofluorescent studies, and PAS-positive intranuclear inclusions were present in an unusual pulmonary lymphoplasmacytoid infiltrate. Two additional cases of Sjogren's syndrome and lymphoma, or reticulum cell sarcoma

and Hodgkin's disease, have been observed by others. It may be significant that three of our four patients received X-ray therapy to the enlarged parotid glands. Only one of the other 54 patients in our series was exposed to radiotherapy. It is suggested that the chronic state of lymphoid proliferation and excessive plasma cell activity predisposes to this malignant transformation or possible mutation. If the malignant cells retain the capacity to synthesize immune globulins or if enough nonmalignant plasma cells remain, hypergammaglobulinemia and macroglobulinemia may be present. If functioning plasma cells are replaced by malignant cells incapable of synthesizing immune globulins, hypogammaglobulinemia may appear.

6350

Talal, N.; Bunim, J.J. 1964. The development of malignant lymphoma in the course of Sjogren's syndrome. Amer. J. Med. 36:529-540.

Of 58 patients with Sjogren's syndrome reticulum cell sarcomas developed in three, and lesions resembling Waldenstrom's macroglobulinemia in a fourth. These patients have certain clinical and laboratory features in common which distinguish them from patients with the usual benign cases of Sjogren's syndrome. The gamma globulin abnormalities have been investigated by immunoelectrophoretic analysis, ultracentrifugation, and fluorescent antibody techniques. The relationship between connective tissue diseases, gamma globulin abnormalities, thymomas, and malignant lymphomas is discussed. The hypothesis is presented that in Sjogren's syndrome the chronic state of immunologic hyperactivity and the proliferation of immunologically competent cells producing abnormal tissue antibodies predispose to the relatively frequent development of malignant lymphoma.

8 Other Diseases Involving Autoimmune Factors

6351

Ansell, B.M.; Wigley, R.A.D. 1963. Arthritic manifestation in regional enteritis. Arth. Rheum. 6:260-261.

It has been suggested that the arthritis seen occasionally in patients suffering from regional enteritis is similar to that seen in ulcerative colitis. To investigate this further, a survey was undertaken of 101 patients suffering from chronic regional enteritis who had attended three centers during the past 5 years. None of the patients showed a positive sheep cell agglutination test or positive test for antinuclear factor. There was no correlation of either arthritic manifestations or sacro-ileitis with colonic involvement.

6352

Barnhart, M.I.; McCutcheon, S.A.; Riddle, J.M.; Ohorodnik, J.M. 1964. Thrombotic thrombocytopenic purpura as a model of accelerated protein synthesis. Throm. Diath. Haemorrh. 12:211-231.

Proteins concerned in blood coagulation and clot lysis were studied during a fulminating and fatal case of thrombotic thrombocytopenic purpura. One outstanding feature was the enhanced synthesis of several different plasma proteins. Specific fluorescent antibodies were used to detect cellular synthesis and storage of fibrinogen, prothrombin, albumin, profibrinolysin, and gamma globulin. Most liver cells were actively producing fibrinogen, prothrombin, and albumin. Bone marrow eosinophils were synthesizing profibrinolysin. Peripheral blood eosinophils still contained their packages of profibrinolysin as if they were transporting this to intravascular thrombi. Polymorphonuclear leukocytes in the blood were marked with fluorescent antifibrinogen Breakdown products of fibrin contained in these leukocytes most likely accounted for the staining. Polymorphonuclear leukocytes may provide an important defense mechanism for individuals in a thrombotic state.

6353

Beck, J.S. 1965. Autoantibodies in ulcerative colitis. Lancet 2:187.

Experience in comparing the serological findings in 29 ulcerative colitis patients with sex and age matched healthy laboratory and factory workers is reported. Neither speckled nor antinucleolar antinuclear antibodies were detected in any of the patients or controls. The frequency of antigastric and anti-thyroglobulin antibodies was similar to that in the controls. The presence of serological abnormality could not be related to the severity of colonic disease.

6354

Beck, J.S., Anderson, J.R., Gray, K.G., Rowell, N.R. 1963. Antinuclear and precipitating autoantibodies in progressive systemic sclerosis. Lancet 2 1188-1190

The immunological abnormalities in a group of 32 patients with progressive systemic sclerosis (PSS) are described. Antinuclear antibodies, often of high titer, were found in 78 per cent; 19 per cent showed precipitating autoantibodies to saline extracts of human tissues. The autoantibodies detected in PSS have all been found in other connective tissue diseases. Antinucleolar antibodies are seen much more commonly than in the other connective tissue diseases. One of the precipitating antibodies, anti-Lup, was found in two cases; the only other condition in which this antibody has been found is systemic lupus erythematosus.

6355

Benoit, F.L.; Rulon, D.B.; Theil, G.B.; Doolan, P.D.; Watten, R.H. 1964. Goodpasture's syndrome: A clinicopathologic entity. Amer. J. Med. 37:424-444.

As a part of these case studies and review, FA was used in an unsuccessful attempt to demonstrate renal autoantibody.

6356

Beregi, E.; Perenyi, L.; Simon, J. 1963. Immunofluorescence studies in experimental periarteritis nodosa in rabbits of different ages. Gerontologia 8:233-241.

Rabbits of different ages develop experimental periarteritis nodosa. Immunofluorescence studies showed that the immunoglobulin concentration and the intensity of fluorescence do not depend on age, but are correlated with the intensity of the allergic reaction. It is obvious that the histological and histochemical data supply only limited information as to the intensity of the allergic reaction

6357

Bernhardt, H.; Burkett, L.L.; Fields, M.L.; Killian, J. 1965. The diagnostic significance of the parietal cell immunofluorescent test. Ann. Intern. Med. 63:635-641.

The parietal cell immunofluorescent test was done in cases of pernicious anemia, thyroid disease, rheumatoid arthritis, subtotal gastrectomy, Whipple's disease, nutritional macrocytic anemia, nonmacrocytic anemia, diabetes mellitus, and miscellaneous other conditions in an effort to evaluate its diagnostic significance and usefulness as a clinical tool. It is suggested that the test may prove to be of value as a screening procedure to detect cases of chronic atrophic gastritis in an early asymptomatic stage that may then be followed up for the development of intrinsic factor deficiency and carcinoma. The test is of limited value in the differential diagnosis of anemias, since it is indicative of atrophic gastritis and not specifically of pernicious anemia. Its only use in this regard may be in suggesting an early probable diagnosis.

6358

Beutner, E.H.; Jordan, R.E. 1964. Demonstration of skin antibodies in sera of pemphigus vulgaris by indirect immunofluorescent staining. Proc. Soc. Exp. Biol. Med. 117:505-510.

Eight of 13 sera from patients with pemphigus vulgaris were found to contain antibodies to a substance at the surface of the cells of stratified squamous epithelium, particularly in the stratum spinosum, as demonstrated by indirect immunofluorescent (IIF) staining. The reactions of four of these eight sera were deemed to be weakly positive or doubtful, the remaining four sera yielded titers of 1:30 to 1:120 by IIF staining. The reactive antigen was found only in stratified squamous epithelium. Other types of epithelial tissue studied to date do not appear to contain the antigen. None of 88 sera from other individuals contained such activity. A skin biopsy of one of the antibody-producing patients with pemphigus vulgaris revealed the presence of bound gamma globulin on the surface of the epithelial cells in an apparently normal portion of the skin adjacent to a vesicle. This localization was identical to that obtained by IIF staining with the serum of that patient.

6359

Beutner, E.H.; Lever, W.F.; Witebsky, E.; Jordan, R.; Chertock, B. 1965. Autoantibodies in pemphigus vulgaris. J. Amer. Med. Ass. 192:682-688.

Antibodies to an intercellular substance of stratified squamous epithelium were detected by indirect immunofluorescent staining in the sera of eight of 16 patients with pemphigus vulgaris. The autoantibody nature of these antibodies could be demonstrated by testing the skin with the serum of that patient. The sera of three of six patients with chronic bullous dermatosis contained antibodies to a substance in the basement zone of the epidermis. By direct immunofluorescent staining of biopsies, gamma globulin was demonstrated in the intercellular areas of the epidermis of three patients with pemphigus vulgaris and in the basement zone of a patient with chronic bullous dermatosis. Intradermal injection of monkeys with human sera containing antibodies of the intercellular substance resulted in binding of gamma globulin to the intercellular substance of the epidermis.

6360

Bohrer, N.; Churg, J.; Gribetz, D. 1964. Glomerulonephritis in two sets of identical twins: Electron microscopic studies of renal biopsy specimens. Amer. J. Med. 36:787-794.

Case reports of glomerulonephritis in two pairs of identical twins are presented. Renal biopsies were performed. Three were studied by electron microscopy, one by FA. In one pair, one twin had typical chronic glomerulonephritis, the other had persistent proteinuria and glomerular changes that were interpreted as mild glomerulonephritis on the basis of electron microscopic and FA studies. In the second pair, both twins gave a history of acute glomerulonephritis and showed mild but unmistakable changes of glomerular disease by electron microscopy. Genetic factors may predispose to the development of glomerulonephritis in some instances.

6361

Bonomo, L.; Tursi, A. 1963. Study by the immunofluorescence method of elimination of cells containing gamma globulin and a rheumatoid factor in the sputum. Boll. Soc. Ital. Biol. Sperim. 39:1379-1381. In Italian.

Immunofluorescence techniques aided in the demonstration of the presence of cells containing gamma globulin in a sizable percentage of 11 patients with chronic bronchitis, especially in those in which marked dysproteinemia with hyper-gamma-globulinemia existed. Cells were also observed containing an anti-gamma-globulinic factor of rheumatoid type. The morphology of these cells was studied. The results confirm the presence of foci of chronic pulmonary inflammation and the wealth of reticulohystiocytic elements in the lungs. The importance of chronic expectoration as a dysproteinemizing mechanism is emphasized.

6362

Bonomo, L.; Tursi, A.; Minerva, V. 1963. Rheumatoid factor in liver shown by immunofluorescence method. Boll. Soc. Ital. Biol. Sperim. 39:1372-1375. In Italian.

The results of a study of a small group of patients show the presence of gamma globulins fixed to the tissue of liver biopsies in cases of rheumatoid arthritis, chronic liver disease, and chronic bronchitis. The cellular location of the fluorescence is noted.

6363

Burkholder, P.M. 1965. Malignant nephrosclerosis: An immunohistopathologic study on localized gamma globulin and fixation of guinea pig complement in human kidneys. Arch. Pathol. 80:583-589.

By use of immunohistologic techniques it has been shown that human immunoglobulins bound in the walls of damaged arteries and focally in only an occasional glomerulus in kidneys of patients with malignant nephrosclerosis take up components of guinea pig complement in vitro under conditions established for complement-fixation. Localized immunoglobulins may be antibodies in immune or immune-like complexes. The vascular lesions could be due to an immunopathogenesis.

6364

Coghill, N.F.; Doniach, D.; Roitt, I.M.; Mollin, D.L.; Williams, A.W. 1965. Autoantibodies in simple atrophic gastritis. Gut 6:48-56.

Forty-seven chronic gastritis patients were examined. In nine it was superficial and in 38 atrophic. Patients with pernicious anemia were excluded, and in only two was there a doubtful family history of pernicious anemia. A relatively high incidence of autoantibodies to the parietal cells of the stomach was found. In no case, however, were there antibodies to intrinsic factor, although these were detected in nearly 60 per cent of patients with pernicious anemia. Interesting correlations were found between the parietal cells antibodies and such factors as the degree of loss of parietal cells, the severity of the gastric secretory defect, and the presence of thyroid antibodies. ANF and thyroid autoantibodies were determined by FA

6365

Cotran, R.S. 1963 Retrograde Proteus pyelonephritis in rats: Localization of antigen and antibody in treated sterile pyelonephritic kidneys. J Exp Med 117.813-822

Rats with retrograde Proteus pyelonephritis were treated with antibiotics until their kidneys became sterile. Using FA, specific P. mirabilis antigen was found in some sterile pyelonephritic kidneys 20 weeks after cessation of treatment and presumed renal sterilization. Persistent antigen was associated with interstitial chronic inflammation but not with acute inflammation or progressive scarring. Rat gamma globulin and Proteus antibody were localized in plasma cells of the renal inflammatory infiltrates. Persistent antigen in chronic pyelonephritis may lead to the continued local appearance of antibody-producing cells.

6366

Del Giacco, G.S.; Luporini, G. 1964. Possible relationship between antinuclear factor and rheumatoid factor in malignant dermatoviscerítis. Reumatismo 16:272-275. In Italian.

Six patient sera were tested for the presence of antinuclear antibodies by FA and other techniques and found to be positive. In two cases the antibodies were removed by treatment with gamma globulin aggregates.

6367

Duncan, D.A.; Drummond, K.N.; Michael, A.F.; Vernier, R.L. 1965. Pulmonary hemorrhage and glomerulonephritis: Report of six cases and study of the renal lesion by the fluorescent antibody technique and electron microscopy. Ann. Intern. Med. 62:920-938.

Six cases of pulmonary hemorrhage and glomerulonephritis are presented. The pulmonary disease appeared to precede the nephritis. By the fluorescent antibody technique, 7S gamma globulin was found on the capillary basement membrane of the kidneys of the three patients studied. Beta-1C globulin was found on the capillary basement membrane of one of two patients studied, suggesting that the deposited gamma globulin may be the result of an immunological reaction. Gamma and beta-1C globulins were not found in the lung of one patient studied. In two of four patients, virus-like particles were found in the glomerular epithelial and endothelial cells. Their possible significance is discussed, and a hypothesis is advanced that could relate many of the intriguing facets of this syndrome.

6368

Gotoff, S.P.; Fellers, F.X.; Vawter, G.F.; Janeway, C.A.; Rosen, F.S. 1965. The beta-1C globulin in childhood nephrotic syndrome. New Engl J. Med. 273:524-529.

An immunochemical assay was used to estimate the serum and urine concentration of beta-1C globulin in patients with renal disease. The serum beta-1C globulin was low in patients with acute glomerulonephritis, returning to normal 1 or 2 months after the onset of symptoms. In seven of 56 children with the nephrotic syndrome the serum content was persistently low. With fluorescent antibody, beta-1C globulin and gamma globulin were localized in the glomeruli of all five children with progressive glomerulonephritis in whom these studies could be made

6369

Greenberg, M.S.; Wong, H. 1963. Production of purpura in dogs by heterologous antiendothelium serum. Federation Proc. 22:1602:429.

An autoimmune basis for non-thrombocytopenic vascular purpura analogous to that in thromobocytopenic purpura but with the autoantibody directed against endothelium rather than platelets has been suspected. To determine the effects of antiserum to endothelium, five dogs were injected intraperitoneally with heat-inactivated rabbit antidog endothelium serum, ADES, absorbed with dog red blood cells. The dogs were sacrificed either 6 or 24 hours after injection and examined for visceral purpura and localization of rabbit globulin by the immunofluorescent technique. Thrombocytopenia was observed in two dogs 24 hours after injection. All the dogs had visceral petechiae; in two, this was present 24 hours after injection in the absence of thrombocytopenia. Normal rabbit serum did not cause purpura. Rabbit globulin was found in the glomeruli of the dogs given either ADES or antidog platelet serum. No such localization was found after injection of normal rabbit serum. These data suggest antigenic similarities between platelets and endothelium but also indicate that visceral purpura may be caused by reaction between endothelium and antibody without coexisting thrombocytopenia. Complete article.

6370

Harders, H.; Dolle, W. 1963. The lupoid hepatitis syndrome: A contribution to the problem of the nosological classification of liver diseases with antinuclear serum factors: Four personal cases and review of the literature. Gastroenterologia 100:220-248. In German.

The so-called lupoid hepatitis is a chronic liver disease, predominantly of young females. The syndrome is characterized by the combination of arthralgia, skin rashes, fever, and jaundice. Antinuclear antibodies, hyperproteinemia with hypergammaglobulinemia, positive flocculation tests, and strongly increased ESR are found. The role of antinuclear factors in chronic liver disease is discussed.

6371

Harrison, W.J. 1965. Autoantibodies against intestinal and gastric mucous cells in ulcerative colitis. Lancet 1:1346-1350.

The fluorescent antiglobulin technique was used in a study of the prevalence of organ-specific autoantibodies in ulcerative colitis. An antibody reacting with small and large intestinal mucous cells was detected in 27 of 200 cases. All control sera were negative. The prevalence of this antibody is unrelated to the duration or severity

of the disease or to the extent of inflammation of the large bowel. Four of the sera containing intestinal antibody also contained a separate antibody against superficial gastric mucous cells.

6372

Holborow, E.J.; Asherson, G.L.; Johnson, G.D.; Barnes, R.D.S.; Carmichael, D.S. 1963. Antinuclear factor and other antibodies in blood and liver diseases. Brit. Med. J. 1:656-658.

Tests for antibodies commonly associated with autoimmune disease were carried out on sera from patients with blood and liver disorders. Antinuclear factor was definitely present in one case of scleroderma with agranulocytosis, one case of thymoma with aplastic anemia, and one case of infectious mononucleosis.

6373

Hung, W.; Migeon, C.J.; Parrott, R. H. 1963. A possible autoimmune basis for Addison's disease in three siblings, one with idiopathic hypoparathyroidism, pernicious anemia and superficial moniliasis. New Engl. J. Med. 269:658-663.

A family in which Addison's disease was present in three siblings is described. One of the siblings also had superficial moniliasis, idiopathic hypoparathyroidism, and pernicious anemia. Circulating antiadrenal antibodies were present in the two living siblings. The occurrence of Addison's disease in three siblings and the presence of circulating antiadrenal antibodies strongly suggests that a genetic predisposition to autoimmunity of the adrenal glands may be present in familial idiopathic Addison disease. The association of idiopathic hypoparathyroidism and pernicious anemia with Addison's disease suggests the possibility of polyglandular autoimmunization.

6374

Ignatova, M.S. 1963. Some problems of nephritis pathogenesis. Nephritis as an autoimmunic affection. Vop. Okhrany Matern. Detstva 8 44-51. In Russian.

Experimental and clinical observations of the role played by the autoimmune changes in nephritis are reviewed. A report is presented on 123 children with diffuse glomerulonephritis, the majority of whom possessed foci of chronic infection (chronic tonsillitis, adenoiditis, etc.). A clinical picture of nephritis followed some disease in 10 to 15 days, after a period that, in experimental conditions, was required for the development of autoimmune changes. In the greater proportion of the children examined, there was a rise in the gamma globulin blood fraction. Freely circulating antirenal autoantibodies were revealed in the blood, indicating an important role of autoimmune changes in diffuse glomerulonephritis. Detection of a precipitate-complex antigen-antibody in electron microscopy also confirmed the autoimmune processes occuring in the development of nephritis.

6375

Johnson, G.D.; Holborow, E.J.; Glynn, L.E. 1965 Antibody to smooth muscle in patients with liver disease. Lancet 2:878-879.

The demonstration by FA of a serum factor reacting with smooth muscle is described. The presence of the factor was apparently associated with a possible diagnosis of lupoid hepatitis, whereas all 16 cases of systemic lupus erythematosus studied were negative. Only four positives were found in a collection of sera taken from 93 patients with other diseases of probable autoimmune origin, and all 25 normal controls were negative.

6376

Jordan, R.E.; Beutner, E.H.; Aquilina, J.T. 1964 Immunofluorescent studies of pemphigus vulgaris. Federation Proc. 23.1449:342.

Pemphigus vulgaris is suspected of being an autoimmune disease. Indirect immunofluorescent, IIF, staining with human sera was utilized to determine whether skin-specific antibodies occur in association with this disease. Human and rhesus monkey tissue served as antigen. Of the four sera from patients with pemphigus vulgaris tested, two yielded IIF staining of an intercellular substance in the epidermis of human and monkey skin. Staining occurred predominantly in the stratum spinosum and was negative in the stratum granulosum. Staining of the stratum germinativum is questionable. This reaction occurred at serum dilutions as high as 1:60. Similar reactions occurred in human and monkey oral mucosa and in the monkey esophagus. No reactions could be demonstrated in other epithelial tissues tested to date. Of the other 30 sera tested, four SLE, five normal, and 21 other skin diseases, none yielded positive reactions. A skin biopsy of one of the serologically positive pemphigus patients revealed positive localization of human gamma globulin in the normal portion of skin in the intercellular space. It appears probable that autoantibodies specific for an intercellular substance of squamous epithelium occur in pemphigus vulgaris. Complete article.

6377

Klavins, J.V. 1963. Cytoplasm of colonic mucosal cells as site of antigen in ulcerative colitis. J. Amer. Med. Ass. 183:547-548.

It was demonstrated by FA that sera from three of seven adults and from one of four children contained gamma globulin that combined with colonic mucosal cells. The mucosal cell cytoplasm was identified as the site of the antigen.

6378

Kniker, W.T.; Cochrane, C.G. 1965. Dependence of cardiovascular lesions in serum sickness on polymorphonuclear leukocytes. Federation Proc. 24:1336:371.

Serum sickness is induced in rabbits by the deposition of circulating antigen-antibody complexes. The observed cardiovascular and renal inflammatory lesions result from the interaction of these complexes with host mediators. The purpose of this study was to evaluate the role of the polymorphonuclear leukocyte, PMN, as a mediator of the vascular lesions. This was accomplished by depleting the circulating PMN during immune elimination, when lesions in normal rabbits were developing. In PMN-depleted animals, necrotic vascular lesions were never seen and endothelial proliferation in arteries was largely inhibited. Fibrinoid deposits did not occur. By contrast, over two-thirds of control animals exhibited arterial endothelial proliferation, and half had necrosis of arterial walls. The medial inflammation was associated with disruption of the internal elastic lamina beneath zones of intimal proliferation. This disruption of the internal elastic lamina was only found in the presence of polymorphs. With fluorescent antibodies, bovine serum albumin, rabbit gamma globulin, and complement were localized in vascular lesions. The essential role of the PMN as a mediator of vascular injury following the localization of antigen-antibody complexes is strongly suggested. Complete article.

6379

Koffler, D.; Paronetto, F. 1965. Immunofluorescent localization of immunoglobulins, complement, and fibrinogen in human diseases: II. Acute, subacute, and chronic glomerulonephritis. J. Clin. Invest. 44:1665-1671.

Gamma-2 and gamma-1M globulins in association with complement and fibrinogen have been localized in the glomeruli of kidneys showing acute, subacute, and chronic glomerulonephritis. Gamma-1A globulin was present in tubular epithelium in the absence of complement. Complement was also fixed to glomeruli in vitro. The similarity of immunological findings in systemic lupus erythematosus and glomerulonephritis implies a common injury induced by antigen-antibody complexes and possibly by fibrinogen

6380

Kraft, S.C.; Rimpila, J.J.; Fitch, F.W.; Kirsner, J.B. 1964. Immunofluorescent studies of ulcerative colitis colon. Specificity and nonspecificity. Gastroenterology 47:747-748.

The authors endeavored to evaluate the tissue localization of antigen, or antibody, or both, in ulcerative colitis, by utilizing autologous, homologous, and heterologous serum and colon tissue, in direct and indirect immunofluorescent studies. The specificity of the occasional fluorescence observed in association

with colonic epithelial cells could not be confirmed. The staining seemed attributable to inappropriate concentrations of fluorescein, protein, or both, and other physical-chemical interreactions, rather than to antigen-antibody union. Present studies confirmed the presence of increased numbers of lymphocytes and plasma cells containing gamma globulin in the cellular infiltrate of the lamina propria and in the submucosa of colitis colon. Observations also suggest the presence of both antigen and antibody in the cytoplasm of eosinophils and neutrophils in the colitis cellular infiltrate, although at least part of the fluorescence associated with these leukocytes seems to be nonspecific This finding may represent local antigen-antibody union, or the presence of antigen-antibody complexes or components thereof, undergoing phagocytosis.

6381

Lambie, J.A.; Duff, I.F. 1963. Familial occurrence of dermatomyositis: Case reports and a family survey. Ann Intern Med. 59:839-847

An instance of familial occurrence of dermatomyositis is reported that appears to be the fourth recorded case A family survey was undertaken to determine the presence of connective tissue diseases and serum protein abnormalities in other family members Borderline or definitely elevated serum gamma globulins were found in one proposita and six relatives. The other proposita had low total serum proteins and low normal gamma globulins Two relatives had weakly reactive latex slide tests for the rheumatoid factor A positive antinuclear factor test was noted in one relative One proposita and six relatives had equivocal antinuclear factor tests Other test results are listed These findings may reflect a relationship between rheumatoid arthritis, dermatomyositis, and certain serum protein abnormalities

6382

Lange, K., Treser, G ; Wachstein, M.; Wasserman, E. 1964 Routine immunofluorescent histology as an aid in the diagnosis and prognosis of renal diseases Amer J. Pathol 44.14a-15a

Kidney biopsy specimens from normal individuals and 53 patients with renal diseases were studied by FA for human gamma globulin, undivided complement, complement component C'1, complement component C'3, and human albumin Antigens were deposited by immune processes and not by transudation into inflamed tissues. In acute glomerulonephritis there was intense staining for gamma globulin and all complement components. The staining was diffuse on or near the basement membrane and in the area of the mesangium With clinical healing, the staining became segmental and disappeared with complete clinical healing Even slight urinary abnormalities were accompanied by segmental hyperfluorescence In chronic glomerulonephritis there was diffuse intensive staining of the basement membrane Segmental

or no staining appeared in the fibrotic and hyalinized regions. Intense staining was associated with active SLE. It receded or disappeared after prolonged glucocorticoid treatment. In active pure nephrosis, staining for gamma globulin and complement components was in the basement membranes. Staining of the mesangium was lacking. A patient with severe Bence Jones proteinuria exhibited no staining. Complete article.

6383

Lange, K.; Wachstein, M.; Wasserman, E.; Alptekin, F.; Slobody, L.B. 1963. The congenital nephrotic syndrome: An immune reaction? Amer. J. Dis. Children 105:338-345.

The interpretation of the congenital nephrotic syndrome as a tubular malformation does not explain the presence of erythrocytes, granular casts, and a severe proteinuria in these newborns. In a newborn infant with a congenital nephrotic syndrome the presence of gamma globulin, complement components C1 and C3, and undivided complement could be shown on many but not all glomerular loops by immunofluorescent methods. This indicates a tissue-destroying, complement-binding antigen-antibody reaction. The finding of many albumin reabsorption droplets in the tubular epithelium refutes the explanation that the proteinuria is due to a lack of protein reabsorption by the malformed tubuli. The possible origin of the antibody against the fetus kidney in the mother and the possible reasons for its formation are discussed.

6384

Maclachlan, M.J.; Rodnan, G.P.; Cooper, W.M.; Fennell, R.H., Jr. 1965. Chronic active ('lupoid') hepatitis: A clinical, serological, and pathological study of 20 patients Ann Intern. Med 62:425-462.

Clinical, serological, and pathological observations on 20 cases of chronic, active hepatitis were reviewed. Antinuclear factors were demonstrated in 16 of 18 patients by FA

6385

Mancini, R.E.; Andrada, J A ; Saraceni, D ; Bachmann, A.E.; Lavieri, J.C ; Nemirovsky, M. 1965 Immunological and testicular response in man sensitized with human testicular homogenate J. Clin Endocrinol Metab. 25:859-875.

Three different groups composed of 18 patients having prostatic carcinoma were studied. None had had previous treatment or metabolic diseases The first group was sensitized with autologous or homologous testicular homogenate plus complete Freund's adjuvant Patients of the second group were separately sensitized with testicular homogenate plus incomplete

Freund's adjuvant, testicular homogenate alone, or with complete or incomplete Freund's adjuvant alone. The third group was not sensitized and served as a control for the preceding groups. In all cases complement fixation, hemagglutination, passive cutaneous anaphylactic and antiglobulin consumption tests were performed. FA using the patient's own sera and testis sections showed a positive reaction in the germinal cells, presumably spermatids and spermatozoa. This immunological and testicular response was present in two of four patients of the first group, and it was absent in the control groups. Blood group iso-antigens ascribed to spermatozoa were incapable of interfering with the anti-spermatic antibodies.

6386

McCombs, R.P. 1965. Systemic 'allergic' vasculitis. Clinical and pathological relationships. J. Amer. Med. Ass. 194:1059-1064.

A review of 72 cases of vasculitis indicated that hypersensitivity was a probable cause in the majority, but in five cases there were associated lymphoproliferative diseases. Corticosteroids controlled nearly all of the manifestations of vasculitis if administered in early, adequate dosage. Complete recovery was noted in one-fourth and partial recovery in one-third of the patients. Most deaths were due to renal failure. The terms periarteritis or polyarteritis nodosa should be reserved for those cases with nodular arteritis and the term systemic allergic vaculitis be used for those cases with only small vessel involvement. In these the prognosis is much better. FA anti-nuclear tests were negative in five seriously ill patients.

6387

Meiselas, L.E.; Fierst, S.; Levin, E. 1963. Studies of incidence and type of arthritis and abnormal antiglobulins in ulcerative colitis following ileostomy. Arth. Rheum. 6:287-288

Recently there has been an increased awareness of the incidence of arthritis accompanying ulcerative colitis and ileitis. This study reports the incidence of arthritis and abnormal antibodies in 31 patients with ileostomy. The following antibody systems were studied: rheumatoid factor, antinuclear antibodies, lupus preps, anti-thyroglobulin antibodies, antiglobulins, and anti-Brucella antibodies following stimulation by a single dose of Brucella vaccine.

6388

Mellors, R.C. 1965. Autoimmune disease in NZB-BL mice: I. Pathology and pathogenesis of a model system of spontaneous glomerulonephritis. J. Exp. Med. 122:25-40.

This study, based upon 528 laboratory examinations and 16 complete autopsies of NZB-BL mice, deals with autoimmune manifestations. These were hypergammaglobulinemia, Coombs positive hemolytic anemia, and the occasional presence of lupus- and rheumatoid-like factors. The study deals mainly with the pathology and pathogenesis of glomerulonephritis in these mice, a model system of membranous glomerulonephritis with spontaneous and insidious onset, progression through chronic stages, and almost certainly induced by immunological and autoimmune mechanisms. The earliest and lasting histological change was hyaline thickening of the capillary walls and adjacent intercapillary regions of the glomerular tufts, corresponding in location to polysaccharide-rich capillary basement membrane and mesangial materials. When distributed focally and diffusely in the glomerular tuft and eventually sparing no glomerulus, the hyaline, granular, and fibrillar materials produced narrowing of capillary lumens by concentric or eccentric encroachment upon them. In the latter stages, hyaline lobulation and sclerosis of the glomerular tufts occurred. Glomerular tufts contained selective localizations of mouse immunoglobulins corresponding in distribution to that of the hyaline and polysaccharide-rich materials in focal and diffuse membranous and lobular lesions and in amounts increasing with the severity of glomerular disease. The spleen was identified at the cellular level as the main site of formation of autoantibodies to red cells, as well as the main site of red cell destruction.

6389

Miescher, P.A ; Paronetto, F.; Koffler, D. 1965. Immune studies in vasculitis associated with 19S-7S type of cryoglobulinemia. Arth Rheum 8:457

This study was performed on two patients with recurrent vascular purpura and arthralgia of several years duration. Antinuclear antibodies were not detectable Histologic examination of skin biopsies from the two patients revealed a similar picture of vasculitis in the superficial dermis associated with neutrophils, some mononuclear cells, and red cell extravasates Utilizing the fluorescent antibody technique, gamma-G and beta-1C globulin and fibrinogen were localized in the vessel walls and surrounding connective tissue Gamma-M globulin was detected only in one case exhibiting fresh vascular lesions. In contrast, a case of erythema nodosum vasculitis and of factitious dermatitis did not exhibit positive staining for immunoglobulins and beta-1C globulin. It appears that complement-fixing cryoprecipitates are formed, resulting in a decrease of serum complement and in local vasculitis upon deposition of precipitates on the vascular endothelium.

6390

Okuda, R.; Kaplan, M.H.; Cuppage, F.E.; Heymann, W. 1965. Deposition of autologous gamma globulin in kidneys of rats with nephrotic renal disease of various etiologies. J. Lab. Clin. Med. 66:204-215.

The deposition of autologous gamma globulin in the kidney was examined by the immunofluorescent technique in rats with autoimmune nephrosis, nephrotoxic serum nephrosis, and aminonucleoside nephrosis. Diffuse abundant deposition in glomeruli was noted in all animals with autoimmune nephrosis, and persisted for at least 5 months. The intensity of staining bore no relation to the severity of renal disease. In certain animals, gamma globulin deposits were present in the absence of disease. The deposition of gamma globulin in glomeruli in autoimmune disease was of a characteristic granular distribution in capillary loops distinct from the smooth linear pattern of deposition in nephrotoxic disease, suggesting that different antigenic materials may be involved in the pathogenesis of these diseases. In rats injected with aminonucleoside of puromycin, only slight staining for gamma globulin was noted up to 7 days after the onset of proteinuria. Subsequently, increased deposition became apparent, generally localized to segments of the glomerular tufts. Such deposition was in contrast to the diffuse involvement of glomeruli observed in the experimental immune diseases, and may possibly be related to a nonspecific adherence of the host gamma globulin to injured renal tissue.

6391

Parish, W.E.; Rhodes, E.L. 1965. Investigation of nodular vasculitis by means of the fluorescent antibody technique. Brit. J. Dermatol. 77:529.

Biopsy specimens of nine cases of nodular vasculitis were examined for gamma globulin using FA. Gamma globulin was found in and around the vessel walls of two patients only, one of whom had developed her leg lesions 2 weeks after quinsy. The nine cases were then examined for streptococcal complexes with rabbit anti-streptococcal serum of both Group A and Group D streptococci, tracing any fixation of this serum with a fluorescent labeled goat anti-rabbit serum. Fluorescence with the streptococcal Group A antigen was found only in the section of the patient who had had quinsy and then in the areas where gamma globulin had been found previously. Sections were also examined for tubercle antigen using rabbit antihuman tubercle serum and tracing fixation with fluorescent goat anti-rabbit serum. Specific fluorescence indicating tubercle antigen was found in the sections from two patients, both of whom had active tuberculous glands in their necks. The lesions on the legs in all nine patients looked very similar.

6392

Paronetto, F. 1965. Immunocytochemical observations on the vascular necrosis and renal glomerular lesions of malignant nephrosclerosis. Amer. J. Pathol. 46:901-915.

The effects of phenacetin (or acetophenatidin) and the contaminant present in phenacetin, acetic-4-chloranilide (A4CA), were observed in young male Osborne-Mendel rats fed diets of these drugs for 22 weeks. The results suggest that the principal offending agent producing the changes observed is not phenacetin but is the contaminant A4CA. There was evidence of a hemolytic process, Heinz bodies appeared in red cells, and there were sulfhemoglobinemia, prominent splenic enlargement, an increase of hemosiderin in the spleen, and hemosiderin and hemosiderin-negative pigment in the renal tubular epithelium and in Kupffer cells. The treated animals showed no evidence of interstitial nephritis or necrosis of the renal papillae, both of which may be associated with excessive and prolonged ingestion of phenacetin-containing analgesic agents in human subjects. Frozen sections were stained by FA methods for portions of this study.

6393

Paronetto, F.; Koffler, D. 1965. Immunohistochemical observations in systemic lupus erythematosus and glomerulonephritis. Federation Proc. 24:3059:683.

To assess the relationship of protein deposition to tissue damage, kidneys of 16 patients with SLE, and of 15 patients with acute, subacute, and chronic glomerulonephritis were studied with the fluorescent antibody technique as to: type of immunoglobulin; complement, beta-1C globulin; and deposition of other plasma proteins, fibrinogen, alpha-2 macroglobulin and albumin. Patients with SLE with lupus nephritis exhibited gamma-2 and gamma-1M globulin, complement, and fibrinogen in the renal glomeruli and arterioles with fibrinoid necrosis. Gamma-1A globulin, alpha-2 macroglobulin, and albumin were rarely seen. Three cases showed immunoglobulin staining of nuclei of renal epithelial cells. In acute glomerulonephritis fibrinogen, gamma globulin and complement were localized in renal glomeruli. In subacute and chronic glomerulonephritis glomeruli exhibited plasma proteins similar to that seen in SLE but in decreased amounts. Gamma-2 and gamma-1M globulin are the immunoglobulins present in the lesions of SLE and glomerulonephritis. The localization of complement suggests the presence of immune complexes in the lesions. Renal glomerular deposits of fibrinogen indicate that alterations in blood coagulation may play a role in the pathogenesis of these diseases. Complete article.

6394

Pasternack, A.; Linder, E.; Kuhlback, B. 1965. Glomerulonephritis with initial pulmonary hemorrhage. Acta Med. Scand. 177:601-605.

A surviving patient with Goodpasture's syndrome (pulmonary hemorrhage with glomerulonephritis) is reported. The etiology is discussed in the light of immunological studies, revealing the presence of a circulating antibody against kidney and lung.

6395

Pizzi, F.; Carrara, P.M. 1964. Fluorescent antibody technique applied to immunologic thrombocytopenias. Haematol. Lat. 7:147-156. In Italian.

With the fluorescent antibody technique thrombocytopenic syndromes of presumable immunological origin have been studied. Protein material in cytoplasm of megakaryocytes from patients with chronic thrombocytopenic purpura was found in much greater amount than in normal subjects. The immunological pathogenesis of this condition seems to be supported by this finding.

6396

Raskin, J. 1964. Fluorescent antibody studies of certain dermatoses. Arch. Dermatol. 89:569-578.

FA was used to investigate the possible role of autoimmune factors in certain dermatoses. Serum, sections of cutaneous lesions, and specimens of uninvolved skin were studied. Specific antigen-antibody reaction was not detected in the skin of patients who had one of the following conditions: psoriasis, erythema multiforme bullosum, pemphigus vulgaris, or dermatitis herpetiformis. Antibodies for calf thymus nucleohistone extract were detected in serum of two patients who had both psoriasis and arthropathy and of another patient who had had severe cutaneous manifestations of psoriasis for 17 years. Non-species-specific antinuclear antibodies were detected in sera of patients who had discoid lupus erythematosus. The importance of autoimmune factors in the pathogenesis of discoid lupus erythematosus is discussed. Antinuclear antibody was demonstrated in the serum of patients who had scleroderma. An autoimmune reaction may be a factor in the development of enlarging annular lesions. A correlation could not be made between the presence of serum antinuclear antibodies and abnormal serum protein fractions.

6397

Sanders, V.; Ritts, R.E., Jr. 1965. Ventricular localization of bound gamma globulin in idiopathic disease of the myocardium. J. Amer. Med. Ass. 194:59-61.

The myocardium of nine patients with idiopathic disease of the myocardium (IDM) and 16 control subjects were examined for bound gamma globulin. Five of the IDM subjects were found to have marked accumulation of gamma globulin bound to and extending beneath the sarcolemmal sheath. Two of the control group showed small amounts of gamma globulin in a similar location but without significant histological findings.

6398

Scheer, R.L.; Grossman, M.A.; Seegal, B.A. 1964. Immune aspects of the glomerulonephritis associated with idiopathic pulmonary hemorrhage. Ann. Intern. Med. 61:816.

Reports of the syndrome of recurrent pulmonary hemorrhage with glomerulonephritis (Goodpasture's syndrome) have emphasized the fulminant nature of the disease. Two cases are presented with studies designed to explore a possible immune mechanism. Sections of the patients' kidneys and lungs were examined for fluorescence after being stained with three FIC-labeled antisera: antihuman gamma globulin, antihuman albumin, and anti-Group A streptococcus. Tests for serum antibodies against Type 12, Group A streptococci and anti-streptolysin 'O' titers were done. A search for anti-kidney antibiotics was made by staining normal kidney and lung with labeled patients' sera, and by agglutination tests with kidney extract adsorbed into latex particles. Gamma globulin was present in the glomerular capillary basement membranes, but not in the lungs. No circulating antibody could be demonstrated in the sera. These studies suggest that the glomerulonephritis in Goodpasture's syndrome represents an immune reaction unrelated to streptococcal infections. A possible mechanism is suggested

6399

Scheiffarth, F.; Warnatz, H. 1965. Pathogenesis of chronic hepatitis as an immunological problem. Klin. Wochensch. 43:473-479. In German.

Clinical, morphological, and serological features in the course of chronic hepatitis suggest a definite role of an immunopathological mechanism in this autonomously progressive disease. The circulating antibodies detectable in chronic inflammatory liver disease are defined as autoantibodies, as they are demonstrated by serological and fluorescent microscopic techniques to be bound to autologous and homologous liver tissue. However, based on present evidence, the pathogenicity of these antibodies is unlikely. Different indications denote that immunological factors in the sense of

an autosensitivity process participate in the development of chronic hepatitis. On the basis of clinical and experimental pathologic data, no definite evidence of an autosensitivity process has been produced.

6400

Sharpstone, P.; James, D. 1965. Pernicious anemia and thyrotoxicosis in a family. Lancet 1:246-248.

Two sisters in a family had both pernicious anemia and thyrotoxicosis. Both sisters had thyroid and gastric antibodies, and autoantibodies were also present in several of their relatives. Tests for non-organ-specific antibodies, the antinuclear factors test, and the rat liver complement fixation test were all negative. The mother, who had a normal blood picture, showed gastric parietal cell antibodies and intrinsic factor antibodies, and was found to have latent pernicious anemia. An asymptomatic sister with thyroid antibodies showed evidence of impaired thyroid reserve. The findings have relevance to the autoimmune concept of the etiology of pernicious anemia and thyroid disorders.

6401

Shimizu, T.; Kaga, T.; Matsumoto, T.; Matsumura, N. 1963. Clinical studies on Behcet's syndrome with special reference to epidemiology and predisposing factors. Naika (Hokan) 12:526-536. In Japanese.

Follow-up study of the clinical course of 63 cases of Behcet's syndrome is presented. The disease takes graver course in males than in females and usually reaches its worst stage in 5 to 7 years. Mortality rate is 2.7 to 4.8 per cent and incidence of blindness among males is 39.6 per cent and in females 9.4 per cent. Prognosis of neuro-Behcet's syndrome is grave. In Japan this disease is responsible for 6 to 7 per cent of all acquired blindness. The relationship between the disease and the menstrual cycle, which often is a predisposing factor of a characteristic syndrome, is discussed, and results of biometric study of this disease are presented. As to the cause of the disease, the results obtained by an indirect fluorescent antibody technique similar to that applied to rheumatism and other collagen diseases are presented.

6402

Shimizu, T.; Katsuta, Y.; Oshima, Y. 1965. Immunological studies on Behcet's syndrome. Ann. Rheum. Dis. 24:494-500.

In ten to 30 of 132 cases of Behcet's syndrome seen in our clinic, immunochemical studies were carried out, using an immediate intradermal reaction, a hemagglutination test with heat-aggregated human gamma globulin, and FA. A high incidence of strong positives in the former reactions and

the demonstration of a cytoplasmic fluorescence in the leukocytes of peripheral blood and cells of aphthous ulcers of the cases tested suggest that there may be a related autoimmune mechanism in Behcet's syndrome and in collagen diseases. The rise in the serum sialic acid level and changes with the clinical course of the disease also suggest that the systemic connective tissue damage is similar to that found in the collagen diseases.

6403

Sturgill, B.C.; Westervelt, F.B. 1965. Immunofluorescence studies in a case of Goodpasture's syndrome. J. Amer. Med. Ass. 194:914-916.

Goodpasture's syndrome occurred in a 17-year-old boy, with a fatal course. Gamma globulin was demonstrated by FA in the alveolar septa, as well as in the basement membrane of glomeruli. Pulmonary lesions in this syndrome may result from the same mechanism as that responsible for the renal lesions.

6404

Taylor, K.B. 1965. Role of immune responses in the gastrointestinal tract. Federation Proc. 24:23-28.

FA was used to determine antinuclear antibodies in portions of this report. Diseases such as pernicious anemia, gastritis, ulcerative colitis, celiac disease, nontropical sprue, and regional enteritis were considered. Various factors, largely autoimmune phenomena, involved in these diseases were discussed. Family studies and association studies of the gastrointestinal tract and other organs, using serologic tools, may help elucidate causative factors in these chronic diseases.

6405

Thayer, W.R.; Spiro, H.M. 1963. Protein abnormalities in ulcerative colitis patients and their families. Gastroenterology 44:444-447.

The sera of 98 members from 34 families with ulcerative colitis have been studied for antinuclear factor, rheumatoid factor, thyroid antibodies, and for positive LE latex tests. These were compared with a series of sera from 55 individuals of 27 families of patients with gastric cancer. An antinuclear factor was found in 23 per cent of the ulcerative colitis family members but in only 3 per cent of the controls. The incidence of rheumatoid factor, thyroid antibodies, and positive LE latex was about the same in the two groups.

6406

Turner-Warwick, M.; Doniach, D. 1965. Auto-antibody studies in interstitial pulmonary fibrosis. Brit. Med. J. 1:886-891.

Auto-antibody studies were performed in 48 patients with interstitial pulmonary fibrosis, of whom 14 also had rheumatoid arthritis. Positive results in one or more tests were obtained in 32, most of the antibodies being of non-organ-specific nature. Rheumatoid factors were present in 49 per cent of the cases, including all the patients with arthritis, but also in 11 of 34 cases without joint manifestations, and in these the differential agglutination titer was more often positive than the latex F II test. Antinuclear factors were detected in 28 per cent compared with 4 per cent of matched controls, and two-thirds of positive reactors had negative differential agglutination titers. Non-organ-specific complement fixing antibodies were found in 19 per cent of patients (controls 2 per cent), all of whom also had antinuclear factors or rheumatoid factors. Auto-antibodies specific to lung could not be detected by immunofluorescent techniques in the patients' sera, nor could fixed antigen-antibody complexes be demonstrated in bipsies on two cases of interstitial pulmonary fibrosis.

6407

Vaughan, J.H., Barnett, E.V. 1965. Increased production of circulating L chains in acute serum sickness. Arth. Rheum. 8.476

A patient who received a large dose of horse serum was observed before, during, and after development of serum sickness. At the time of the serum sickness the patient developed a pruritic morbilliform eruption that progressed into an inflammatory purpura. Treatment with corticosteroids brought the patient into good control. His serum proteins and complement titers were recorded before, during, and after the reaction. Antedating the development of serum sickness a moderate number of gamma globulin - containing lymphoid cells were detected in buffy coat preparations of peripheral blood by immunofluorescence techniques. At the time of the serum sickness there was an increased amount of euglobulin precipitable from the serum in 0.02 M sodium chloride. Only at that time was horse serum demonstrable in the euglobulin precipitate. Free L chains, demonstrable by quantitative complement fixation tests, appeared in the serum at the time of the maximal symptomatic reaction. Immunologic events have been documented in a patient with serum sickness. An increase in euglobulins, interpreted as antigen-antibody aggregates, was noted, along with increased serum L chain levels.

6408

White, F.N.; Grollman, A. 1964. Experimental periarteritis nodosa in the rat. Arch. Pathol. 78:31-36.

Rats immunized with extracts of isologous arterial tissue incorporated in Freund complete adjuvant developed precipitating antibody to the antigen and severe periarteritis nodosa of their mesenteric vessels. Fluorescein-conjugated globulins of the immunized animals were bound specifically by normal arteries, and the presence of adsorbed globulin was demonstrated in the periarteritic lesions that appear to be autoimmune in origin. Severe hypertension did not accompany the development of the periarteritis as it does when this disorder is induced by infarction of the kidney. The extrarenal vascular lesions accompanying renal infarction appear less contributory to the development of the hypertension that follows this procedure than are the renal vascular and parenchymal changes that it induces.

B. EXPERIMENTAL AUTOIMMUNE KIDNEY DISEASE

6409

Andres, G.A.; Seegal, B.C.; Hsu, K.C.; Rothenberg, M.S; Chapeau, M.L. 1963 Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody. Localization of antigen-antibody complexes in rabbit glomeruli following repeated injections of bovine serum albumin. J. Exp. Med. 117:691-704

Acute, subacute, and chronic glomerulonephritis, similar in certain features to human glomerulonephritis, has been produced in rabbits by repeated injections of bovine serum albumin. The ratio of antigen to antibody was the factor determining the development and type of glomerulonephritis. Antigen aggregates are present in the blood, cross the endothelium and the basement membrane, and accumulate as dense deposits between the basement membrane and the epithelial cytoplasm. In the deposits electron-dense aggregates formed by antigen or by antigen-antibody complexes and material that might be other endogenous proteins may be identified. In rabbits dead of anaphylactic shock following injection of bovine serum albumin, dense material was found within glomerular capillaries, presumably formed by the embolic deposition of antigen-antibody complexes, because the immunofluorescein and immunoferritin techniques demonstrated the presence of both BSA and rabbit globulin.

6410

Boss, J.H. 1964 Membrane antigens in human and rat tissues. Penn. Med J. 67 25-30

Argyrophile and PAS-positive, membranous, and fibrillar structures of all tissues and organs contain a number of cross-reacting antigens. It is suggested that they be termed membrane antigens. The nephrotoxic serum antigens belong to this group, and hence the diverse anti-organ sera produce glomerulonephritis when injected intravenously. However, administration of anti-heart, anti-liver, anti-spleen, and anti-skeletal muscle sera is not followed by a demonstrable host response in the form of glomerular fixation of the animal's own gamma globulin, although such a host reaction is consistently observed after the injection of anti-kidney, anti-lung, and anti-placenta sera. Human membrane antigens are heterogenic insofar as the corresponding antisera cross-react with membrane antigens of other species.

6411

Boss, J.H. 1964. Microscopic immunofluorescence of glomeruli in chronic nephrotoxic serum nephritis. J. Histochem. Cytochem. 13:350-354.

The microscopic immunofluorescence of the glomeruli differs in acute and chronic nephrotoxic serum nephritis in the distribution pattern of the injected nephrotoxic globulin and the host's gamma globulin. In acute nephritis specific glomerular fluorescence, denoting fixation of nephrotoxic and recipient's gamma globulin, is sharply limited to regular, thin and delicate lines corresponding to the capillary basement membranes. On the other hand, specific glomerular fluorescence in chronic nephritis appears as irregular, broad and tortuous loops consisting of coarse, partially coalescing specks of differing brightness. This difference is possibly related to the profound alterations in chronic nephritis resulting in reconstruction of the glomerular architecture

6412

Boss, J.H. 1965. A comparative study of kidney and muscle membrane antigens in the human and rat. Exp. Mol. Pathol. 4:416-430

Argyrophile, PAS-positive, membranous and fibrillar structures of the diverse organs share a number of common antigens, referred to as membrane antigens. Antisera obtained by heteroimmunization with organ homogenates contain a variety of antibodies. Homologous antigens are partly common to many organs and partly organ-specific. The number of membrane antigens is unknown. Striated muscle contains fewer membrane antigens than the kidney, but at least one nephrotoxic serum antigen is present. The injection of rabbit anti-kidney and anti-skeletal muscle serum in the rat causes glomerulonephri[illegible]. Glomerular localization of rabbit antibody globulin is readily demonstrable in both cases, but glomerular fixation of the rat's own gamma globulin is found only in animals given anti-kidney serum. Nephrotoxicity of anti-kidney serum cannot be neutralized by absorption with muscle material. It is postulated that renal and muscular nephrotoxic serum antigens differ in some as yet unknown qualitative or quantitative properties

6413

Boss, J.H. 1965. Isolation of a potent nephrotoxic serum antigen preparation from rat heart. Brit. J. Exp. Pathol. 46:630-634.

Isolation of a potent nephrotoxic serum antigen preparation from rat heart is described. Nephrotoxic anti-heart serum is distinct from anti-kidney serum in that it does not induce the second phase of experimental glomerulonephritis. The injection of anti-heart serum caused nephritis and glomerular fixation of antibody globulin, but an immune response in the form of glomerular localization of the recipient's own gamma globulin cannot be demonstrated immunohistologically. Inoculation material for sensitization was prepared by differential centrifugation of sonically disintegrated heart homogenate. Antiserum against the 1,500 gravity sediment proved to be essentially similar to anti-kidney serum eliciting a characteristic immune response. Thus, rat heart contains the same or very similar nephrotoxic serum antigens as the kidney.

6414

Cochrane, C.G., Unanue, E.R.; Dixon, F.J. 1965. A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. J. Exp Med. 122 99-116.

In acute nephrotoxic nephritis (NTN) polymorphonuclear leukocytes, or polymorphs, accumulated in large numbers in the glomeruli in the first 12 hours The endothelial cells were dislodged by the polymorphs, which then came to lie immediately adjacent to the glomerular basement membranes. Ultrastructural changes were not observed Depletion of polymorphs prevented the develoment of proteinuria This occurred when doses of nephrotoxic globulin were employed that produced severe proteinurias. Measurable glomerular damage was frequently averted until the onset of the secondary stage of NTN. Polymorph-depleted animals exhibited minimal nonspecific changes in the blood, the ability of their vascular beds to react to stimuli was not affected, and deposition of nephrotoxic antibody and complement in the glomeruli was not inhibited Elimination of polymorphs from the circulation was only partially effective in preventing glomerular damage when large doses of nephrotoxic globulin were used An indirect role of complement in bringing about glomerular injury in first stage NTN was apparent When rabbit nephrotoxic globulin was injected into rats depleted of complement, or when duck nephrotoxic globulin that fixed complement poorly was injected into normal rats, complement failed to bind with the antibody along glomerular basement membranes, and polymorphs did not accumulate

6415

Dixon, F.J. 1965. Renal injury induced by antigen-antibody complexes and other immunologic means. Federation Proc. 24:98-99.

FA technique clearly demonstrates sites of antigen-antibody reaction in various nephritides. Several mechanisms for renal disease due to antigen-antibody reactions are defined. The kidney seems doubly susceptible to immunologic injury. It has relatively organ-specific antigens that may react with anti-kidney antibody. Also, its large circulation and filtering function appears to make the kidney vulnerable to circulating serologic complexes. The exact relation of these points to kidney disease is not known. A role for circulating complexes is suggested in lupus nephritis and glomerulonephritis. If extra capillary deposits of complexes along the basement membrane are specific for induced nephritis, then pathogenesis of autoimmune kidney in adjuvant-induced nephritis may require new interpretation. The finding of host gamma globulin in diseased glomeruli is consistent with an immunologic lesion but does not define the pathogenetic mechanism.

6416

Fennell, R.H., Jr.; Pardo, V.; Gibson, L.L. 1965. Experimental glomerulonephritis in rats as related to antigen dosage. Federation Proc. 24:3057:682.

Holtzman rats hyperimmunized to human serum albumin were given weekly intravenous injections of varying doses of antigen. The amounts of protein varied from 0.1 to 10 mg. When examined at 25 weeks a spectrum of changes was evident, with the most severe alterations in the animals receiving largest amounts of antigen. These showed, by light microscopy, cellular proliferation, synechiae, and scarring of glomeruli. The animals that received smallest amounts had no visible lesions by light microscopy, but by electron microscopy small deposits were detectable in basement membranes and the lamina densa was swollen. Foot processes were fused. Fluorescent antibody studies revealed antigen and rat gamma globulin in the glomerular loops. Although hemagglutinating antibody titers varied, the severity of the glomerular changes was consistently related to increased amounts of antigen when groups of animals were considered. These results may be interpreted as evidence in support of the theory that material in glomerular basement membranes in this type of glomerulonephritis is the result of deposition of antigen and antibody and not synthesis of material by glomerular epithelial cells. Complete article

6417

Fisher, E.R.; Fisher, B. 1964. Nephrotoxic serum nephritis in thymectomized rats. Proc. Soc. Exp. Biol. Med. 115.156-160.

Clinical, biochemical, morphological, and immunohistochemical characteristics of renal disease induced in rats with injections of anti-rat-kidney rabbit serum (NTS) were similar in adult rats that were thymectomized at birth and in intact members of comparable age. The development of chronic glomerular lesions in thymectomized, NTS-treated rats indicates that progression of the disease is not necessarily dependent upon those immunologic functions related to thymic function, at least during the time interval studied.

6418

Fujimoto, T. 1964. The nature of Masugi nephritis. Acta Pathol. Jap. 14:143-146.

The nature of this experimental kidney disease remains in dispute. FA was one method used to study the histopathology of the disease. Three mechanisms of disease are suggested and discussed.

6419

Hammer, D.K.; Dixon, F.J. 1963. Experimental glomerulonephritis: II. Immunologic events in the pathogenesis of nephrotoxic serum nephritis in the rat. J. Exp. Med. 117.1019-1034.

The primary phase of nephrotoxic serum nephritis produced by rabbit nephrotoxic serum appears to be dependent to a great extent, but not completely, upon the participation of serum complement. On the other hand, duck nephrotoxic serum produces its primary renal injury without detectable utilization of or dependence upon serum complement. The secondary phase of nephrotoxic serum nephritis appears to be largely or entirely dependent upon the host antibody response to the heterologous gamma globulin fixed in the glomeruli. No evidence could be obtained for the existence of an autoimmune antikidney response by the host in this experimental model.

6420

Hammer, D.K.; Vazquez, J.J.; Dixon, F.J. 1963. Nephrotoxic serum nephritis in newborn rats. Lab. Invest. 12.8-15.

Rabbit anti-rat kidney serum injected into newborn rats at birth produced acute proliferative glomerulonephritis associated with proteinuria. Rabbit gamma globulin and rat complement localized promptly in the basement membranes of the more mature glomeruli in the inner part of the

renal cortex but not in the periphery. This suggests that renal antigen is present in the basement membrane of glomeruli already differentiated at birth. Renal function returns to normal within 4 to 6 weeks. Glomeruli injured at birth do not recover but progress to subacute and chronic stages of disease more slowly than in adult rats. Slow development of chronic glomerular changes is probably related to slowly developing antibody response to rabbit globulin on the part of the young rat. The return to normal glomerular function apparently reflects the large number of new, normal nephrons that make their appearance during the first 3 postnatal weeks and escape injury from the rabbit anti-rat kidney serum.

6421

Hess, E.V.; Ashworth, C.T. 1964. Further studies in auto-immune nephrosis in rats. Federation Proc. 23:2088:449.

A nephrotic syndrome is produced in 80 per cent of rats injected intraperitoneally with rat kidney in Freund adjuvant. The effect of the adjuvant alone and in combination with extracts of liver, spleen, heart, and lung has been further studied in 55 rats. Fractions obtained by differential ultracentrifugation of rat kidney extracts in sucrose have been tested in another group of 54 rats to determine the distribution of the antigen involved. The antigenic material has distributed itself over the nuclear, mitochondrial, and microsomal fractions. The antigenic activity of preparations of rat kidney and lung basement membrane has been investigated in 48 rats. Sera have been examined for the presence of antibody by the Ouchterlony agar diffusion and immunofluorescent methods. Precipitation lines against whole-kidney supernatant have been observed after immunization with kidney supernatant, differential ultracentrifugal fractions, and basement membranes. Complete article.

6422

Hinton, W.E.; Evers, C.G ; Brunson, J.G. 1964 The influence of adrenal medullary hormones on nephrotoxic nephritis in rabbits Lab Invest 13:1374-1380

A state of tolerance to large intravenous doses of a 50.50 mixture of epinephrine and levarterenol markedly decreases the incidence of chicken antirabbit kidney nephritis. Fluorescent antibody studies reveal glomerular localization of both injected antikidney gamma globulin and host antibodies to these heterologous nephrotoxic globulins. The differences in precipitating antibody titers to injected serum and differences in serum complement levels between the nephrotoxic control animals and those treated with adrenal medullary hormones suggest the final antigen-antibody reaction may be of lesser magnitude in the treated animals.

6423

Kantor, F.S. 1964. Glomerular lesions induced by intravenous injection of streptococcal M protein. J. Clin. Invest. 43:1251-1252.

Crude M protein extracts of Group A streptococci have been previously shown to contain a fibrinogen precipitating factor. The nature of this factor, the stoichiometry of the reaction with fibrinogen, and the biological effects of M protein injection into mice and rats are reported. Type I streptococcal M protein was partially purified and precipitated with human fibrinogen. The precipitate was used to immunize rabbits. The resulting sera were shown to contain bactericidal and mouse protective antibodies against Type I streptococci, indicating the identity of the M protein and fibrinogen precipitating factor. The stoichiometry of the reaction between purified M protein and fibrinogen was studied by trace labeling. Within 24 hours after injection of 3 mg of M protein into mice and 10 mg into rats, renal lesions were observed consisting of eosinophilic, hyaline depositions of material within the glomerular capillary bed. This material was identified as M-fibrinogen complexes by FA. One day after injection, rats developed proteinuria and urea retention, but both anomalies disappeared by the 6th day. However, on the 8th to 10th days animals developed secondary proteinuria and blood urea retention, at which time circulating anti-M antibodies were demonstrable. Complete article.

6424

Kleinsmith, L.J.; Pierce, G.B., Jr.; Midgley, A.R., Jr. 1963. Nephrotoxic roles of antibodies against epithelial and endothelial components of glomerular basement membranes. Federation Proc. 22:576:256.

Rabbit antisera against the hyaline secretion, NH, of a mouse yolk sac carcinoma were characterized and, after suitable absorptions, yielded an antibody that reacted only with basement membranes and reticulin of the mouse. When this anti-NH was injected intravenously in mice, acute glomerulonephritis with proteinuria was produced. Using fluorescence microscopy, the antibody was localized in the thickened basement membranes of the inflamed glomeruli. Since it has been shown that glomerular basement membranes consist of endothelial and epithelial components that lie in juxtaposition but are antigenically distinct, the role of these antigens in the development of glomerulonephritis was questioned. Epithelium-specific antibody was produced by absorbing the anti-NH with splenic pulp until endothelial basement membranes no longer reacted. By elution of the splenic pulp, antibody was obtained that reacted only with endothelial basement membranes. When epithelium- and endothelium-specific antibodies were injected in mice, both were localized in the glomerular basement membranes by immunofluorescence, but only the epithelium-specific produced severe glomerulonephritis. Complete article.

6425

Kolker, I.I. 1964. Concerning pathogenesis of burn injuries and of glomerulonephritis in the light of immunological data. Vestn. Akad. Med. Nauk SSSR 19:42-49. In Russian.

Anti-organ antibodies were studied using FA. Intravenously injected anti-kidney serum rapidly localizes in the kidney, producing cytotoxicity. Biopsies of human kidneys revealed gamma globulin deposition in kidneys of persons with glomerulonephritis. Gamma globulin was also located similarly in burn cases.

6426

Lidsky, M.D.; Sharp, J.T. 1965. Studies on the role of collagen in the glomerulus. Federation Proc. 24:1693:434.

Isolated beef glomeruli contain hydroxylysine, 1.11 mol per 100 mol, and 4-hydroxyproline, 3.35 mol per 100 mol, amino acids that have been found in mammalian tissue only in collagen. The hylys-hypro ratio of 0.331 is higher than reported for other mammalian collagens, 0.35 to 0.218, and might be explained by the following: the presence of a distinctive collagen with a hylys-hypro ratio not previously observed in mammalian tissue; the presence of collagen and a new protein containing hylys; or the occurrence of a non-collagenous protein containing hylys and hypro. Sections of isolated glomeruli revealed reticulin, suggesting the presence of collagen. Reports indicate that collagen is antigenic. Experiments were conducted to ascertain whether collagen determines localization of anti-mouse kidney serum, AMKS, on glomerular basement membrane, GBM. In control studies fluorescent staining revealed localization of AMKS on GBM. Antigens in tryptic digest of kidney prevented localization of AMKS. Soluble collagen extracted from mouse skin did not prevent localization of AMKS. Tryptic and peptic digests of the soluble collagen and reconstituted collagen fibers also did not prevent AMKS localization. It is concluded that neither skin collagen nor its telopeptide is the antigen in the GBM responsible for localization of nephrotoxic serum antibodies. Complete article.

6427

Mohos, S.C. 1965. The protective aspect of complement in immunological tissue injury. Federation Proc. 24:2707:620.

In testing different components of complement in conjunction with anti-mouse-kidney serum, it was observed that two antibodies are responsible for the two-phase, first and second peak, proteinuria, the first removable by absorption with mouse red blood cells, diminishing also 50 per cent of the second peak. This first-peak proteinuria is accentuated by conglutinating complement-fixation reaction requiring non-hemolytic complement. In contrast, hemolytic complement protects from the second-peak proteinuria and this protection follows the sequence of C component attachment and the kinetics found with in vitro hemolytic systems. Initially, fluorescent kidney antibodies localize equally in both groups but disappear more rapidly from the glomeruli in the complement-protected group; this is reflected in an increased excretion of unbound dye in the urine. These findings suggest that, in the absence of hemolytic complement, antibody continues to be deposited on tissue-bound antigens, leading to continuous tissue injury. In contrast, in the presence of hemolytic complement this antigen-antibody complex is eliminated and further damage cannot occur. Complete article.

6428

Muller-Ruchholtz, W.; Kraus, E.; Federlin, K. 1965. Studies on the transmission of Masugi nephritis in the rat: VII. Further fluorescence microscopic examinations of parabiotic and nephritic individual animals. Z. Immunol. Allergieforsch. 128:137-160. In German.

Female CFN Wistar rats were treated with rabbit antisera to rat kidneys and, in the control groups, with rabbit antisera to ovalbumin and to horse serum, or with normal rat serum. The kidneys, adrenals, and spleens of these animals, rinsed free of blood, were incubated with fluorescein-labeled antisera to rabbit globulin, rat globulin, and complement. Comparative examinations of the organs were performed. Only in the primarily nephritic rats was fluorescence following treatment with antirabbit globulin observed. Fluorescence was absent both in primarily healthy parabiotic partner animals and in the rats treated with control serum. The capillaries of the renal glomeruli, of the adrenal cortex, and of the hepatic lobuli were the sites at which fluorescence occurred. In both of the latter organs fluorescence was demonstrable only during the first week after injection. No fluorescence following treatment with anticomplement was demonstrable in the adrenals or in the liver but only in the glomeruli.

6429

Nagasawa, T.; Narikyo, T.; Shibada, S. 1965. Studies on nephritis by fluorescent antibody technique with special reference to the method involving IV injection of labeled antibody. Jap. J. Allergy 14:43-53. In Japanese.

A method useful for the study of nephritis, which consisted of intravenous injection of labeled antibody, was developed. With this method nephrotoxin was found to be fixed to the basement membrane of the glomerulus. Specific fluorescence was also observed in Bowman's capsules, renal tubules, and blood vessel walls.

6430

Pfeiffer, E.F.; Mosler, I.; Federlin, K. 1964. Studies on the transfer of Masugi nephritis in rats: VI. Limitation of the localization of heterologous rabbit anti-rat-kidney serum to the glomeruli of primary nephritic parabions. Z. Ges. Exp. Med. 138:385-398. In German.

Elucidation of the immunologic mecharism of transfer of experimental glomerulonephritis to originally healthy partners of primary nephritic parabiotic rats was attempted by immunofluorescence studies in 15 parabiotic pairs of one inbred strain. Sheep anti-rabbit-globulin antiserum labeled with fluorescein isothiocyanate was used to demonstrate the localization of tissue-bound rabbit anti-rat-kidney antiserum injected into the primary nephritic parabions. Specific staining of the rabbit nephrotoxins was restricted completely on the basement membranes of the glomerular loops of the single rats and the rats of the parabiotic pairs, i.e., the animals injected with the rabbit antiserum, whereas in the originally healthy partners, despite positive histologic evidence of glomerulonephritis, fluorescent staining was completely absent. Rabbit nephrotoxins injected primarily into one of the latter parabiotic partners are not involved in the transfer phenomenon.

6431

Schafer, H E 1964. Possibilities of diagnosing immunologically induced renal diseases. Munchen Med. Wochensch. 106:715-719. In German.

Various renal diseases can be explained immunopathogenetically, although a specific classification of antigen and antibody has not been established up to now. In the serologic diagnosis of glomerulonephritis we consequently depend on so-called indicative reactions. Among all indirect methods of demonstration, however, it is the fluorescent-optic demonstration of antibody and complement in the biopsy sample that holds a special position, since it yields the most nearly accurate demonstration of an immunologically developing renal disease.

6432

Sherwin, A.L.; Leznoff, A.; Richter, M.; Rose, B. 1963. Studies on the immunological specificity of autologous gamma globulin in the glomeruli of rabbits with experimentally induced glomerulonephritis. Can. J. Biochem. Physiol. 41:897-904.

Experimental glomerulonephritis was induced in rabbits by the intravenous administration of chicken antirabbit glomerulus serum. These rabbits were simultaneously immunized, either actively or passively, with bovine serum albumin or ovalbumin. Autologous gamma globulin was identified in the glomeruli of these nephritic rabbits by the fluorescent antibody technique. It was not possible to demonstrate the presence of an anti-BSA or anti-OA component in the gamma globulin even though these antibodies were present in high titer in the blood. This suggests that the autologous gamma globulin present in the lesions is entirely specific antibody rather than an accumulation of serum gamma globulin.

6433

Shibada, S.; Nagasawa, T. 1965. Experimental nephritis and the fluorescent antibody technique - with special reference to the importance of the selection of proper methods. Saishin Igaku 20:135-146. In Japanese.

Effective application of fluorescent antibody technique to the study of glomerulonephritis relies heavily on the selection of proper methods. The detection of fluorescence at the expected site has been over emphasized in the past, and results obtained by entirely unrelated methods have been compared with each other without careful evaluation. The introduction of the method which involves the injection of labeled antibody will solve some of the problems. It seems to be promising for the future study of nephritis.

6434

Tanaka, N.; Nishimura, T.; Tada, T.; Okabayashi, A. 1964. Autoimmune phenomenon occurred in the course of prolonged stimulation of heterologous protein. Jap. J. Exp. Med. 34:53-57.

An accumulation of gamma globulin was observed by the fluorescent antibody method in the renal glomerulus of the rabbits, which were injected with ovalbumin in an adjuvant and observed for more than 150 days; these showed protein in bladder urine. Circulating autoantibody against the renal basement membrane was demonstrated in the sera of the same animals.

6435

Teodoru, C.V.; Brancato, P. 1963. Free and tissue-bound heteroantigens and antibodies during the development of nephrotoxic serum nephritis in rabbits. Arth. Rheum. 6:794-795.

Duck antirabbit kidney serum (DARKS) and normal duck serum (NDS) were precipitated with goat anti-DARKS serum (GADARKS) by double diffusion on agar plates. DARKS yielded several precipitation bands from which one, cDG, was characteristic and was not yielded by NDS. A similar band was obtained by immunodiffusion of isolated DARKS immune globulins. Immunofluorescence techniques showed duck globulin affixed to the basement membranes and interstitial connective tissue of rabbit kidneys coincidental to the disappearance of cDG from circulation. Later, by the time duck heteroantibodies appeared in the circulating blood, rabbit gamma globulin appeared affixed at the site of the sessile duck globulin, suggesting that both cDG and cDG-heteroantibodies missing from circulation are linked to rabbit tissues. In contrast with GADARKS or rabbit anti-NDS, fluorescent rabbit anti-DARKS immune globulins failed to stain the DARKS nephrotoxic globulin affixed to rabbit glomeruli. This indicates the lack of circulating heteroantibodies against the nephrotoxic fraction in rabbits injected with DARKS and suggests the possibility that the cDG isolated by immunodiffusion represents the nephrotoxic principle of DARKS.

6436

Teodoru, C.V.; Brancato, P. 1964. Nephrotoxic, NTX, nephritis without NTX precipitin titer. Federation Proc. 23:2448:509.

Duck anti-rabbit-kidney serum, DARKS, given iv in rabbits remained in circulation for 5 to 7 days as demonstrated by immunodiffusion. However, one protein fraction shown by diffusion against goat anti-DARKS serum as a characteristic DARKS globulin, CDG, vanished from the rabbit blood within the first hour after injection if overdosage was avoided. Simultaneously, duck globulin became demonstrable by immunofluorescence in the glomerular basement membrane. As a consequence of CDG disappearance, CDG heteroantibody failed to appear in the circulation of the rabbit. The resulting rabbit anti-DARKS serum had antibodies precipitating various DARKS protein fractions but not the CDG, which remained in the supernatant. Such supernatant provoked belated glomerulonephritis but poor or no antibody titer when injected iv in rabbits. This indicates CDG as a possible nephrotoxic globulin and suggests that the host antiduck precipitin titer, although instrumental in lesions associated with the circulating heteroprotein, may have no bearing on the belated injury elicited by the tissue-linked NTX fraction. Complete article.

6437

Treser, G.; Sagel, I.; Lange, K. 1964. Specificity of immunofluorescence in experimental and human renal diseases. Proc. Soc. Exp. Biol. Med. 117:842-844.

Severe proteinuria with considerable gamma globulin content was produced in rats by aminonucleoside injections. Sections of their kidneys at intervals between 4 and 12 days after onset of proteinuria did not show immunofluorescent staining for gamma globulin and complement. Rats made nephritic by injection of anti-rat-kidney rabbit serum showed immediate morphologic damage and proteinuria with considerable gamma globulin content. Rabbit gamma globulin can be demonstrated immediately on the glomeruli, but rat gamma globulin does not appear before the 7th day of disease. A kidney biopsy of a patient with multiple myeloma and advanced renal damage did not show immune staining for gamma globulin and complement, in spite of a severe proteinuria with large amounts of albumin, Bence Jones protein, and gamma globulin in the urine. Positive immune staining for gamma globulin or complement and its components in renal diseases therefore can not be attributed to trapping or unspecific transudation in the damaged kidney.

6438

Treser, G.; Sagel, I.; Lange, K. 1964. Specificity of immunofluorescence in experimental and renal diseases. Federation Proc. 23:2450:510.

Severe proteinuria with considerable gamma globulin content was produced in rats by aminonucleoside injections. Cryostat sections of their kidneys at various intervals after the onset of proteinuria did not show immunofluorescent staining for gamma globulin and the third component of complement, beta-1A. Rats made nephritic by injections of anti-rat-kidney rabbit serum showed immediate morphologic damage and proteinuria with considerable gamma globulin content. Rabbit gamma globulin and beta-1A can be demonstrated immediately on the glomeruli, but rat gamma globulin does not appear before the 4th day of disease. Cryostat sections of a kidney biopsy of a patient with multiple myeloma and advanced renal damage did not show immune staining for gamma globulin, complement, or beta-1C or -1A in spite of a severe proteinuria with large amounts of albumin, Bence Jones protein, and gamma globulin in the urine. Positive immune staining for gamma globulin or complement and its components in renal diseases can therefore not be attributed to trapping or unspecific transudation in the damaged kidney. Complete article.

6439

Unanue, E.; Dixon, F.J. 1964. Experimental glomerulonephritis: IV. Participation of complement in nephrotoxic nephritis. J. Exp. Med. 119:965-982.

Rats with nephrotoxic serum nephritis may show fixation of complement (C) in the glomeruli during two periods. The first period occurs immediately after the injection of the antisera and extends until the host response occurs. The second period is related to the deposit of rat gamma globulin in the glomeruli, presumably occurring at the time of the immune response to the heterologous nephrotoxic serum (NTS). This second period terminates about 2 to 3 weeks after injection of NTS, presumably at the time the rat stops making antibodies to the nephrotoxic gamma globulin fixed in the glomeruli. The fixation of C in the glomerular lesions was studied with FA. The detection of host beta-1C globulin and gamma globulin in the glomerulus pointed to an immune reaction but not necessarily an active one. The tissue complement fixation test using kidney slices and guinea pig C proved to be a more sensitive method of detecting tissue reactants capable of fixing C, but its relationship to in vivo events is not certain.

6440

Unanue, E.R.; Dixon, F.J. 1965. Experimental glomerulonephritis: V. Studies on the interaction of nephrotoxic antibodies with tissues of the rat. J. Exp. Med. 121:697-714.

A direct correlation between the amount of kidney-fixing antibody and the degree of associated renal injury was demonstrated for rabbit and duck nephrotoxic antibodies. No evidence for a qualitative difference among nephrotoxic antibodies of a given type was obtained. It appeared that duck nephrotoxic antibody was directed against a wider spectrum of rat renal antigens than was rabbit nephrotoxic antibody. In order to produce immediate proteinuria an amount of rabbit or duck gamma-2 kidney-fixing antibody capable of occupying approximately 45 per cent or more of the capillary filtration surface was needed. Nephrotoxic antibodies in hyperimmune rabbit nephrotoxic sera were gamma-2. Nephrotoxic antibodies in duck nephrotoxic sera were gamma-2 and gamma-1M. Gamma-1M duck nephrotoxic antibody was 60 times more potent a nephritogen than gamma-2 duck nephrotoxic antibody on a molecular basis. Mercaptoethanol abolished the nephrotoxicity of gamma-1M duck antibody and reduced that of gamma-2 duck antibodies, but had no effect on rabbit gamma-2. In no case did mercaptoethanol treatment have an effect on the kidney-fixing properties of the antibodies involved. After injection of nephrotoxic antibodies there appeared to be a prompt fixation of a majority of the antibody to tissue antigens, primarily in the kidney.

6441

Unanue, E.R.; Dixon, F.J. 1965. Experimental glomerulonephritis: VI. The autologous phase of nephrotoxic serum nephritis. J. Exp. Med. 121:715-726.

Nephrotoxic serum nephritis was studied in rats receiving various amounts of kidney-fixing antibody and active or passive immunization to the heterologous gamma globulin of the species supplying the nephrotoxic antibody. Rats injected with a moderate to a large amount of kidney-fixing antibody, 180 to 380 ug, and immunized to the heterologous gamma globulin, developed a more severe nephritis than rats receiving similar amounts of antibody without further immunization. Rats injected with minimal amounts of kidney-fixing antibody and immunized to the heterologous gamma globulin developed a moderate nephritis in contrast to rats that received similar amounts of antibody with further immunizations and showed no evidence of renal injury. In the presence of excess circulating antibody, antigens occupying a few per cent of the glomerular capillary surface can provide an antigen-antibody interaction that will cause detectable morphological and functional alterations of the glomerulus.

6442

Unanue, E.R.; Lee, S.; Dixon, F.J.; Feldman, J.D. 1965. Experimental glomerulonephritis: VII. The absence of an autoimmune anti-kidney response in nephrotoxic serum nephritis. J. Exp. Med. 122:565-578.

Rats with nephrotoxic serum nephritis were studied for the presence of a possible autoimmune response to renal antigens formed and/or liberated during the immunologic reactions taking place in the glomeruli. The experiments consisted of transplantation of a normal isologous kidney to a nephritic rat and parabiosis of a normal rat to a nephritic rat. Neither functional nor morphologic abnormalities were noted in the normal kidneys in either situation. Transfer of the rabbit nephrotoxic antibody to the normal kidneys was noted in both experiments, indicating a continual dissociation and reassociation of nephrotoxic antibody with the tissue antigens of the host. Some of the nephritic rats showed a decrease in proteinuria and a slowing in the progression of the nephritis during the period in which the normal kidney was transplanted or a normal rat was united in parabiosis.

6443

Watson, J.I.; Dixon, F.J. 1965. Studies on the transfer of autoimmune nephrosis in rats. Federation Proc. 24:1321-1368.

Several experiments have been performed to investigate whether the nephrosis induced in rats by the intraperitoneal injection of rat kidney in complete Freund adjuvant can be transferred among isologous rats. Lymphoid cell transfers of from 100 to 600 million viable cells from nephrotic, intact or splenectomized, donors were made to normal isologous recipients under a variety of experimental conditions. Thirty-five recipients of such transfers were followed for up to 100 days and in none did proteinuria nor any other manifestations of renal injury appear. Kidneys from these recipients showed no evidence of injury or immunologic reactions by electron microscopy or immunofluorescence. However, these cell recipients had an increased susceptibility to this disease, since only one injection of kidney in adjuvant induces nephrosis in about 1 month. Parabiosis of nephrotic and normal isologous rats has also failed to transfer renal disease during intervals up to 1.5 months. These failures to transfer this disease among isologous rats raises a question about its proposed autoimmune anti-kidney pathogenesis. Complete article.

C. EXPERIMENTAL AUTOIMMUNE DISEASES (NOT KIDNEY)

6444

Barnett, E.V.; Dumonde, D.C.; Glynn, L.E. 1963. Induction of autoimmunity to adrenal gland. Immunology 6:382-402.

Rabbits immunized with homologous and heterologous adrenal homogenates responded with CF heat-stable autoantibody directed against adrenal. Rabbits responded with autoantibody production against cytoplasm of adrenal cortex and of ovarian and testicular cells as detected by FA. Antibody, with sedimentation similar to 7S gamma globulin, was directed against a heat-labile antigen in rabbit, guinea pig, and rat adrenal. Antibody was absorbed by an ultracentrifugal sediment of adrenal or ovary. Guinea pigs immunized with heterologous adrenal homogenates developed more extensive adrenal infiltrates than did guinea pigs immunized with homologous adrenal. Antibody from guinea pigs immunized with heterologous adrenal reacted with autologous organ antigens as well as with rabbit and rat adrenal. The detection of autoantigens in fetal rabbit adrenal and ovary suggests that rabbits should be tolerant to such antigens. The greater efficacy of immunization with heterologous adrenal in eliciting autoantibody and autoimmune adrenalitis may be a feature of experimental autoimmunity induced by those organ-specific antigens to which the animal is tolerant. The presence of autoantibody in the serum, bound gamma globulin at the site of adrenal lesions, and the absence of delayed-type hypersensitivity to homologous adrenal suggest that the humoral immune mechanisms play a role in experimental autoimmune adrenalitis.

6445

Brown, P.C.; Glynn, L.E.; Holborow, E.J. 1963. The pathogenesis of experimental allergic orchitis in guinea pigs. J. Pathol. Bacteriol. 86:505-520.

Guinea pigs were immunized with guinea pig testis homogenate or with some of its fractions emulsified with Freund's complete or incomplete adjuvant. The development of the lesion obtained when complete adjuvant was used is described. The absence of cellular infiltration in many of the lesions, especially in the early stages, makes it difficult to accept a direct role for cellular hypersensitivity in their production. The fluorescent antibody method was used to demonstrate uptake of antibody by the more mature cells of normal seminiferous tubules in tissue sections, and also to show loss of antigen as the lesion develops. The lesion was prevented by prior immunization with testis and incomplete adjuvant and by repeated injection of antigen alone from birth. Animals that had recovered from a previous lesion were resistant to subsequent challenge.

6446

Hashiguchi, T. 1964. Patholcgical research of arthritis experiment: Parallel use of fluorescent antibody technique. Nippon Seikeigakkai Zasshi 38:695-696. In Japanese.

Human serum was injected into the knee joint of female rabbits, bringing about Arerugi-type arthritis. The changes due to Arthus disease and rheumatic disease after various intervals of time were compared. Gross observations, pathology, and FA studies are reported. Using FA, the fate of human gamma globulin, the movement of rabbit globulin, and the relationship between fibrinoid change and bound globulin were investigated. Human gamma globulin first is found in the injection site, but it soon collects around blood vessels. It is subsequently taken up by large circular cells and is located in the cytoplasm. It disappears in 6 days. Rabbit gamma globulin is at first located similarly, but soon the amount increases markedly, and it may be demonstrated in the circular cells for 2 weeks.

6447

Heller, P.; Yakulis, V.J. 1963. Auto-antigenicity of connective tissue extracts. Proc. Soc. Exp. Biol. Med. 112:1064-1069.

Rabbit tendon extracts, reinjected into the donor animals, produced circulating complement-fixing antibodies, skin reactivity of the delayed type, and fixation of antibody to synovia and interstitium of several organs. The growth of the animals was stunted, but no other abnormalities were demonstrable. Isologous antibodies were produced in a similar way, but the effects were not as marked.

6448

Holborow, E.J.; Asherson, G.L.; Wigley, R.D. 1963. Autoantibody production in rabbits: VI. The production of autoantibodies against rabbit gastric, ileal, and colonic mucosa. Immunology 6:551-560.

Rabbits injected with a homogenate of rat gut mucosa produce autoantibodies reacting with their own gut mucosa. Different organ-specific antigens present in the stomach mucosa and colonic and ileal mucosae can be demonstrated in this way. The antigens concerned are in the mucous secretions as well as in certain epithelial cells and may be mucopolysaccharides FA was one of the methods used to detect the antigen

6449

Johnson, G.D.; Asherson, G.L.; Kaklamanis, E.; Dumonde, D.C. 1963. Demonstration by immunofluorescence of autoantibody in the serum of rabbits given injections of rat tissue. J. Pathol. Bacteriol. 86:521-525.

The immunofluorescence procedure showed that the sera of rabbits that had received injections of rat liver, kidney, heart, muscle, and brain in Freund's complete adjuvant contained factors that adhered to rabbit tissue. These factors reacted in vitro with the tissue of the rabbits in which they occurred and were regarded as autoantibodies that occurred in the same sera. Absorption studies showed that the anti-liver and anti-brain sera had a component that reacted with the corresponding rabbit organ and another that reacted with rabbit kidney. There was no evidence of in vivo uptake of gamma globulin by the tissues of the immunized rabbits.

6450

Kniker, W.T.; Cochrane, C.G. 1965. Pathogenic factors in vascular lesions of experimental serum sickness. J. Exp. Med. 122:83-98.

Polymorphonuclear leukocytes (PMN) are essential for the development of cardiovascular lesions in serum sickness. In the absence of PMN, necrotic vascular lesions were never seen, and endothelial proliferation in arteries was inhibited. Zones of fibrinoid deposits did not occur. In arterial lesions that involved the intima and media, the internal elastic lamina was disrupted. This was associated with accumulations of PMN and was prevented when PMN was depleted. The elastic lamina acts as a barrier to the outward spread of inflammation in arteries. It is an important substrate of PMN action. Host complement was localized along with the antigen and rabbit gamma globulin in glomeruli and arteries showing lesions. In the glomeruli these deposits formed a granular lining along the area of the basement membrane. In arteries the fluorescent amorphous particles were in the intima and media of inflamed vessels. The immune response to bovine serum albumin (BSA) and the incidence and severity of cardiovascular and renal lesions were enhanced by the intravenous administration of pooled rabbit antiserum to BSA given 18 hours before BSA antigen and by injecting endotoxin along with the BSA. These additions did not change the quality of the disease. In normal rabbits, the peak incidence of cardiovascular lesions was early in immune elimination of antigen. The severest renal injury was noted several days later, at the completion of immune elimination.

6451

Koffler, D. 1964. Serologic and immunocytochemical studies following immunization with homologous thyroid extract in guinea pigs. Federation Proc. 23:1108:285.

Humoral antibody response to administration of homologous saline thyroid extract was studied in Hartley guinea pigs. Hemagglutinating antibodies were detected within 7 days of injection. At 3 weeks, maximum titers corresponded with the appearance of severe thyroiditis. Indirect fluorescence microscopy demonstrated antibodies binding to colloid in highest titer during the same period. Antibodies to thyroid epithelium were rare. Precipitating antibodies to thyroid extract were present in 2 of 36 guinea pigs. Serum electrophoresis revealed nonspecific elevation of alpha-2 and gamma globulin. The fluorescent antibody technique was used to localize immunoglobulins in the thyroid gland and to ascertain the onset of antibody production to thyroglobulin in axillary, inguinal, and cervical lymph nodes and spleen. Formation of antigen-antibody complexes following deposition of antibody to thyroglobulin in colloid may contribute to the inflammatory process in experimental thyroiditis. Complete article.

6452

Koffler, D.; Paronetto, F. 1965. Transfer of experimental autoimmune thyroiditis in Hartley guinea pigs. Federation Proc. 24:1334:370.

Experimental autoimmune thyroiditis was transferred by lymph node and peritoneal cells in 27 Hartley guinea pigs. Donor and recipient animals were from the same litter. Focal thyroiditis, observed in five of seven recipients at 6 days, was predominantly composed of mononuclear cells. Animals sacrificed 12 days after transfer exhibited increased numbers of lymphocytes and plasma cells with destruction of thyroid follicles Hemagglutinating antibodies to thyroglobulin were detectable at 6 and 12 days. Several guinea pigs demonstrated antibody to colloid by the indirect fluorescent antibody technique. Gamma globulin was rarely detectable in colloid of animals with thyroiditis. No correlation was found between the severity of donor and recipient lesions, nor were significant differences noted in recipients with lymph node or peritoneal cells. Thyroid lesions in the recipients were of lesser severity than in donor animals. Recipients with cells from donors treated with Freund adjuvant also showed small focal infiltrations of mononuclear cells. These studies indicate that autoimmune thyroiditis may be transferred by lymph node or peritoneal cells and that humoral antibodies to colloid constituents are present in recipient animals. Complete article.

6453

Krafe, S.C.; Fitch, F.; Kirsner, J. 1963. Histologic and immunohistochemical features of the Auer colitis in rabbits. Amer. J. Pathol. 43:913-927.

An experimental colitis in rabbits may be produced by a modification of the Auer reaction. The principles seem to involve sensitization to a soluble antigen, a generalized antibody response, an attraction of nonspecifically mildly irritated tissue for circulating antigen and antibody on the basis of an increased vascular permeability in these areas, and a resultant antigen-antibody reaction leading to tissue injury by mechanisms that have not been fully clarified. The histologic features of the Auer colitis include submucosal edema, perivascular round cell accumulations, mucosal-submucosal infiltrations of inflammatory cells, and ulceration. The direct immunofluorescent technique has been used to demonstrate specific antigen and antibody in the identical sites of this cytotoxic reaction characterizing the Auer phenomenon.

6454

Krakower, C.A.; Greenspon, S.A. 1964. The nature of late reactions following intradermal injection of heterologous anti-tissue sera. Proc. Soc. Exp. Biol. Med. 116:301-306.

Late lesions with the qualities of a delayed hypersensitivity reaction occur in the skin of rats injected intradermally with rabbit anti-rat-tissue sera. These lesions are related to the fixation and persistence of antibodies directed against antigens in the epithelial and mesenchymal basement membranes of skin.

6455

Lupulescu, A.; Simionescu, L.; Merculiev, E. 1963. The role of estrogenic hormones in the development of immunological rabbit goiter. Endokrinologie 44.335-346. In German.

Massive immunization with thyroid antigen to rabbits elicits a parenchymatous microfollicular craw with reduced colloid. The craw development seems to be traceable to the hypersecretion of thyreotropic hormone due to blockage of synthesis of thyroid hormone through autoantibody. The serological tests show no significant difference between the test groups; the radio-iodine uptake changes according to the actual test model. The presence of intracellular anti-thyroid antibodies in the immunological craw may be shown with the help of a marking with fluorescein isothiocyanate. The simultaneous dispensation of estradiol benzoate in large amounts leads to an arrest of the craw developing processes

and to a change in development in the direction of a colloid craw. The simultaneous bilateral ovarial extirpation leads to a predominance of lesions similar to those observed in Hashimoto's thyroiditis, which points to the possible role of a gonadotropic hypersecretion in the etiology of this disease. These tests show the role of hormones in the development of immunological thyroid diseases.

6456

Premachandra, B.N.; Berns, A.W.; Blumenthal, H.T. 1965. Fluorescence microscopic studies in the thyroglobulin-immunized guinea pig. Federation Proc. 24:639:242.

The marked effects of circulating antibodies on physiological parameters of the thyroid in thyroglobulin-immunized guinea pigs have been noted in this laboratory; the present report embodies further investigations on the localization of anti-thyroglobulin in the thyroid and the peripheral tissues. Sixteen guinea pigs were immunized by three weekly injections of electrophoretically pure bovine thyroglobulin; 12 to 13 weeks later the animals were sacrificed. Thyroid and the pituitary were sectioned at 4 to 6 microns and prepared for fluorescence microscopy. Fluorescence was observed in the blood vessels of the thyroid and the muscular tissue as well as in epithelial cells of the follicle. Cellular infiltrates in two instances also showed fluorescence. For control studies, fluorescein-conjugated insulin and fluorescein-conjugated snake venom were used and no fluorescence was observed when these were substituted for conjugated thyroglobulin, thereby indicating the specificity of the nature of binding observed. Complete article.

6457

Premachandra, B.N.; Berns, A.W.; Blumenthal, H.T. 1965. Physiological aspects of thyrogobulin immunity: III. Studies of localization of antibodies in vascular tissue and abnormal plasma thyroxine binding in the guinea pig J. Lab. Clin. Med. 66:893-905.

Immunofluorescent and thyroxine I-131 kinetic studies were carried out in untreated, adjuvant-treated, and actively thyroglobulin-immunized arimals. Fluorescent microscopy demonstrated localization of fluorescein-labeled thyroglobulin in the blood vessel and follicular epithelial cells of the thyroid and in the vessels of muscle and adipose tissue. Furthermore, fluorescence studies revealed thyroglobulin-binding coagula in the blood vessels of muscular tissue in the immunized animals. No fluorescence was observed in the adjuvant-treated animals. These investigations in actively immunized animals, along with our previous observations of thyroxine binding by antithyroglobulin, suggest the possible existence of a peripheral immune block that prevents antibody-bound thyroxine from exerting

its full effects because of its inability to diffuse freely from the vascular compartments, thereby affecting other thyroid parameters. Binding of thyroxine by antithyroglobulin may affect hormonal kinetics in other types of thyroiditis, as well as in other circumstances where antibody to thyroglobulin can be detected.

6458

Ridley, A. 1963. Localization of gamma globulin in experimental encephalomyelitis by the fluorescent antibody technique. Z. Immunitats.-Allergieforsch. 125:173-190. In German.

The fluorescent antibody method has been used to investigate, at the cellular level, the possible role of circulating brain antibody in the pathogenesis of experimental encephalomyelitis in the guinea pig. An increase of gamma globulin - containing plasma cells occurred in the regional lymph nodes and spleens of injected guinea pigs, whether or not they developed encephalomyelitis. In the brains of encephalomyelitic animals, gamma globulin was present in lymphocytes and plasma cells of vascular cuffs and in the brain parenchyma around cuffed lesions. Attempts to reveal brain antibody in the cells of regional nodes and spleen, and in brain lesions, were unsuccessful. The significance of the gamma globulin changes is discussed. If circulating brain antibody is responsible for the changes, the findings do not support it as a basic disease mechanism.

6459

Weigle, W.O. 1965. The induction of autoimmunity in rabbits following injection of heterologous or altered homologous thyroglobulin. J. Exp. Med. 121:289-308.

Experimental autoimmunity was produced in rabbits following injection of altered homologous thyroglobulin. The thyroglobulin was altered by coupling to chemically defined haptens and by heating. With some preparations antibody to native thyroglobulin as well as thyroid lesions were produced. Injections of thyroglobulin coupled to the diazonium derivatives of arsanilic acid and sulfanilic acid were effective when given in either soluble form or incorporated into incomplete Freund's adjuvant; injections of the same preparations precipitated by alum had relatively little effect on production of antibody or induction of lesions. The injection of native thyroglobulin in soluble form, incorporated into incomplete adjuvant or precipitated by alum, usually resulted in production of little or no antibody and only rarely in the formation of lesions. The injection of a heterologous thyroglobulin into rabbits resulted in the production of antibody reacting with both the heterologous and rabbit thyroglobulin, but no thyroid lesions were observed.

D. ANTINUCLEAR TESTS

6460

Bardawil, W.A.; Galins, N.; Gavaller, B. 1965. Antinuclear globulins in fowl. Federation Proc. 24:2709:620.

In a study designed to investigate the role of viruses among other infectious agents in the immunogenesis of antinuclear globulins and their relationship to autoimmune diseases, collagen diseases, it was found that the serum from a number of apparently normal chickens, Leghorn variety, gave a positive antinuclear globulin reaction, 12 per cent, against homologous, chicken, and heterologous, human, nuclei as determined by the indirect fluorescent antibody technique. By applying the freeze and thaw techniques described previously from this laboratory it was found that the number of positive antinuclear reactions in the upper levels of the undisturbed thawed serum increased to 79 per cent; the incidence in the lower level of the thawed serum remained unchanged from that of the unfrozen serum. After mixing the upper and lower levels of the thawed serum the original findings were duplicated. These findings correlate with the results obtained with human serum. The serum of chickens immunized against influenza A and influenza A2 viruses, which are predominantly RNA viruses, gave negative results to date. Complete article.

6461

Barnes, R.D. 1965. Thymic neoplasms associated with refractory anaemia. Guy's Hosp. Rep. 114:73-82.

Certain serological reactions noted in four cases of refractory anemia and thymoma are presented with data on the antinuclear factor in certain other diseases that can be associated with thymoma. Circumstantial evidence is reviewed and cross associations are noted to suggest that possibly, through the possession of a thymic tumor and antinuclear factor, a relationship does exist between such cases of refractory anemia associated with thymoma and certain other diseases such as systemic lupus erythematosus and myasthenia gravis, that demonstrate auto-immune phenomena. Thymic pathology should be studied in refractory anemia in the absence of thymoma. Younger patients with refractory anemia may present abnormalities resembling the changes in systemic lupus or myasthenia gravis.

6462

Barnett, E.V.; Vaughan, J.H. 1965. Antinuclear factors in rabbit sera. Federation Proc. 24:3110:692.

The limited success of immunizing experimental animals to elicit antinuclear factors, ANF, has been a major obstacle to the consideration of human ANF as antibodies. The sera of 15 of 18 rabbits immunized repeatedly with whole human serum in Freund adjuvant were shown to contain ANF, titer 1:4 to 1:64, reactive with human white blood cell nuclei in the indirect immunofluorescence test. There was no reactivity with rabbit or chicken nuclei. ANF were detected by fluorescein-conjugated goat antirabbit gamma globulin and were found to sediment as 7S and 19S globulins. The two rabbit sera with ANF in highest titer were shown to contain complement-fixing antibody, titer greater than 1:200, to DNA of human, rabbit, bovine, and bacterial origin in the Wassermann and Levine quantitative complement-fixation test. In every case reactivity was greater with single-strand than with native DNA and reactivity was abolished by treatment of the DNA with DNAase. Pre-immunization sera and sera from rabbits similarly immunized with human Cohn Fractions I, II, and V contained no detectable ANF. Cohn Fractions IV-1, IV-4, and III infrequently elicited ANF. These results are taken to indicate that there are antigenic nuclear materials, DNA, in normal human sera. Complete article.

6463

Barnett, E.V.; Bienenstock, J.; Bloch, K.J. 1964. Antinuclear factors in synovial fluid: Possible participants in the rheumatoid inclusion body. Arth. Rheum. 7:726.

Cytoplasmic inclusions observed in rheumatoid synovial fluid leukocytes may represent ingested deposits of immune complexes or aggregated gamma globulin, plus rheumatoid factor. Fixation of complement by these complexes may account for the depletion of hemolytic complement observed in certain synovial fluids. Presence of ANF was sought in synovial fluid of patients with various rheumatic diseases. Paired sera and synovial fluid samples from ten patients with definite or classical rheumatoid arthritis were tested. The level of hemolytic complement was markedly reduced in the synovial fluid of all patients. Rheumatoid factor was present in serum and synovial fluid from nine patients. ANF of one or more immunoglobulin classes was found in serum of seven patients; five of these patients also had ANF of one or more immunoglobulin classes in synovial fluid. One patient had gamma-1M ANF in synovial fluid only. Among nine patients with other arthritides, gamma-1M ANF was found in serum in two cases and in synovial fluid in one. ANF could participate in one of several antigen-antibody systems in synovial fluid. Failure to detect ANF in all rheumatoid synovial fluids may be attributable to a failure of formation or to removal by excess nuclear antigen.

6464

Beck, J.S. 1963. Auto-antibodies to cell nuclei. Scottish Med. J. 8:373-378.

The FA technique is valuable for detecting antinuclear antibodies. The technique shows an overlap of the immunological abnormalities among the various connective tissue diseases. Antinuclear antibodies have not been found in sera of patients with polyarteritis nodosa and dermatomyositis. Finding low titer antinuclear antibodies in sera of some patients with organ-specific autoimmunity is of interest. Evidence is presented that confirms the previous impression that humoral antibodies are not the main pathogenetic mechanism in the autoimmune diseases, although such antibodies may potentiate the action.

6465

Beck, J.S.; Paterson, J.C. 1965. Nuclear antigens in normal and leukaemic leucocytes: A histochemical study using human auto-immune antinuclear antibodies. J. Pathol. Bacteriol. 90:567-578.

Histochemical observations were made on normal and leukemic leukocytes by FA staining with characterized human antinuclear sera. The homogeneous (DNA-protein) and membranous (DNA) antigens were detected in all leukocyte nuclei. The speckled antigen was absent from all normal polymorphonuclear leukocytes and from a proportion of polymorphonuclear leukocytes from patients with myeloid leukemia, but it was present in all other types of leukocyte. The nucleolar antigen was absent from all normal and leukemic polymorphs, many myeloid leukemic stab cells, and a small proportion of normal lymphocytes, but it was present in all other leukocytes. Antinucleolar antibodies were sensitive and specific reagents for detection of nucleoli in leukocytes. Blood films cannot be recommended for FA tests for the detection of antinuclear antibodies in human sera because the system is much less sensitive than the rat liver test system, two of the antigens are absent from polymorphonuclear leukocytes, and the nuclear staining pattern is less easily recognized in leukocyte nuclei than in the larger rat-liver cell nuclei.

6466

Bonomo, L.; Torsi, A.; Trimigliozzi, G.; Dammacco, F. 1965. LE cells and antinuclear factors in leprosy. Brit. Med. J. 2:689-690.

Serum antinuclear factors as detected by FA were found in 16 of 55 cases of leprosy. LE cells or 'rosettes' were present in the blood specimens of 4 of 10 leprosy patients with serum antinuclear factors.

6467

Bouchier, I.A.D.; Rhodes, K.; Sherlock, S. 1964. Serological abnormalities in patients with liver disease. Brit. Med. J. 1964:592-594.

Serological studies for the presence of antinuclear antibodies, rheumatoid factors, and thyroglobulin antibodies were undertaken in 116 patients suffering from alcoholic, juvenile, cryptogenic, or primary biliary cirrhosis or from virus hepatitis. An increased incidence of positive latex and sheep-cell agglutination tests, compared with normals, was found in all the varieties of liver disease studied. The highest number of positive results were encountered in juvenile, cryptogenic, and primary biliary cirrhosis. In alcoholic cirrhosis and virus hepatitis the rate of positive results was lower. Antinuclear antibodies were present in 42 per cent of cases of juvenile cirrhosis. Compared with normals, their incidence was increased also in cryptogenic cirrhosis. They were not detected in patients with alcoholic cirrhosis and virus hepatitis. The incidence of thyroglobulin antibodies did not differ from that found in normal subjects.

6468

Dry, J.; Kahn, M.F. 1964. Comparative evolution of various auto-antibodies and hetero-antibody levels as a function of age. Gerontologia 9:222-229. In French.

The authors subjected 169 sera obtained from healthy persons to the latex-F2 reaction and to the titration of the antistreptolysin 0. An investigation of antinuclear antibodies was also carried out by the immunofluorescence method for 61 sera obtained from persons over 70 years of age. Antinuclear antibodies detected by the immunofluorescence method have been revealed in 23 per cent of the 61 persons aged over 70, and in only 3.6 per cent of the 166 persons aged between 20 and 60. The difference is significant. They occur more frequently in females than in males.

6469

Faber, V.; Elling, F.; Norup, G.; Mansa, B.; Nissen, N. 1964. An antinuclear factor specific for leukocytes. Lancet 2:344-345.

The pathogenic significance of antinuclear factors has been questioned because of the organ-nonspecificity of the antibodies. From experiments with plasma transfusions it had been suggested that antinuclear globulins might be partly responsible for the leukopenia in Felty's syndrome. In our patient the leukocyte-specific antinuclear factor was found

and titrated to the same degree before and after the operation, and even during convalescence, when the leukocytes were temporarily increasing in number. Thus the direct pathogenic role played by this serum factor is still an open question. Since completing this report we have found another patient, with dermatomyositis and a severe leukopenia, whose serum contained this factor.

6470

Fisher, E.R.; Fisher, B.; Samuelson, J. 1963. Antinuclear factor in sensitized lymph and serum from skin homografted dogs. Proc. Soc. Exp. Biol. Med. 113:872-876.

A factor reacting with epithelial and mesenchymal nuclei of 54 per cent of skin homografts in dogs could be demonstrated in sensitized lymph supernatant of the host by the indirect fluorescent antibody technique. Recipient serum less frequently contained this factor and only against nuclei of donor (14 per cent) and host normal skin (9 per cent). Pretreatment of skin sections with DNAase failed to alter this reaction. Lymph from homografted dogs exhibited elevation of total protein, albumin, and gamma globulin. However, this latter could not be absolutely correlated with the occurrence of antinuclear factor. The precise role of antinuclear factor in homograft rejection remains to be demonstrated.

6471

Friou, G.J.; Teague, P.O. 1963. Autoantibody-like antinuclear globulin in A/J mice. J. Lab. Clin. Med. 62:875.

Various strains of inbred mice were studied in a serum survey. An antinuclear factor was found consistently in 1 of 25 strains.

6472

Friou, G.J.; Teague, P.O. 1963. Autoantibody-like antinuclear globulin in A/J mice. Arth. Rheum. 6:773-774.

A systematic survey for serum antinuclear gamma globulin factors among serum specimens from 25 different strains of inbred mice revealed 24 strains that were consistently negative and one strain in which such activity was regularly detected. In the initial survey, sera from fifty 6- to 12-month-old multiparous A/J mice were tested and 13 were positive. Of males of similar age, 8 of 50 were positive, but sera of young animals have been negative. Sera were tested against mouse peripheral blood leukocytes and calf thymus nucleoprotein spots by the indirect fluorescent antibody technique. Fluorescein-labeled antiglobulin used was highly specific for mouse gamma globulin by immunoelectrophoresis. Starch block electrophoresis confirmed the gamma globulin nature of the factor. Activity was removed

by absorption with calf thymus nucleoprotein. Lupus erythematosus cells of characteristic morphology were produced by positive sera. These findings regarding the antinuclear factors of A/J mice are analogous to properties of antinuclear antibodies found in human sera.

6473

Friou, G.J.; Teague, P.O. 1964. Spontaneous autoimmunity in mice: Antibodies to nucleoprotein in Strain A/J. Science 143:1333-1334.

An antibody to nucleoprotein, which appears to be an autoantibody, occurs in the gamma globulin fraction of serum from mice of the A/J strain. This antibody combines with nucleoprotein of several species but not with calf thymus DNA. The frequency of its occurrence increases with age and is greater in females. Sera that contain the antibody produce typical lupus erythematosus cells in vitro.

6474

Hanson, L.A.; Tan, E.M. 1965. Characterization of antibodies in human urine. J. Clin. Invest. 44:703-715.

A number of antibodies in urine were studied, including antibodies against T2 phages, blood group B substance, and antinuclear antibodies in patients with connective tissue disease. The main portion of the antibody activity resided in the 7S gamma globulin; weak activity was noted in the low-molecular-weight fraction related to gamma globulin (gamma-L globulin) in spite of evidence that the 7S gamma globulin was present in smaller amounts than the gamma-L globulin. The low molecular-weight gamma globulin or urine consisted largely of material identical or similar to L chains, but some material partially identical to H chains of 7S gamma globulin was consistently found. Evidence indicated that the latter finding was not due to contamination with whole gamma globulin. Absorption experiments with antisera showed that this portion of the H chain was essential for antibody activity of the gamma-L globulin fraction, since no activity was present when only the L chains were present.

6475

Hijmans, W.; Schuit, H.R.E.; Mandema, E.; Nienhuis, R.L.F.; Feltkamp, T.E.W.; Holborow, E.J.; Johnson, G.D. 1964. Comparative study for the detection of antinuclear factors with the fluorescent antibody technique. Ann. Rheum. Dis. 23:73-77.

A comparative study was carried out to investigate the influence of a number of variables on the detection of antinuclear factors in human sera. The indirect immunofluorescent test was applied throughout, using a number of different substrates and antisera conjugated with FITC. Positive results were obtained in 0 to 30 per cent in a series of 100 sera obtained from patients suffering from pulmonary tuberculosis. It could be shown that the discrepancies were due not only to quantitative differences in the sensitivity of the techniques but also to the presence of various antigen-antibody systems.

6476

Holborow, E.J.; Barnes, R.D.S.; Tuffrey, M. 1965. Auto immunity in NZB/Bl mice. Ann. Rheum. Dis. 24:401.

Mice of the NZB/Bl strain regularly develop autoimmune hemolytic anemia with red cell autoantibodies detected by anti-globulin test. Another red cell autoantibody in this strain reacts with antigens present in both mouse and human red cells. This is distinct from the mouse-specific antibody already recognized. This second autoantibody is not found in mice younger than 24 weeks and does not readily sensitize normal mouse cells to give a direct antiglobulin reaction. It is more readily absorbed from positive sera by human or mouse red cell stroma than by intact erythrocytes. It is demonstrated by indirect FA of mouse or human tissue sections or of mouse or human blood smears. It is about three times more common in male than in female mice. It is found in about 50 per cent of male mice over 24 weeks old. Complement-fixing activity has been demonstrated. Its association with males may account for the more severe hemolytic anemia that male NZB/Bl mice show. Antinuclear factor was found in the sera of about 20 per cent of mice of this strain over the age of 20 weeks, but showed no relationship to sex or progress of their autoimmune disease.

6477

Holborow, E.J.; Johnson, G.D. 1965. The nature of antinuclear immunoglobulins. Ann. N.Y. Acad. Sci. 124(Pt.2):833-837.

Tests with specific anti-7S and anti-19S conjugates confirm the mainly 7S-character of antinuclear activity in systemic lupus erythematosus sera, and its predominantly 19S character in RA sera. 19S antinuclear factor (ANF) differs from the 19S heterophile antibody of glandular fever in displaying heat-lability at 65 C and probably also in its reactivity with antiglobulin specific for immunoglobulin B(L) chains. Although there is evidence that when ANF and rheumatoid factor are present in the same serum they may interact to form complexes, 19S ANF may be identified independently.

6478

Holzknecht, F. 1965. A female patient with Behcet's disease. Wiener Klin. Wochensch. 77:44-46. In German.

This is a report on a 45-year-old female patient with Behcet's syndrome. FA tests for ANF were negative.

6479

Kough, R.H.; Barnes, W.T. 1964. Thymoma associated with erythroid aplasia, bullous skin eruption, and the lupus erythematosus cell phenomenon: Report of a case. Ann. Intern. Med. 61:308-315.

FA was used to demonstrate antinuclear factor in this case.

6480

Kramar, J.; Strejce, J.; Rejholec, V. 1964. Proof of the LE factor by the immunofluorescence method. Cas. Lek. Ceskych 103:255-258. In Czech.

The method of examination of the antinuclear factor (LE factor) by immunofluorescence is described in detail. Our own experiences with detecting the antinuclear factor by immunofluorescence appear to point to the conclusion that this method is more sensitive than the examination of LF cells, and the risk of false-positive results is small.

6481

Kurose, Y.; Michon, J., Jr.; Leopold, I.H. 1964. Study of serum antinuclear factor in uveitis. Arch. Ophthalmol. 72:844-849.

In this investigation, ANF was detected in only one case of 211 sera of patients with uveitis. This provides no evidence for ANF playing a significant role in uveitis. In other words, the present fluorescent antibody technique for ANF has little to offer as a screening test in uveitis.

6482

Lachmann, P.J. 1964. Reactivity of antinuclear factors with DNA-nucleoproteins. Ann. Rheum. Dis. 23:311-318.

The antibodies giving rise to diffuse nuclear staining in the ANF test have been found to show specificity largely for the native nucleoprotein complex rather than for its constituent parts. The native nucleoprotamine of sperm heads shares some of these determinants with the nucleohistone of somatic nuclei. The failure of sperm heads to stain in the ANF test with a number of positive sera has been found to be due to their inaccessibility to the antibody rather than to the lack of appropriate antigenic determinants or to any nonspecific interference by sperm head protamine. Sections pretreated with protamine provide a test system for detecting nonhomogeneous staining by sera that will, with untreated tissue, give diffuse staining.

6483

Laffin, R.J.; Bardawil, W.A.; Pachas, W.; McCarthy, J.S. 1964. Immunofluorescent studies on the occurrence of antinuclear factor in normal human serum. Amer J Pathol. 45:465-479.

Freezing and thawing of normal human serum and testing of the top fraction of the thawed sample have revealed the presence of antinuclear factor in approximately 76 per cent of the sera tested. This percentage is fairly constant. It is proposed that freezing and thawing produces its effect in uncovering ANF by lowering the salt concentration of the serum. Additional methods such as dialysis and dilution, which are more effective in lowering the salt concentration, are likewise more effective in uncovering ANF. Restoration of the salt concentration of treated serum to physiologic levels abolishes ANF activity. Results of experiments with paired maternal and cord sera suggest that the antinuclear factor in normal serum may be a beta-2A or a beta-2M globulin. A hypothesis is made for the development of the autoimmune state. A parallel between connective tissue disorders and human allergic states is suggested.

6484

Laffin, R.J.; Baumstark, J.S.; McCarthy, J.S. 1965. Effect of ionic strength of the reaction between nucleoprotein and antinuclear factors. J. Immunol. 94:214-219.

It has been demonstrated that at low ionic strength nucleoprotein removes greater quantities of antinuclear factors from serum. These factors can be dissociated from nucleoprotein and obtained in fairly pure form by increasing the ionic strength of the system. A study of these preparations has shown that they contain principally 6.6S gamma globulin and lesser amounts of beta-2 macroglobulin and beta-2A globulin. Antinuclear factor (ANF) activity has been demonstrated by positive indirect fluorescent antibody tests. In a random sample of ten normal sera, 6.6S gamma globulin ANF has been detected in levels ranging from 0.51 to 0.73 mg per ml.

6485

Laffin, R.J.; Pachas, W.N.; Bardawil, W.A. 1963. Detection of antinuclear globulin in normal serum. Federation Proc. 22:342:217.

The spurious finding of an occasional positive antinuclear globulin test in apparently normal individuals prompted an extensive investigation into the occurrence of such globulin in normal subjects. We reasoned that antinuclear globulin might go undetected in some instances because of low concentration or masking by substances in serum or both. Normal human sera known to have given a negative antinuclear globulin reaction by the fluorescent antibody technique were subjected to freezing and thawing without subsequent mixing. After this procedure only the upper portions of such sera, which appear to be lighter in color and less viscous than lower portions, very frequently gave a positive reaction. Because of the nature of the substrate this finding suggested that antinuclear globulin in normal individuals might be bound with DNA protein. To test this hypothesis it was decided to treat such sera with DNAase. Exposure of whole normal sera to the enzyme at a concentration of 10 ug per ml for 1 hour at 37 C rendered them positive. This finding lends additional support to the suggestion that normal serum contains bound globulin that is prevented from reacting with nuclear antigen in conventional fluorescent antibody tests. Complete article.

6486

Leopold, H.C.; Rynes, S.; Stoloff, I.L. 1965. Fluorescent antibody study for antinuclear antibodies in bronchial asthma. J. Allergy 36:175-177.

Fluorescent antinuclear antibody studies in LE cell tests for antinuclear antibodies were completely negative in a series of cases of chronic bronchial asthma. The formation of antinuclear antibodies, as found in collagen diseases, does not occur in bronchial asthma.

6487

Myers, L.; Friou, G.J. 1965. Histopathology of A/J mice: A laboratory model with spontaneous occurrence of antinucleoprotein antibody. Arth. Rheum. 8:459.

A/J mice known to develop spontaneous antinucleoprotein antibody and elevated gamma globulin levels were compared with DBA mice known not to develop these abnormalities. Both groups of 20 mice each were sacrificed at approximately 2 years. Antinucleoprotein antibody was found in serum of 75 per cent of A/J mice and none of the DBA strain. Organs were weighed and sections stained with routine, histochemical, and fluorescent antibody stains. All A/J mice had kidney disease characterized by uniform thickening of the glomerular capillary wall and low grade proteinuria. Lymphoid hyperplasia was found to a greater extent in A/J mice, along with splenic involvement and scattered foci of mononuclear cell cuffing of arteries and veins in lung, liver, and other organs. Lung adenomas, usually multiple, were found in 30 per cent of A/J mice. The findings of glomerular capillary wall thickening and lymphoid hyperplasia, in mice spontaneously developing antinucleoprotein antibody and elevated gamma globulin levels, suggests an immune disease. The association in these mice of a high incidence of lung adenoma and hepatitis is of interest as possible triggering mechanisms in the production of this disease.

6488

Neblett, T.R.; Merriam, L.R.; Burnham, T.K.; Fine, G. 1964. A source of false-positive fluorescent treponemal antibody reactions. J. Invest. Dermatol. 43:439-440.

Non-syphilitic sera that demonstrated antinuclear factor with tumor imprints also produced a reactive fluorescent treponemal antibody (FTA) test whose intensity seemed to parallel that of nuclear immunofluorescence. Such reactive FTA results were rendered negative by absorption with human tumor homogenates; they were diminished partially by normal tissue homogenate absorption, but remained unaffected by animal tissue powder absorptions. Patients furnishing such sera were considered non-syphilitic. Known syphilitic sera absorbed

with tumor or normal tissue homogenates could not be rendered negative to the FTA, and these did not produce nuclear immunofluorescence. False-positive FTA test results may indicate the presence of an autoimmune disorder.

6489

Nienhuis, R.L.F.; Mandema, E.; Jansz, A. 1963. Antinuclear factors and antithyroid antibodies. Ann. Rheum. Dis. 22:431-434.

The population of a small island was investigated twice for the presence of rheumatic disease. About 25 per cent of the residents had been born on the island. The results of the two surveys were about the same. About 25 per cent complained of rheumatism, and of these about 6 per cent had rheumatoid arthritis. About 50 per cent had osteoarthritis, and about 44 per cent had nonarticular rheumatism. Positive serological reactions were not more common in relatives up to the third generation of patients. Tests for antinuclear factor and antithyroid antibodies gave a number of positive reactions, but there was no correlation among the immunofluorescent technique, the Coombs' consumption test, and the Boyden's passive hemagglutination technique, or between the presence of antibodies and the diagnosis of clinical rheumatic disease.

6490

Norins, L.C.; Holmes, M.C. 1964. Antinuclear factor in mice. J. Immunol. 93:148-154.

The natural incidence of antinuclear factor (ANF) has been examined in several mouse strains by a standard fluorescent anti-globulin technique. NZB mice with autoimmune disease had an ANF incidence of about 40 per cent; the normal strains C3H and C57-B1 showed delayed onset of about 15 per cent incidence. F1, NZB X C3H, showed a distinctive sex difference, females eventually showing about the NZB incidence and males the C3H incidence. The apparently normal Hall Institute outbred mice showed a steady increase to an ANF incidence of about 85 per cent. The murine ANF appeared to be all or mainly a 7S gamma globulin with nucleoprotein specificity. In NZB mice, an increased ANF incidence was associated with severe renal disease, early splenectomy in males, partial or complete thymectomy, and receipt of a thymus graft; a decreased ANF incidence was associated with late splenectomy in females.

6491

Pachas, W.N.; Bardawil, W.A.; Laffin, R.J. 1963. Occurrence of antinuclear globulin in normal human serum. Arth. Rheum. 6:292.

The occasional finding of a positive antinuclear globulin test in apparently normal individuals suggested that such globulins might be found to be more widespread in occurrence if methods could be devised to detect their presence. Sera from hospitalized patients known to have given a negative antinuclear globulin reaction were frozen in test tubes and subsequently thawed without mixing. Testing of fractions extending from the top to the bottom of the sample revealed antinuclear globulins in the uppermost fraction in approximately 75 per cent of sera thus treated. Similar results were obtained with sera from individuals who were disease-free. Reading of fractions at 380 mu in the Beckman DU spectrophotometer revealed a progressively greater amount of serum protein from top to bottom of the sample. There is no preferential concentration of gamma globulin in the topmost fractions. Antinuclear globulins are present in many normal sera. Their presence is masked by other serum constituents. Freezing and thawing appears to result in a concentration of these inhibitory substances in the lower portions of a container.

6492

Ritchie, R.F.; Bayles, T.B.; Harter, J.G. 1964. Non-gamma antinuclear globulin and its 7S antibody. Arth. Rheum. 7:748-749.

In the course of antinuclear inhibition experiments, it was noted that many sera were able to increase the binding of fluorescein-labeled human antinuclear factors (ANF) to fixed tissue nuclei. Studies were made to determine if this was an increase in the number of nuclear sites available or a different antigen-antibody reaction. Conjugate preparation procedures are described that resulted in greater stability. Seventy-five human, rabbit, and rat sera were examined. All sera tested increased the ability of the labeled globulin to bind the nuclei. Cytoplasmic staining was nonexistent and did not appear after the addition of sera that produced markedly increased nuclear fluorescence. Augmentation is dependent on two components. One present in all sera tested is a non-gamma globulin. The other is a 7S gamma globulin found only in sera with a high titer of ANF. Separate sites are involved in augmentation. It is not the equivalent of a prozone phenomenon of inhibition. The differences in percentage of ANF positivity in reported family and population studies might be due to poorly specific commercial antisera.

6493

Seligmann, M. 1963. DNA antibodies. Arth. Rheum. 6:542-557.

Panel discussion of this paper included mention of FA tests for antinuclear staining. Fluorescent labeling of nucleoprotein inactivates the nucleoprotein.

6494

Teague, P.O.; Friou, G.J. 1964. Antinuclear antibodies in A/J mice: Induction in serologically negative animals. Federation Proc. 23:1451:343.

An autoantibody-like antinuclear gamma globulin factor has been found in A/J mice but not in serum pools of 15 other inbred strains. The factor was detected with the indirect immunofluorescent technique, using calf thymus nucleoprotein spots and the fluorescent homogeneous reaction pattern produced with leukocyte nuclei from mice, rabbits, and man. These sera did not react with calf thymus DNA. Some sera containing the factor produce typical lupus erythematosus cells in vitro. The factor appears spontaneously, the frequency increasing with age. It is not found in young virgin animals before 5.5 months. It occurs in 16 per cent of males and 34 per cent of females at 6 to 12 months, and later increases to nearly 100 per cent. Serologically negative intermediate-aged males and females were immunized. Injection of calf thymus nucleoprotein, native or denatured DNA in Freund adjuvant, or adjuvant alone, resulted in the prompt appearance of antinuclear activity with the same spectrum of reaction as the spontaneously occurring factor. Retired breeders of the DBA-1J strain immunized under identical conditions were unresponsive. Complete article.

6495

Teague, P.O.; Friou, G.J.; Casals, S.P.; Myers, L.L. 1964. Antinuclear antibodies in mice: Further studies of A/J and control animals, including experimental induction. Arth. Rheum. 7:349.

Several strains of mice demonstrate ANF and one does not. Comparison of A/J and DBA strain sera by zone electrophoresis has revealed the former to have gamma globulin levels very significantly higher than the latter. Less striking differences have been found between serologically positive and negative A/J animals. Studies of the histopathology have not revealed characteristic lesions. Induction of antinuclear antibodies in serologically negative A/J animals has been tested by immunization. A majority have responded following use of calf thymus nucleoprotein, but a number of other antigens have also been successful, suggesting that autologous nuclear antigens released by tissue damage may have been responsible. Identically immunized DBA controls have

consistently failed to produce antinuclear antibodies. Results suggest that antinucleoprotein may appear in A/J animals as a result of breakdown of self-recognition mechanisms with aging, possibly related to somatic mutations.

6496

Thivolet, J.; Kratchko, A. 1964. Antinuclear antibodies: Experimental data. Ann. Inst. Pasteur Paris 106:679-692. In French.

This reports an experimental study of certain biological properties of antinuclear antibodies. The antigenicity of cell nuclei of various tissues (blood, lymph nodes, striated muscle, liver, kidney, spleen, buccal mucous membrane, sperm) from different animal species (man, monkey, rabbit, guinea pig, mouse, chicken, fish, selachian, crustacea, paramecia), of cell cultures, either cultured cell lines (HeLa, T, Hep-2) or obtained by means of trypsination (young rabbit kidney), has been systematically and comparatively studied and demonstrated. No significant difference was observed, but certain cells of chicken blood had a very poor antigenicity. The thermostability of antinuclear antibodies was also studied: they are inactivated at 70 C only. A prolonged contact of these antibodies with cell cultures did not show any fixation on the nuclei; the impermeability of living cells to antibodies is therefore confirmed. Finally, the immunofluorescence technique did not demonstrate any favorable influence of complement on the fixation of antinuclear antibodies.

E. MISCELLANEOUS STUDIES

6497

Adams, J.F.; Glen, A.I.M.; Kennedy, E.H.; Mackenzie, I.L.; Morrow, J.M.; Anderson, J.R.; Gray, K.G.; Middleton, D.G. 1964. The histological and secretory changes in the stomach in patients with autoimmunity to gastric parietal cells. Lancet 1:401-403.

Twenty patients whose serum contained gastric parietal-cell antibody, but who did not have pernicious anemia, were investigated by biopsy of the mucosa of the body of the stomach, augmented histamine test of acid output, serum-vitamin B12, Schilling test, hemoglobulin, and serum-iron levels. All the biopsy specimens showed chronic gastritis, varying in degree from superficial gastritis in two cases to atrophic gastritis with complete loss of parietal cells in nine cases. Parietal-cell antibody was found to react with autologous gastric mucosa. The presence of parietal-cell autoantibody in the serum is associated with chronic gastritis, demonstrated by indirect FA. When considered together with the known immunological associations of pernicious anemia, these findings suggest that chronic atrophic gastritis and the gastric lesion of pernicious anemia are related.

6498

Anonymous. 1964. Rheumatoid factor. Brit. Med. J. 2:1613-1614.

Evidence indicates that rheumatoid factor may be antibody against exposed antigenic groups on gamma-G globulin. FA studies support this view.

6499

Ball, J.; Bahgat, N.E.D.; Taylor, G. 1964. Effect of aldehyde fixation on cellular rheumatoid factor and certain tissue antigens. J. Histochem. Cytochem. 12:737-739.

Rheumatoid factor, nuclei, and gamma-2 globulins can be identified by immunofluorescence titers on alcohol-fixed paraffin-embedded sections, although wax of low melting point is required for preservation of nuclear antigens. Rheumatoid factor also resisted aldehyde fixatives; hence, it should be possible to investigate the ultrastructural characteristics of cells containing it.

6500

Holborow, E.J.; Barnes, R.D.S.; Tuffrey, M. 1965. A new red-cell autoantibody in NZB mice. Nature 207:601-604.

In addition to the mouse-specific autoantibodies against red cells already known, NZB mice may also develop a different autoantibody, reactive with antigens present in both human and mouse red cells. Antigenic sites for this second antibody are not abundantly present on the surface of intact erythrocytes but are readily accessible in dried smears or in stromata. Various other possible explanations for this phenomenon are discussed. When the spleen cells are obtained from donors positive for the new antibody described here, the ability to produce this new antibody is transferred. After a few days the sera of the young recipient mice begin to give positive immunofluorescent test with human thyroid sections.

6501

Holborow, E.J.; Barnes, R.D.S.; Tuffrey, M. 1965. A new red-cell autoantibody in NZB/B1 mice. Arth. Rheum. 8:446.

Mice of the NZB/B1 strain regularly develop spontaneous autoimmune hemolytic anemia associated with the presence of red-cell autoantibodies. This report describes a different red-cell autoantibody, occurring in NZB/B1 mice, that reacts with both mouse and human red cells. It is not found in mice younger than 24 weeks. It does not sensitize normal mouse red cells to give direct antiglobulin reactions. It is three times commoner in male mice than in females, and is present in about 50 per cent of male mice examined over the age of 24 weeks. The antibody is complement fixing when tested by an immunofluorescent method.

6502

Hulka, J.F.; Brinton, V. 1963. Antibody to trophoblast during early postpartum period in toxemic pregnancies. Amer. J. Obstet. Gynecol. 86:130-134.

A total of 32 sera from 20 patients with toxemia of pregnancy was collected during the antepartum, labor, and immediate postpartum periods. The globulins of these sera were tagged with fluorescein isothiocyanate, then used to stain frozen sections of placenta. An antibody localizing in the syncytiotrophoblastic cytoplasm was found early in the puerperium, and in some cases even during labor. Its strength reached a peak around the 3rd postpartum day and diminished, but persisted thereafter until at least the 6th week postpartum. This is in contrast to normal pregnancies, where the phenomenon does not appear until about the 4th postpartum day. The significance of this antibody and its possible role in the etiology of the glomerular lesions of toxemia of pregnancy are discussed.

6503

Jacobs, A.; Entwistle, C.C.; Elwood, P.C. 1964. Prevalence of parietal-cell antibodies. Lancet 2:313.

Parietal-cell antibody was detected by FA in 37 of 96 women. Reasons for this high prevalence are discussed.

6504

Jeffries, G.H. 1965. Parietal cell antibodies. Ann. Intern. Med. 63:717-719.

This is a discussion of intrinsic factor and the associated antibodies. FA is a valuable tool for demonstration of the serologic factors involved.

6505

Johnson, G.D.; Holborow, E.J. 1963. Immunofluorescent test for rheumatoid factor in serum. Lancet 2:1142-1143.

A method for demonstrating rheumatoid factor by immunoflurescence, using sheep blood smears sensitized with hemolysin, is described. Correlation was found between positive staining and differential agglutination titer.

6506

Kyle, J.; Bell, T.M.; Porteous, I.B ; Blair, D.W. 1963. Factors in the etiology of regional enteritis. Bull. Soc. Int. Chir. 22:575-584.

Regional enteritis has been studied during an 8-year period in a relatively closed community in northeast Scotland. There has been a striking increase in the incidence in recent years The condition is more common in females. Thirty patients were tested for the presence of adenovirus and Coxsackie Group B virus antibodies. There was no significant difference between the titers recorded in the patients with active disease and those encountered in the general population. Small portions of affected intestinal wall and lymph nodes were cultured for viruses but with negative results. The immunofluorescent technique has been used in an attempt to demonstrate the presence of a specific serum autoantibody. The sera from 27 proven cases of regional enteritis were tested; no anti-intestinal antibody could be demonstrated. The double diffusion technique also gave negative results.

6507

Litwin, S.D.; Singer, J.M. 1965. Studies of the incidence and significance of anti - gamma globulin factors in the aging. Arth. Rheum. 8:538-550.

The anti - gamma globulin factors found in 30 per cent of an aging population reacted poorly with rabbit gamma globulin and anti-D coats. Their reactivity was like that of anti - gamma globulin factors most frequently encountered in non-rheumatoid disorders. After age 70, distribution of these factors was approximately equal in those with and without evident chronic disease. However, in the group between 50 and 70, there was a much higher incidence of anti - gamma globulin factors in subjects with various unrelated chronic conditions. The presence of these factors could not be correlated with a high incidence of hemagglutinating antibodies to thyroglobulin, an increased number of weakly positive VDRL slide tests, or an upward trend in mean gamma globulin levels found in this aging group.

6508

Malik, G.B.; Watson, W.; Murray, D.; Cruickshank, B. 1964. Immunofluorescent antibody studies in idiopathic steatorrhoea. Lancet 1:1127-1129.

Sera from patients with the malabsorption syndrome and other gastrointestinal disorders were tested by the indirect fluorescent antibody method against jejunal biopsies. In patients with idiopathic steatorrhoea on an ordinary diet a reaction was observed between the sera and the cytoplasm of epithelial cells in the crypts of Lieberkuhn and occasionally those of the surface villi This reaction was absent or diminished in patients on a gluten-free diet. It is suggested that this shows that the intestinal epithelium absorbs antigenic derivatives of gluten.

6509

McCormick, J.N. 1963. An immunofluorescence study of rheumatoid factor. Ann. Rheum. Dis. 22:1-10.

Fluorescent aggregated human gamma globulin and normal or immune rabbit globulins have been used to trace the distribution of rheumatoid factor in rheumatoid lymph nodes and synovium. Rheumatoid factor was detected by each class of reagent in plasma cells, in lymph nodes and synovium, and in the intrinsic cells of reactive follicles. The labeled rabbit globulin reagents also reacted with rheumatoid factor at various extracellular connective tissue sites. Mixed-staining experiments with contrastingly labeled aggregated human gamma globulin and the rabbit globulins showed that some plasma cells and intrinsic cells reacted with either the aggregate or the rabbit globulins and that others reacted with both. Various combinations

of each fluorescence reaction were found in individual cells and germinal centers. Mixed-staining procedures with contrastingly labeled anti-7S and a specific anti-19S antisera indicated that 7S gamma globulin was associated with macroglobulin at similar sites. Postulations on the source and action of immune globulins are made.

6510

Melin, H. 1964. An atrophic circumscribed skin lesion in the lower extremities of diabetics. Acta Med. Scand. Suppl. 176:1-75.

A survey is presented of the so-called long-term diabetic vascular changes and of skin lesions associated with diabetes. A previously unknown skin lesion in diabetes is decribed. It consists of small, rounded, brownish, atrophic and circumscribed lesions localized in the lower extremities. A fluorescence-microscopic examination of skin from diabetics with atrophic lesions with antihuman gamma globulin revealed fluorescence, partly in the capillary walls immediately under the epidermis and partly in the basal cell layer of the epidermis. The latter fluorescence was similar to that demonstrated by former investigators in skin from patients with discoid and systemic lupus erythematosus. As in systemic lupus erythematosus, this fluorescence was not only observed in the skin lesions, but also in apparently intact skin. The possibility of the atrophic skin lesions in diabetes being due to changed immunological conditions is discussed.

6511

Michael, A.F.; Drummond, K.N. 1965. Diffuse glomerular disease immunologic considerations. Marquette Med. Rev. 31:1-8.

Evidence is summarized that points to an immunologic mechanism in various forms of glomerulonephritis. This evidence includes: experimentally induced immunologic renal disease, the participati n of complement in immunologic reactions, the presence of anti-kidney antibodies, and the deposition of gamma globulin and complement. The diseases in which immunologic mechanisms are considered are acute post-streptococcal, subacute, and chronic glomerulonephritis; lupus erythematosus; anaphyl-lactoid purpura; hemolytic - anemia - uremia syndrome; Goodpasture's disease; periarteritis nodosa and the glomerulitis of serum sickness; nephrotic syndrome; and certain diseases such as pyelonephritis and polycystic disease.

6512

Myhre, B.A. 1963. Cytochemical and immunochemical changes produced by rheumatoid factor. Amer. J. Pathol. 43:2a-3a.

Rheumatoid factor extracted from active sera by elution from a diethylaminoethyl cellulose column was used to damage human liver and synovial cells suspended in a source of complement. Various characteristic morphologic and histochemical features were present. There was a twofold increase in nuclear and cytoplasmic areas as compared with cells exposed to control serum fractions. The damaged cells usually assumed an eccentric shape when compared with the controls. There was a decrease in the concentration of DNA in the nucleus but no decrease in total DNA. These findings are best explained by cellular swelling. White blood cells when exposed to rheumatoid factor assumed eccentric cytoplasmic shapes with numerous cytoplasmic vacuoles and eventually became lysed. FA studies using anti-macroglobulin and anti-gamma globulin showed the antibody to be a macroglobulin of mainly anti-nuclear specificity. Complete article.

6513

Paronetto, F.; Schaffner, F.; Mutter, R.D.; Kniffen, J.C.; Popper, H. 1964. Circulating antibodies to bile ductular cells in various liver diseases. J. Amer. Med. Ass. 187:503-506.

Sera were examined by immunofluorescence for circulating antibodies to cytoplasm of bile ductular cells in order to ascertain the role of these antibodies in progression or self-perpetuation of liver diseases. Cirrhotic livers were examined with positive sera to identify the nature of the antigen and the type of ductules to which the sera bind. Antiductular antibody was found most frequently in primary biliary cirrhosis (75 per cent) viral hepatitis (67 per cent), and postnecrotic cirrhosis (47 per cent). The incidence roughly reflected the extent and severity of the bile ductular reaction. The antigen is presumed to be a carbohydrate-protein complex occurring in larger ductular cells with basophilic cytoplasm surrounded by mesenchymal reaction.

6514

Schaefer, H.E.; Schaefer, A. 1965. Aspects of immunopathogenesis of malignant hypertension. Med. Weltkongr. 21:1144-1150. In German.

Eleven patients with a malignant hypertonia were studied by clinical evaluations and immunofluorescent histological findings. The diseases included: hypertonia, chronic glomerulonephritis, pyelonephritis, and scleroderma. Clinical diagnoses were confirmed histologically by demonstration of vessel lesions of malignant nephrosclerosis. FA demonstrated gamma globulin in areas of arteriole necrosis. In chronic glomerulonephritis the gamma globulin is evenly distributed,

whereas in essential hypertonia, pyelonephritis, and scleroderma only focal deposits of gamma globulin were seen. Complement was also found in the areas of gamma globulin deposit. The immunologic nature of the malignant phase of essential and renal hypertonia is indicated.

6515

Schroeder, W.F.; Ristic, M. 1965. Anaplasmosis: XVIII. An analysis of autoantigens in infected and normal bovine erythrocytes. Amer. J. Vet. Res. 26:679-682.

Free-serum autoantibody was absorbed by a soluble antigen obtained from normal and Anaplasma-infected erythrocytes and by normal erythrocytes treated with trypsin. A reaction between free-serum autoantibody and trypsin-treated erythrocytes from Anaplasma-free cattle was shown by serologic absorption, hemagglutination, and indirect fluorescent antibody techniques.

6516

Serafini, U.; Torrigiani, G.; Masala, C.; Serafini, F.; Spanedda, M. 1964. Antibodies against gastric mucosa. Minerva Med. 55:1784-1789. In Italian.

Sections of gastric mucosa were obtained with the cryostat from patients subjected to gastric resection. A total of 1,088 sera from apparently healthy patients were examined for antibodies against gastric mucosa by the FA technique. Positive results were obtained in 5.7 per cent of the subjects.

6517

Vazquez, J.J. 1963. The fluorescent antibody method in the study of immunopathologic conditions. Can. Med. Ass. J. 88:483-487.

Histochemical studies of immunopathologic conditions were carried out by FA. Experimental conditions studied were serum sickness, generalized anaphylaxis, the Arthus reaction, and experimental glomerulonephritis. Human diseases studied were those referred to as collagen diseases. Specific immunologic reactants were localized in the lesions of all experimental conditions studied, thus offering objective evidence of a possible immunologic pathogenesis of the lesions. In human diseases, gamma globulin was localized in the lesions of rheumatic fever, rheumatoid arthritis, systemic lupus erythematosus, and amyloidosis. Although the finding of gamma globulin in human lesions might suggest that it is an antibody, such an interpretation should be made with care. The tissue-localizing properties of sera from different disease states

showed appreciable variability within a given disease, as well as similar localizing properties among sera of different diseases. Serum factors (autoantibodies) might result as a host response and not be primarily involved in the pathogenesis of the diseases.

6518

Vigliani, E.C. 1964. Human and experimental rheumatoid factor in silicosis. Riv. Ist. Sieroterap. Ital. 39:173-178. In Italian.

The fluorescent antibody technique was used to study a possible association of the rheumatoid factor with silicosis. Out of 1,500 cases of silicosis the test was positive in only five or six instances.

6519

Wright, R.; Morton, J.A.; Taylor, K.B. 1965. Immunological studies in multiple sclerosis: Incidence of circulating antibodies to dietary proteins and auto-antigens. Brit. Med. J. 1965:491-492.

Specimens of serum from 51 patients with multiple sclerosis have been tested for antinuclear factor, rheumatoid factor, thyroid and gastric antibodies, and non-organ-specific complement fixation. When compared with a healthy control group matched for age and sex there was no significant increase in the incidence of these antibodies. In addition, the sera have been tested for antibodies to several dietary proteins. Sera from patients with multiple sclerosis showed a significantly greater incidence of antibodies to two of the purified proteins of cows' milk, alpha-lactalbumin and beta-lactoglobulin, than sera from the controls, but high-titer reactions were infrequent in both groups. The antibody titers to casein, ovalbumin, and gluten Fraction III closely resembled the levels in a control group. The significance of the findings in relation to current theories of an immunological disturbance as the underlying cause in multiple sclerosis is discussed.

II. BLOOD FORMED ELEMENTS

A. BLOOD GROUPS

6520

Beattie, K.M.; Zuelzer, W.W.; McGuire, D.A.; Cohen, F. 1964. Blood group chimerism as a clue to generalized tissue mosaicism. Transfusion 4:77-86.

Blood group studies disclosed mosaicism of the red cells in a healthy male Negro donor who was subsequently found to have a mosaicism of skin and bone marrow with respect to XX-XY chromosomal constitution and whose skin showed unequal pigmentation. The two red cell populations were unequal in numbers with a ratio of approximately 10:1. The major population was Group A, Jka negative, and showed the sickling trait in wet films and by electrophoresis. The minor population was Group B, Jka positive, did not sickle, and contained only hemoglobin A. Both red cells were Lea-positive despite the fact that the propositus was a secretor. However, only A substance was secreted and it was demonstrated by salivary studies and with the help of complete family studies that the minor genetic product that had produced the Group B erythrocytes represented the gene contribution per se and was the source of Lea substance sufficient to coat both red cell populations. The family studies showed that the propositus had received a 2 allele contribution from each parent and was therefore the result of double fertilization of a double egg nucleus, presumably an ovum and a polar body. FA determined the blood type.

6521

Branditzaeg, P. 1965. Localization of blood-group substances A and B in alcohol-fixed human gingivae by indirect immunofluorescence technique. Acta Odontol. Scand. 23:335-345.

Blood-group substances A and B were disclosed in gingival epithelium by an indirect immunofluorescence technique. The antigens were resistant to water extraction and were not affected by alcohol fixation. They were present in the stratum spinosum of the surface epithelium and in the superficial layers of the epithelium of the sulcus area but not farther apically in the pocket epithelium. This may support the concept that the pocket epithelium at all times consists of young cells, since the biosynthetic capacity of producing blood-group antigens seems to depend on a certain maturity of the epithelial cell.

6522

Burstein, R.; Berns, A.W.; Hirata, Y.; Blumenthal, H.T. 1963. A comparative histo- and immuno-pathological study of the placenta in diabetes mellitus and in erythroblastosis fetalis. Amer. J. Obstet. Gynecol. 86:66-76.

In study comparing the normal placenta with that in diabetes and erythroblastosis, proliferative vascular lesion was demonstrated that binds fluorescent insulin specifically in the diabetic placenta and fluorescent anti-Rh serum in the erythroblastotic placenta. Neither the vascular endothelium nor the syncytial knots bind the fluorescent reagents; the antigen-antibody complex evidently diffuses through these cells and is trapped in basement membrane structures. Serological studies indicate that in both disease states maternal antibodies cross the placental barrier and enter the fetal circulation. Binding of fluorescent reagent by placental tissue in cases with negative titers indicates the presence of fixed tissue antibody. Pathological and clinical evidence suggests that the latter is more directly related to the occurrence of placental lesions than is circulation antibody. In the case of diabetic subjects who have never received insulin, the binding of fluorescent insulin may be indicative of an autoimmune phenomenon.

6523

Cohen, F.; Zuelzer, W.W. 1965. Interrelationship of the various subgroups of the blood Group A: Study with immunofluorescence. Transfusion 5.223-229.

The various subgroups of A studied with immunofluorescence seemed to form a single antigenic continuum with the A-1 bloods at one end, and the weak A bloods at the other. Within this continuum each blood except the strongest samples of A-1 was characterized by its own individual spectrum of reactivity, which ranged from 4 plus to no fluorescence. These findings support a quantitative basis for the differences between the various subgroups of A. It is suggested that weak or A-w be used for the various bloods comprising the weak end of the continuum, rather than the numerous designations currently used, since with the fluorescent antibody technique, the differences within this group were no greater than those within the single category of A-1 bloods.

6524

Cohen, F.; Zuelzer, W.; Evans, M. 1964. Fluorescent antibody technique and the Lyon hypothesis. Lancet 1:1392-1393.

Our results indicate that at the present time the fluorescent antibody technique for demonstration of the Xg2 blood factor cannot be used as evidence either for or against the Lyon hypothesis.

6525

Fenton, J.W., II; Duggleby, C.R.; Otten, C.; Stone, W.H. 1965. Isolation and fluorescent labeling of Ulex europaeus anti-H lectin. Vox Sang. 10:208-211.

Fluorescent anti-H lectin was prepared. The labeling method is given. Specific fluorescent staining was observed with both fluorescein and RB 200 labeled reagents. A positive correlation was found between strength of agglutination and degree of fluorescent staining. Cells of Types A and B stained weakly with labeled reagents used undiluted or diluted 1/2. However, at higher dilutions, staining of Type O cells only was observed.

6526

Hammarstrom, S.; Lagercrantz, R.; Perlmann, P.; Gustafsson, B.E. 1965. Immunological studies in ulcerative colitis: I. Colon antigen and human blood Group A- and H-like antigens in germfree rats. J. Exp. Med. 122:1075-1086.

Sera from patients with ulcerative colitis contain antibodies that hemagglutinate sheep red cells sensitized with phenol-water extracts from colon, cecum, or feces of germfree rats. Minor concentrations of such antibodies are also present in a certain fraction of normal human sera. Hemagglutination and hemagglutination inhibition experiments with human erythrocytes and with the rat extracts showed that the latter contained an antigen similar to human blood Group A antigen. In contrast, a blood antigen like Group B could not be detected in these extracts. However, experiments with eel serum indicated that these extracts also contained an antigen similar to the H antigen of the human ABO system. Hemagglutination inhibition experiments indicated that A, H, and colon antigen were widely distributed throughout the gastrointestinal tract of the germfree rats. The colon antigen was enriched in the extracts from colon, cecum, and feces. Fluorescent antibody staining provided evidence that both the colon antigen and the A antigen were present in similar sites of the colon and cecum mucosa, particularly in goblet cells of the crypts, and in the mucus.

6527

Hanson, L.A.; Tan, E.M. 1965. Characterization of antibodies in human urine. J. Clin. Invest. 44:703-715.

A number of antibodies in urine were studied, including antibodies against T2 phages, blood group B substance, and antinuclear antibodies in patients with connective tissue disease. The main portion of the antibody activity resided in the 7S gamma globulin; weak activity was noted in the low-molecular-weight fraction related to gamma globulin (gamma-L globulin) in spite of evidence that the 7S gamma globulin was present in smaller amounts than the gamma-L globulin. The low-molecular-weight gamma globulin or urine consisted largely of material identical or similar to L chains, but some material partially identical to H chains of 7S gamma globulin was consistently found. Evidence indicated that the latter finding was not due to contamination with whole gamma globulin. Absorption experiments with antisera showed that this portion of the H chain was essential for antibody activity of the gamma-L globulin fraction, since no activity was present when only the L chains were present.

6528

Halpern, B.; Zweibaum, A.; Oudea, P.; Veyre, C. 1965. Immunology: Immunofluorescent demonstration of a tissue antigen similar to the human A erythrocyte agglutinogen in the colic glands of various mammals. Compt. Rend 260:3195-3198. In French.

Use of immunofluorescent technique on colon sections from monkey, dog and cat demonstrated the presence of a cellular antigen, specifically located in the terminal portion of the digestive tube, and immunologically similar to the human A erythrocyte agglutinogen. In man, however, the salivary and gastric mucous glands of some group A and AB subjects are rich in A agglutinogen, while the colic mucous gland only contains very small quantities.

6529

Hosoi, T 1965. Studies on hemoglobin F within a single erythrocyte by fluorescent antibody technique. Exp. Cell Res. 37:680-683.

The present fluorescent antibody method for the detection of HbF in the red cells has high sensitivity and validity as long as the antisera have high specificity and high precipitin content for HbF. There was no difference in fluorescence of cells in adult or cord blood. HbF, however, was present in 70 per cent of cord blood and 1 per cent of adult blood. HbF is unevenly distributed in the cells.

6530

Ishizuka, T.; Morishita, S.; Kano, I.; Tomoda, T. 1963. Detection of fetal hemoglobulin by fluorescent antibody technic. J. Jap. Obstet. Gynecol. Soc. 15:1181-1182. In Japanese.

Intra-erythrocytic Hb F was detected by FA technique. This technique has more extensive applicability than any other method hitherto employed.

6531

Johnson, G.D.; Holborow, E.J. 1965. The demonstration of red cell antigens by immunofluorescence. Proc. 10th Congr. Int. Soc. Blood Transfus. 1964:492-493.

Thin dried blood smears are suitable for the demonstration of a variety of red cell antigens, with a high degree of specificity. The method also offers a convenient means of studying the nature of the immunoglobulins involved in the reactions.

6532

Kent, S.P. 1963. The demonstration of water-soluble blood group H-O-antigen in tissue sections using a fluorescein-labeled extract of Ulex europaeus seed. Federation Proc. 22:214:196.

Extracts of Ulex europaeus seed have been shown by Cazal and Lalaurie to react with blood group H-O- antigen. As anti-H serum suitable for demonstrating H antigen in tissue is difficult to obtain, the use of an extract of Ulex europaeus seed for this purpose was investigated. The seeds were ground and extracted with saline. The extract was concentrated, and a portion of the concentrate was labeled with fluorescein isothiocyanate. Samples of tissue from various organs were obtained at autopsy from patients of known blood type and secretory status. The tissues were fixed in formalin, dehydrated, and embedded in paraffin. Sections of tissue were incubated with the fluorescein-labeled extract and examined with a fluorescence microscope. H antigen was demonstrated in the epithelial mucin of individuals of A, B, and O blood types. In secretors the antigen was widely distributed. In nonsecretors the H antigen was largely confined to the mucosa of the stomach and duodenum. Complete article.

6533

Kent, S.P. 1964. The demonstration and distribution of water-soluble blood Group O (H) antigen in tissue sections using a fluorescein-labeled extract of Ulex europeus seed. J. Histochem. Cytochem. 12:591-599.

A fluorescein-labeled extract of Ulex europeus seed was satisfactory for demonstrating water-soluble H antigen in formalin-fixed paraffin-embedded tissue sections. Commercially available anti-A typing serum was labeled with fluorescein and used to demonstrate water-soluble A antigen in formalin-fixed paraffin-embedded tissues. The reactivity of A and H antigen after neutral buffered formalin fixation was superior to that observed after Zenker's, Helly's, or Bouin's fixation; reactivity in paraffin sections of formalin-fixed tissues was equal to that of fresh-frozen sections. Autolysis did not prevent the demonstration of blood group antigen A or H in fixed paraffin-embedded tissues but was associated with some diffusion of the antigens. The distribution of H antigen in formalin-fixed paraffin-embedded tissue sections for patients with Types A, B, and O blood also was studied. In the secretor, blood group substances were widely distributed in the epithelial mucins of the body, but in nonsecretor blood the antigens are largely confined to the deep portion of the pyloric mucosa of the stomach and Brunner's glands. The distribution of A and H antigen within a gland, such as Brunner's glands, was not homogeneous. That is, some acini contained A antigen but no H antigen; a few contained both A and H antigen. The significance of this mosaic distribution was discussed.

6534

Massey, B.W.; Klein, S.J.; Reilly, E.B. 1965. Enhancement of immuno-fluorescent staining of erythrocytes by saponin hemolysis. Transfusion 5:434-439.

The demonstration of human blood group antigens by the fluorescent antibody technique has been improved by hemolyzing the red cells with saponin. Hemoglobin has a quenching effect on fluorescence. Its removal resulted in dramatic enhancement of the stain. Saponin proved more effective for this purpose than several other common hemolytic agents, all of which caused nonspecific staining. Antigens A, B, M, and N were readily demonstrated when the saponin step was incorporated into the usual two-layer staining procedure. Antigen Rh-O-(D) required the additional enhancement provided by the triple sandwich technique.

6535

Matej, H. 1962. The use of fluorescent antibodies in the study of blood groups. Arch. Immunol. Therap. Exp. 10:877-907.

A technique for conjugating sera used for blood group studies with FITC was elaborated. Human anti-A, anti-AB, anti-B, anti-Rh (anti-DC, anti-D, anti-E) sera and rabbit anti-A and anti-human globulin sera were conjugated. Direct and indirect FA was successful. The various reactions and cross-reactions are described. A search for natural mixed erythrocyte populations was conducted successfully in pregnant women.

6536

Moeller, E.; Eklund, A.E. 1965. Cytotoxic effect of iso-antibodies directed against ABO and Rh antigens on human lymph node cells. Nature 206:731-732.

Iso-antibodies of the human ABO and Rh system can be cytotoxic for lymph node cells in vitro. A combination of sera of different specificities showed a synergistic effect, suggesting that the density of reactive sites on the cell surface is the critical factor determining cellular sensitivity. It seems probable that iso-antigens that determine cellular sensitivity to cytotoxic antibodies can act as transplantation antigens. ABO and Rh antigens localized on tissue cells might contribute to the transplantation immunity responsible for graft rejection in humans.

6537

Myhre, B A. 1965. Blood group differentiation using fluorescent antibodies. Proc. Soc. Exp. Biol. Med. 120:712-714.

Human antisera against isologous blood groups have been combined with fluorescent chemicals and used in the study of reactions of red cells and antiserum. The decrease in fluorescence of the antiserum measured after addition of red cells is used as an indication of the amount of antibody combining with the cells. By this technique, cells have been differentiated by ABO group, subgroup, and Rh type.

6538

Posrovitz, J. 1964. Collective antigens and Rh factor in human spermatozoa. Dapim Refuiim 23:445-446. In Hebrew.

The serological methods described allowed determination of human blood type of the subject in the smallest quantity of human spermatozoa. This is of great significance in juridicial medicine. It was also determined that the Rh factor is present in the sperm.

6539

Reed, T.E. 1964. Letter to the editor regarding blood-group locus. Lancet 1:1393.

In a previous communication my colleages and I reported that a fluorescent antibody technique demonstrated that essentially all red cells of each of five women heterozygous at the Xg2 blood-group locus were Xg(a plus). This report must now be withdrawn, since it appears that the fluorescent goat antihuman globulin used was contaminated with anti-A. This was not detected at the time, in part because the Xg(a plus) control and the five heterozygotes were all group A, but the Xg(a minus) control was group O. Useful fluorescence, with other antiglobulins, has not yet been obtained. This error is my own. I regret any inconvenience it may have caused.

6540

Reed, T.E. 1964. The frequency and nature of blood group A-3. Transfusion 4:457-468.

A special survey of 20,826 random blood donor specimens collected in Ontario, Canada, yielded one A-3 blood; a review of blood grouping worksheets of 158,000 individuals in this area also yielded one A-3 blood. These frequencies are much lower than that found in Denmark, perhaps because of the present availability of high-titered immune anti-A serum. Elution studies on these two A-3 bloods show that both the agglutination-positive and the agglutination-negative cells absorb anti-A, indicating that A-3 is not always an A-2 plus O mixture as has been suggested. Fluorescent antibody studies revealed a marked cell-to-cell variability in fluorescence and therefore, presumably, cell A antigen content. This variability, together with a threshold value of A antigen required for agglutination, could explain the characteristic appearance of A-3 as agglutinates and free cells.

6541

Szulman, A.E. 1964. The blood group antigens A, B, and H in tissues of human embryos. Bibl. Haematol. 19:575-577.

The antigens A, B, and H have been found by immunofluorescence in the earliest human embryos available, in the 6th week of ovulation age. The antigens apparently form an integral part of the cell wall of the endothelium throughout the cardiovascular system, in which they persist into adulthood, and of cell walls in a variety of epithelia, some but not all of which retain the antigens permanently. The water-soluble antigens appear late, at about 8 weeks' ovulation age, thus allowing the embryo to go unprotected from maternal transplacental antibodies in heterologous pregnancies. The early, clinically inapparent wastage of incompatible A, B embryos of group 'O' mothers could be interpreted in terms of the data presented.

6542

Szulman, A.E. 1964. The histological distribution of the blood group substances in man as disclosed by immunofluorescence. III. The A, B, and H antigens in embryos and fetuses from 18 mm in length. J. Exp. Med. 119:503-516.

The ABH antigens have been mapped out in the tissues of embryos and fetuses. The H and A, B antigens have the same distribution. The cell-wall antigens are present in their maximal distribution in the youngest specimens available. They outline the endothelium of the cardiovascular system and the cells of most of the epithelia throughout the body. The exceptions are the liver, the adrenal, and the nervous system. The antigens of the stratified epithelia, together with the endothelial antigens, are permanent and persist throughout adult life. All other cell-wall antigens disappear at a time characteristic for each organ. The antigenic recession coincides with recognizable steps of morphological advancement and often with assumption of function by the organ concerned. It is completed at about the end of the first trimester. The secretion-borne antigens first appear at the 35- to 40-mm stage in the salivary glands and in the stomach, to be followed in a constant sequence by the rest of the gastrointestinal tract, respiratory system, and pancreas. The early presence and wide distribution of the cell wall A, B antigens render them likely targets for maternal anti-A, B antibodies in heterologous pregnancies.

6543

Szulman, A.E.; Krenis, L.J. 1964. The A-like antigen in tissues of rabbits. J. Histochem. Cytochem. 12:16-17.

Rabbits are grouped into those that have 'spontaneous' anti-A antibodies, and are capable of hyperimmunization, and those that possess an extractable A-like antigen in their tissues, and are devoid of anti-A antibodies. Rabbits were grouped on the basis of their possession of agglutinins for human Group A erythrocytes. Frozen sections from freshly killed animals were employed, and the histologic distribution of the antigen was investigated by FA using hyperimmune rabbit and human anti-A sera. Control reagents were provided by absorption of the sera with Group A human erythrocytes or secretor saliva. The antigen was found in the mucus of the gastrointestinal tract and tracheobronchial tree, and in the secretions of the salivary glands and the pancreas. The antigen was also found in the epithelial cells lining the gastrointestinal and tracheobronchial tracts, as well as in the urinary transitional epithelium. Specific outlining of cell envelopes and various degrees of cytoplasmic staining suggest the possibility that the cell antigen may be derived from the secreted specific substance. A negative animal with spontaneous anti-A antibody disclosed no antigen in either secretions or tissues. Complete article.

6544

Thiede, H.A.; Choate, J.W.; Gardner, H.H.; Santay, H. 1965. Immunofluorescent examination of the human chorionic villus for blood Group A and B substance. J. Exp. Med. 121:1039-1050.

The chorionic villi of term placentas were examined for A and B blood group substance, using the FA technique with heterologous and homologous antisera. No specific fluorescence was found in either the villous trophoblast or vessels of the chorionic villi. The implications of these findings in relation to the question of trophoblastic antigenicity are discussed.

6545

Yunis, J.J.; Yunis, E. 1963. Cell antigens and cell specialization: I. A study of blood group antigens on normoblasts Blood 22:53-65.

This article describes the application of four different models to the study of the presence of specific isoantigens on normoblasts. The findings demonstrate the A, B, and H receptors on human normoblasts. These receptors can be shown to be present on the surface of normoblasts in all stages of development and on mitotic normoblasts. These findings suggest that cell isoantigens appear early in cell formation and independently of cell maturation.

6546

Yunis, J.J.; Yunis, E. 1964. A study of blood group antigens on normoblasts. Bibl. Hematol. 19:238-243.

A report on the demonstration of A, B, and H receptors on human normoblasts is presented. Findings establish the presence of such red cell antigens during erythropoiesis. Nucleated cells of the bone marrow were identified by use of phase contrast and bright microscopy. Visualization of antigen receptors on nucleated cells present in mixed populations was realized by applying the principles of direct agglutination method, Coombs' mixed agglutination method, Jones' minor population technique, and direct FA.

B. ERYTHROCYTES

6547

Cohen, F.; Zuelzer, W.W. 1964. Identification of blood group antigens by immunofluorescence and its application to the detection of the transplacental passage of erythrocytes in mother and child. Vox Sang. 9:75-78.

FA was used to demonstrate antigenically different minor cell populations in vivo and in vitro as well as to differentiate weak antigens from blood group chimerism. Fetal erythrocytes were demonstrated. Favorable comparisons between FA and other methods were achieved. No massive invasion of maternal circulation by fetal cells was seen, but some maternal cells were demonstrated in fetal blood. Significance of the findings is discussed.

6548

Cohen, F.; Zuelzer, W.W.; Gustafson, D.C.; Evans, M.M. 1964. Mechanisms of isoimmunization: I. The transplacental passage of fetal erythrocytes in homospecific pregnancies. Blood 23:621-646.

A critical evaluation and discussion are presented of certain technical aspects of the acid elution method and the immunofluorescent technique for the demonstration of fetal erythrocytes in maternal blood. Close agreement between the two methods was obtained. Base-line data concerning transplacental passage were obtained in 622 unselected women, compatible with their offspring in the ABO system and not sensitized against the Rh factor. The intermittent entry of fetal erythrocytes into the maternal blood in small quantities was found to be a physiologic event. Postpartum, fetal erythrocytes were demonstrated in 50 per cent of the mothers. In approximately 10 per cent of the series, large fetal losses, estimated to range from 0.5 to 40 ml, were observed. Massive transplacental hemorrhage was detected in nearly 1 per cent of the cases by examination of the maternal blood, and usually but not always by overt anemia in the fetus. The evidence suggested that fetal hemorrhages usually began well before the onset of labor. Although labor itself under pathologic circumstances may be associated with transplacental hemorrhage, under ordinary conditions it was found to be of little or no significance with regard to the entry of fetal erythrocytes into the maternal circulation. Survival of fetal erythrocytes in the maternal circulation after delivery corresponded to the expected life span of red corpuscles.

6549

Holborow, E.J.; Barnes, R.D.S.; Tuffrey, M. 1965. A new red-cell autoantibody in NZB mice. Nature 207:601-604.

In addition to the mouse-specific autoantibodies against red cells already known, NZB mice may also develop a different autoantibody, reactive with antigens present in both human and mouse red cells. Antigenic sites for this second antibody are not abundantly present on the surface of intact erythrocytes but are readily accessible in dried smears or in stromata. Various other possible explanations for this phenomenon are discussed. When the spleen cells are obtained from donors positive for the new antibody described here, the ability to produce this new antibody is transferred. After a few days the sera of the young recipient mice begin to give positive immunofluorescent test with human thyroid sections.

6550

Lee, R.E.; Maxwell, N.G.; Gaffney, P.C.; Hutchinson, D.L. 1965. Red blood cell studies in the newborn following intrauterine transfusions. Amer. J. Clin. Pathol. 44:564.

In a current series, three of seven infants who have received intrauterine transfusions have been delivered. Cord blood from each infant was typed and examined by the direct Coombs test. The percentage of fetal hemoglobin was determined, and the fluorescent antibody technique was used for detection of minor cell populations. In one infant the direct Coombs test was negative, and the concentration of fetal hemoglobin was 2.5 per cent. No Type A cells were detected using fluorescent anti-A serum. Six weeks later, 10 per cent of the cells were Type A, and the fetal hemoglobin concentration rose to 6.5 per cent. The second infant showed a smaller percentage of adult red cells at the time of delivery, with a fetal hemoglobin concentration of 9.5 per cent. The Coombs test was positive, and fluorescent antibody serum demonstrated 10 per cent of the cells to be Rh-positive. These results offer proof for the success of this technique in providing the fetus with a population of erythrocytes resistant to destruction by maternal antibody. Complete article.

6551

Matuhasi, T. 1962. Fluorescent blood cells and anti-fluorescein antibodies. Proc. Congr. Int. Soc. Blood Transfus. 9:578-581.

Blood cells can be directly conjugated with FITC. Anti-fluorescein antibodies are produced in rabbits by injection of fluorescent blood cells or serum globulin conjugated with FITC. The fluorescent blood cells can be so clearly distinguished from normal blood cells that they are detected in normal blood cells for a long time even after they are reinjected into the blood stream and that the life span of the fluorescent blood cells can be easily measured. The life span of the fluorescent red blood cells was about 35 days in normal rabbits, but it was much reduced in rabbits having anti-fluorescein antibodies.

6552

Streiff, F.; Peters, A.; Duheille, J.; Vincent, D. 1964. Demonstration of transplacental passage of erythrocytes by fluorescent group antibodies. Comp. Rend. Soc. Biol. 158:629-633. In French.

Maternal blood was incubated with antiserum corresponding to the erythrocytic antigens of the infant in 20 cases. When the infant was group A and the mother group O, the erythrocytes passed into the maternal circulation appeared fluorescent. Many technical difficulties, here discussed, present themselves in this demonstration.

6553

Streiff, F.; Peters, A.; Genetet, B.; Vincent, D. 1964. Technique of marking red blood corpuscles by fluorescent antibody: Application to fetal-maternal transfusions. Transfusion 7.383-387. In French.

Anti-A, anti-B, and anti-D sera were conjugated with FITC. Excess color was removed by passage through Sephadex and amberlite columns. A 2 per cent suspension of red blood cells 'rbc' of a Type O mother with an A or B child was incubated with an equal volume of labeled A, B, or D antisera. A highly fluorescent aggregate of labeled rbc occurred. Fluorescence did not occur when the corresponding antigen was absent. Fluorescent cells were visible in experimental mixtures of 1/1000 of O, D, AB, and D that correspond to the transplacental passage of 0.5 ml of fetal blood. Eighteen cases of Type O women at the time of delivery were negative by this technique, but the children were O. Maternal-fetal incompatibility occurred in ten additional cases. Of the total 28 cases, fluorescent cells occurred five times, including a Group B child. BA-46-99405.

6554

Streiff, F.; Peters, A.; Vincent, D. 1964. Placental permeability to erythrocytes. Pathol. Biol. 12:963-972. In French.

The placental permeability to blood is a fact that can be studied today by modern research. The methods used in this aim are numerous: anatomical, clinical, experimental, isotopic, biochemical, cytochemical, serological, and in particular the use of fluorescent antibodies. The various techniques are described, as well as personal variations used in this work.

6555

Tomoda, Y. 1964. Demonstration of foetal erythrocyte by immunofluorescent staining. Nature 202:910-911.

FA was used to distinguish fetal from adult erythrocytes. The antiserum used was against fetal hemoglobin. The method may have value in studies of placental passage of fetal erythrocytes, hemoglobin changes, and synthesis.

6556

Trouet, A.; Stadtsbaeder, S. 1964. Demonstration of group antigens by immunofluorescence technique. Rev. Belge Pathol. Med. Exp. 30:53-64. In French.

Group antigens, particularly D antigens, can be identified by indirect technique FA. The reaction is specific for the majority of red cells, but a variable percentage (1/2,000 to 1/20,000) fluoresce aspecifically. The aspecific reactions were eliminated by use of a fluorescent anti-globulin fraction obtained by chromatography on DEAE-cellulose and adsorbed with normal human red cells and with the supernatant fraction of human serum precipitated with 50 per cent saturated ammonium sulfate. The causes of aspecific reactions are discussed.

C. LEUKOCYTES

6557

Barnhart, M.I. 1965. Importance of neutrophilic leukocytes in the resolution of fibrin. Federation Proc. 23:864:853.

Immunofluorescent studies in thrombotic states revealed the existence of fibrin, fibrinogen, or their split products within circulating neutrophils. These intravascular neutrophils functioned to pick up fibrin-like material in experimental microthrombosis in dogs and in humans suffering the stresses of either cerebrovascular thrombosis or myocardial infarction. Hypotheses have been presented that outline the possibilities for neutrophil contribution to thrombolysis. The value of neutrophils in thrombotic disease may reside in the mobility and appetite of the neutrophils for intravascular fibrin deposits.

6558

Cohn, Z.A.; Benson, B. 1965. The in vitro differentiation of mononuclear phagocytes: III. The reversibility of granule and hydrolytic enzyme formation and the turnover of granule constituents. J. Exp. Med. 122:455-466.

Mouse mononuclear phagocytes cultivated in 50 per cent newborn calf serum medium pinocytize actively and form large numbers of phase-dense granules as well as three hydrolytic enzymes. When such cells are then placed in 1 per cent newborn calf serum they show a low level of pinocytic activity, a shrinkage in granule size, and a loss in cell protein, acid phosphatase, beta-glucuronidase, and cathepsin. Examination of the extracellular medium revealed no detectable hydrolase activity. The reintroduction of cells into high levels of serum again resulted in granule and enzyme formation. Cells rapidly incorporated fluorescein-conjugated calf serum proteins into the phase-dense granules. The fluorescence of labeled granules was lost during an 18-hour period in non-fluorescein-containing medium Crystalline egg white lysozyme was concentrated in the macrophages. Approximately 80 per cent of the cell-associated enzyme was lost during a 24-hour washout period in either 1 or 50 per cent serum medium.

6559

Faber, V.; Elling, P.; Norup, G.; Mansa, B.; Nissen, N. 1964. An antinuclear factor specific for leukocytes. Lancet 2:344-345.

The pathogenic significance of antinuclear factors has been questioned because of the organ-nonspecificity of the antibodies. From experiments with plasma transfusions it had been suggested that antinuclear globuline might be partly responsible for the leukopenia in Felty's syndrome. In our patient the leukocyte-specific antinuclear factor was found and titrated

to the same degree before and after the operation, and even during convalescence, when the leukocytes were temporarily increasing in number. Thus the direct pathogenic role played by this serum factor is still an open question. Since completing this report we have found another patient, with dermatomyositis and a severe leukopenia, whose serum contained this factor.

6560

Fuerst, D.E.; Jannach, J.R. 1965. Autofluorescence of eosinophils: A bone marrow study. Nature 205:1333-1334.

Autofluorescence of eosinophils obtained from patients with various diseases was studied. Degrees of fluorescence varying with disease were obtained. No correlation with drug therapy was found. The results of this study may clarify some problems of autofluorescence obscuring specific FA reactions.

6561

Hartl, W. 1963. Fluorescent serological demonstration of leukocyte-specific antibodies. Acta Hematol. 30:288-304.

Technical directions for carrying out a fluorescent antiglobulin test on human leukocytes are given, with a report on experience with this test in an immunological investigation. The advantages of this method for serological diagnosis of leukocyte immunopathy are discussed.

6562

Hartl, W. 1964. Possibilities of an immune hematological serodiagnosis of drug-allergy agranulocytosis (Schultz' disease). Deut. Med. Wochensch. 89:81-87. In German.

To date, clinical immune hematology can contribute only to a moderate degree in the diagnosis of agranulocytosis.

6563

Litt, M. 1964. Studies in experimental eosinophilia: Uptake of immune complexes by eosinophils. J. Cell Biol. 23:355-361.

A method is described whereby immune complexes may be visualized in a single cell. Bovine serum albumin labeled with a red-fluorescing dye was joined to a rabbit antiserum labeled with a green-fluorescing dye to yield an immune complex that fluoresced yellow when illuminated by ultraviolet light. Such yellow-fluorescing immune complexes were injected into the peritoneal cavity of guinea pigs, and the peritoneal exudates

were examined subsequently. Yellow fluorescent particles were seen in eosinophils obtained from guinea pigs sensitized to hemocyanin and from normal animals. Eosinophils of the blood and of the bone marrow could also take up the complexes in vitro. Neither antigen nor antibody alone was taken up by eosinophils, nor was a mixture of labeled antigen and labeled normal globulin. Similar observations were made with human blood eosinophils. These experiments suggest that eosinophils act as part of the defense against the pathogenic effects of certain immune complexes.

6564

Riddle, J.M.; Barnhart, M.I. 1965. The eosinophil as a source for profibrinolysin in acute inflammation. Blood 25:776-794.

The emigration sequence and cytology of acute inflammation produced by a variety of stimulants was studied in dogs by a modified skin window procedure. Fibronogen, fibrin, or proteolytic enzymes that promote fibrin formation selectively attracted eosinophils into the inflammatory site. The fluorescent antibody technique was employed to assess the level of cellular profibrinolysin. Only inflammatory eosinophils and bone marrow eosinophils were marked with rhodamine antiprofibrinolysin. Staining was confined to intra- and extra-cellular eosinophil granules and coalesced masses. The intensity of fluorescence varied somewhat and perhaps reflected profibrinolysin release into the inflammatory exudate. Exudative eosinophils, like all members of the bone marrow eosinophilic series, contain profibrinolysin localized in the specific granules. Eosinophils that migrate and collect at an inflammatory site clearly transport profibrinolysin to an area of fibrin deposition. Granule release of profibrinolysin provides one mechanism for fibrinolysis that probably facilitates wound repair.

D. PLATELETS AND MEGAKARYOCYTES

6565

Borzman, M. 1963. Immunohistochemical evidence of thrombocyte antigens. Cas. Lek. Ceskych 102:880-884. In Czech.

Using the method of fluorescent antibodies the author identified platelets as well as fibrinogen and serum proteins in organs of experimental animals in which thrombotic processes were produced by administering thromboplastin or thrombin and in animals where embolic processes were induced by the administration of amniotic fluid or a suspension of meconium. Simultaneously thrombolizing processes developed in these animals to a different extent. The experiments confirmed the existence of antigens common to platelets and fibrinogen, which is not always taken into account when providing evidence of structures that are difficult to identify by histological methods. In the discussion the authors analyze some findings and discuss the application of this method to investigation of thrombotic processes, atherosclerosis, and some other diseases.

6566

Carstairs, K.C. 1965. The identification of platelets and platelet antigens in histological sections. J. Pathol. Bacteriol. 90:225-231.

A modification of the picro-Mallory technique is given that enables the three major components of thrombi to be distinguished by staining fibrin red, platelets blue, and erythrocytes yellow. The details of production of a rabbit anti-human-platelet globulin, and its use in an immunofluorescence technique to demonstrate platelet antigens in tissues, are described. With this method the retention of the antigenic identity of a platelet long after a thrombotic episode has occurred is shown. It is also noted that platelet antigens may be found in some human atherosclerotic lesions.

6567

Carstairs, K.C.; Woolf, N.; Crawford, T. 1964. Immunohistochemical cross-reaction between platelets and fibrin. J. Pathol. Bacteriol. 88.537-540.

The reactions of an anti-human fibrin serum (AF) and an anti-human platelet serum (AP) with the fibrin and platelets of artificial thrombus have been studied by two-layer (sandwich) FA. The AP reacts only with the platelets of the thrombus, leaving the strands of fibrin unstained. The AF reacts strongly with the fibrin, but gives a less intense reaction also with the platelet clumps. Absorption of the AF with platelets finally

abolishes the platelet staining, but it so weakens the fibrin reaction as to render it useless. In analyzing the extent to which platelets and fibrin contribute to the structure of complex lesions such as atherosclerotic plaques, it is therefore necessary to use both antiplatelet and antifibrin sera.

6568

Gokcen, M.; Yunis, E. 1963. Fibrinogen as a part of platelet structure. Nature 200:590-591.

Direct FA was used to supply evidence that fibrinogen is part of the platelet structure. Enzyme treatment of the platelets helped establish this. Fibrinogen was present in the cytoplasm of the megakaryocytes, suggesting the site of production.

6569

Santos-Buch, C.A.; Campbell, W.G., Jr. 1964. Immunofluorescent study of necrotizing arteritis induced in rabbits. Federation Proc. 23:814:236.

Necrotizing arteritis and hypertension were induced in rabbits by unilateral silk-and-turpentine perinephritis followed in 7 days by contralateral nephrectomy. Early arterial lesions were seen in each of the nine rabbits of Group I sacrificed from 5 to 8 days following nephrectomy; healing lesions were found in each of the five rabbits of Group II sacrificed 15 days after nephrectomy. Quick-frozen blocks of diaphragm and ileum were serially sectioned and studied by immunofluorescent technique, using fluorescein-conjugated guinea pig antibodies prepared against rabbit washed platelets, purified fibrin, and gamma globulin. In Group I, intense, immunologically specific localization of each of the conjugates was seen in the intima and inner portion of the media of the early arterial lesions and little or no appreciable uptake of guinea pig complement was demonstrated by the method of Klein and Burkholder. In Group II, healing lesions showed an intense concentration of anti-rabbit-fibrin and anti-rabbit-platelet conjugates. In contrast, these healing lesions demonstrated a less intense reaction with anti-rabbit gamma globulin and no uptake of guinea pig complement. Complete article.

6570

Stefanini, M. 1963. The occurrence and significance of antibody for platelets. Amer. J. Clin. Pathol. 40:428.

Studies were performed with specimens of serum from 252 patients with idiopathic thrombocytopenic purpura of the acute or chronic variety, and also with more than 500 specimens of serum from patients with various other disorders, with or without thrombocytopenia. Procedures used for the identification of antiplatelet agents in human serum included warm and cold agglutination, lysis, CF, precipitation, direct and indirect FA tests, inhibition of clot retraction, and release of 5-hydroxyptamine and of thromboplastic factor. Warm agglutination had the chief value as a screening procedure, whereas cold agglutination was nonspecific. Antibody detected by means of other techniques seems to be more significant. It is usually found in patients with chronic thrombocytopenic purpura, and it can be reduced in titer by means of relatively large doses of glucocortico-steroids or antimetabolites. Antibody for platelets also affects the activity of megakaryocytes and the biochemical activity of platelets. Complete article.

6571

Wakisaka, G.; Yasunaga, K.; Kuramoto, J.; Ohguma, H ; Hurukawa, Y. 1964. Determination of the life span of platelets and the application of the fluorescent antibody technique in idiopathic thrombocytopenic purpura Jap J. Allergy 13:669-672. In Japanese.

In nine cases of ITP, the life span of platelets determined by Cr^{51} technique was shorter than normal. Antiserum prepared against normal human platelets and conjugated with tetramethyl rhodamine isothiocyanate stained cytoplasm of megakaryocytes in smears of bone marrow. However, it was difficult to determine whether the activity of megakaryocytes was increased in ITP.

III. BLOOD PLASMA PROTEINS

A. ORIGIN AND DISTRIBUTION

6572

Allansmith, M.; Buell, D.N. 1965. Immunoglobulins in the skin of subjects with and without atopy. Federation Proc. 24:2782:632.

The presence and distribution of immunoglobulins in the skin are not known. The purpose of this investigation has been to study these points in the skin of allergic and nonallergic subjects. Skin biopsies were obtained from seven individuals with hay fever and positive skin tests to mixed grass, and from nine normal subjects with negative skin tests. Tissues were lyophilized and embedded in paraffin. Sections were stained for gamma-2, gamma-1A, and gamma-1M globulin by the indirect immunofluorescence technique. The three globulins were present in all specimens, and no difference in amount or distribution was detected between the allergic and normal groups. Serum albumin was also detected in all specimens and was present in the same distribution as were the immunoglobulins. Specific fluorescence indicating the presence of an immunoglobulin appeared as a green band in the papillary and upper reticular dermis, gradually decreasing in intensity into the deeper dermis. The fluorescence extended to but was not present in the basement membrane. It was not seen in the epidermis, nor was it associated with any particular cell or structure of the dermis. We conclude that the serum immunoglobulins are normally present in the extravascular tissues of the dermis. Complete article.

6573

Allerand, C.D.; Yahr, M.D. 1964. Gamma-globulin affinity for normal human tissue of the central nervous system. Science 144:1141-1142.

FA staining of the serum and cerebrospinal fluid of patients with and without neurological disease demonstrates an affinity of the gamma globulin fraction for glial cells and myelin sheaths in normal human nervous tissue. This affinity appears to be specific for the 7S gamma globulin fraction and is not seen with the other major protein fractions of serum and cerebrospinal fluid or with the FA conjugate alone.

6574

Back, N.; Hiramoto, R.; Ambrus, J.L. 1965. Immunohistochemical study of thrombolytic mechanisms. Blood 25:1028-1036.

The indirect immunofluorescent staining technique has been employed to study the localization onto fibrin clots of components of the fibrinolysin system and its activators. Immunodiffusion techniques revealed the heterogeneity of the various enzyme preparations used. The activated fibrinolytic enzyme preparations localized onto and diffused into the matrix and core of the clots. A high degree of localization was seen with streptokinase-, urokinase-, and spontaneously activated human plasmin, as well as with human plasminogen. Chloroform-activated bovine plasmin localized to a lesser extent. No differences were observed in the results whether the fibrin clots were of human, canine, or bovine origin.

6575

Barnhart, M.I.; Ferar, J.; Aoki, N. 1963. Demonstration of Ac-globulin in bovine hepatocytes. Federation Proc. 22:19:164.

The fluorescent antibody technique revealed that Ac-globulin, Ac-G, was localized in liver parenchymal cells, or hepatocytes. Spleen, lung, or other cell types in the liver did not stain specifically. About 35 per cent of the hepatocyte population contained sufficient quantities of Ac-G to stand out from neighboring cells. Such a localization in the liver probably indicates the cellular sites for synthesis of Ac-G, an important procoagulant. An unequivocal answer to whether the liver localization reflects storage as well or synthesis alone is difficult to obtain with bovine material. The multiplicity of hepatocyte function was clearly indicated. Coincidental with the study on Ac-G, other fluorescent antibodies were used to identify cells containing prothrombin, fibrinogen, and albumin. Using serial sections of frozen liver it was apparent that most hepatocytes contained small amounts of fibrinogen and large amounts of albumin. In addition, some of the hepatocytes contained Ac-G, while fewer hepatocytes contained prothrombin rather than Ac-G. Complete article.

6576

Barnhart, M.I.; Forman, W.B. 1963. The cellular localization of fibrinogen as revealed by the fluorescent antibody technique. Vox Sang. 8:461-473.

The liver parenchymal cell is the source of fibrinogen according to data obtained with the fluorescent antibody technique. With both normal and abnormal stimulation of fibrinogen synthesis only liver parenchymal cells responded. Defibrination of dogs by using thrombin stimulated the liver's production of fibrinogen. Fibrinogen appears to be synthesized and released more rapidly than prothrombin. Various physiologic conditions and their effects on fibrinogen production were studied.

6577

Barnhart, M.I.; Riddle, J.M. 1963. Cellular localization of profibrinolysin (plasminogen). Blood 21:306-321.

A previously unrecognized property of the human eosinophil is described; namely, profibrinolysin localization within the eosinophilic granules of bone marrow cells. For this demonstration the fluorescent antibody technique was employed. Highly specific fluorescent antiprofibrinolysin marked all members of the eosinophilic series. As maturation proceeded, the profibrinolysin content increased, with the greatest intensity of fluorescence seen in the mature eosinophils. These findings are compatible with the view that the bone marrow eosinophil is the site of profibrinolysin synthesis. Mature eosinophils in peripheral blood smears consistently stained less intensely. Apparently, profibrinolysin is transported from the marrow and released to the circulation and tissues when needed. These findings may be interpreted as indicating a role of the eosinophil in clot lysis and in maintaining the fluidity of the blood.

6578

Barnhart, M.I.; Riddle, J.M. 1964. Leucocytic phagocytosis of fibrin. J. Reticuloendothelial Soc. 1:361-362.

Following thrombin inoculation into dogs, neutrophils phagocytize fibrin or breakdown products of fibrin. The leukocyte count dropped but later increased two to five times the control level. The rise reflected increased neutrophils. A marked left shift occurred with neutrophilic bands sometimes comprising 50 to 65 per cent of the circulating neutrophils. FA was used to identify and assess cellular fibrin. In the normal physiologic state neutrophils do not contain fibrin, fibrinogen, or related materials. Following defibrination by thrombin, many circulating neutrophils reacted specifically with fluorescent antifibrinogen. The responses of the neutrophilic series in bone marrow occurred at different times than those of circulating neutrophils. There is evidence that neutrophils in the blood were stimulated to phagocytize either fibrin or split products resulting from fibrinolysis and resolution of thrombi. Plasma studies showed conversion of profibrinolysin to fibrinolysin within one hour. Neutrophils, either trapped or attracted, in the microthrombi returned to the circulation with their newly acquired load of fibrin. Leukocytes play an important role as a defense mechanism against naturally occurring protein aggregates like fibrin.

6579

Beneke, G. 1963. The histochemistry of fibrin. Verhandl. Deut. Ges. Pathol. 47:234-237. In German.

In model experiments concerning interstitial fibrinous inflammation, the deposition of plasma proteins (fibrinogen) in the subcutaneous connective tissue has been examined. Histochemical protein reactions have been employed for differentiation of the structured connective tissue and plasma proteins. Deposition, or accumulation of plasma proteins in the connective tissue, has been explained in the following manners: as being a colloid-chemical process resulting from the mutual precipitation of two colloids, mucopolysaccharides and plasma proteins, or as being a process of polymerization occurring between fibrin molecules and collagenous fibers by formation of hydrogen bridges.

6580

Bernier, G.M.; Cebra, J.J. 1964. Polypeptide chains of human gamma globulin: Cellular localization by fluorescent antibody. Science 144:1590-1591.

Fluorescent antibody to antigenically distinct portions of 7S gamma globulin was used to study the occurrence of H-chains and L-chains of Types I and II within single cells. Individual lymphoid cells contained both H- and L-chains, but individual cells did not contain both antigenic types of L-chains.

6581

Bernier, G.M.; Cebra, J.J. 1965. Frequency distribution of alpha, gamma, kappa, and lambda polypeptide chains in human lymphoid tissues. J. Immunol. 95:246-253.

Fluorescent antibody preparations of different colors and specificities have been used to determine the qualitative and quantitative distribution of four kinds of immunoglobulin polypeptide chains in human splenic tissue. Single cells were found to contain one kind of heavy chain and one kind of light chain, but rarely, if at all, did two kinds of light chain, kappa and lambda, coexist in a single cell. Similarly, the heavy chains, alpha and gamma, appeared to be mutually exclusive. The relative frequency of the two kinds of light chains paralleled the relative serum concentrations of these moieties. Cells containing gamma chains were slightly more frequent than cells containing alpha chains, but not to the extent anticipated by the relative serum concentrations of these two immunoglobulins.

6582

Buffe, D.; Burtin, P.; Grabar, P. 1964. Intracellular localization of the low molecular weight chains and high molecular weight chains of gamma globulins. Compt. Rend. 258:4629-4631. In French.

With the aid of specific antisera labeled with FITC and sulforhodamine dyes, new evidence is shown that long- and short-chain gamma globulins are synthesized within the same cell, but in different parts.

6583

Carbonara, A.O.; Rodhain, J.A.; Heremans, J.F. 1963. Localization of gamma-1A globulin (beta-1A globulin) in tissue cells. Nature 198:999-1000.

Patterns of fluorescent antibody localization of gamma-1A globulin were found to be essentially similar to those obtained with specific anti-gamma-ss or anti-gamma-1M antibodies, except that cells staining for gamma-1A globulin were much scarcer than those taking the anti-gamma-ss stain. It may be concluded that gamma-1A globulin shows the same pattern of cellular distribution as has previously been described for gamma-ss globulin, for gamma-1M globulin, or for antibodies as a whole.

6584

Carstairs, K.C.; Woolf, N.; Crawford, T. 1964. Immunohistochemical cross-reaction between platelets and fibrin. J. Pathol. Bacteriol. 88:537-540.

The reactions of an anti-human fibrin serum (AF) and an anti-human platelet serum (AP) with the fibrin and platelets of artificial thrombus have been studied by two-layer (sandwich) FA. The AP reacts only with the platelets of the thrombus, leaving the strands of fibrin unstained. The AF reacts strongly with the fibrin, but gives a less intense reaction also with the platelet clumps. Absorption of the AF with platelets finally abolishes the platelet staining, but it so weakens the fibrin reaction as to render it useless. In analyzing the extent to which platelets and fibrin contribute to the structure of complex lesions such as atherosclerotic plaques, it is therefore necessary to use both antiplatelet and antifibrin sera.

6585

Chiappino, G.; Pernis, B. 1964. Demonstration with immunofluorescence of 19S macroglobulins and 7S gamma globulins in different cells of the human spleen. Pathol. Microbiol. 27:8-15.

Immunohistological localization of 7S and 19S gamma globulins has been performed in the cells of normal human lymphoid tissues with the use of specific antisera conjugated with different fluorochromes. In the red pulp of the spleen a complete differentiation has been observed and the single cells appeared to contain either 7S or 19S gamma globulins, the former being about ten times more numerous than the latter. No correlation was seen between cell morphology and the class of gamma globulins produced: 7S- and 19S-producing cells were equally identified amongst immature blast elements, lymphocytoid plasma cells, and mature, classical plasma cells. In contrast with the cells of the splenic red pulp, the elements of the germinal centers of lymphoid follicles in various tissues appeared to contain mixtures of 7S and 19S gamma globulins.

6586

Choquet, C. 1964. Technique of Coons' method for the detection in smears of intracellular immune globulins. Rev. Franc. Etud. Clin. Biol. 9:1099-1100. In French.

A technique is described for detecting intracellular immunoglobulins in smears. The direct FA shows gamma globulins, the indirect test shows specific antibodies.

6587

Colberg, J.E.; Dray, S. 1963. Cellular studies of rabbit gamma globulin allotypes. Federation Proc 22:1314:380.

Two rabbit 7S gamma globulin allotypic specificities, A4 and A5, are determined by alleles at a single gene locus, b. Using labeled isoantibodies, cellular localization of A4 and A5 was studied in lymph nodes from A4 homozygotes, A5 homozygotes, and A4-A5 heterozygotes. The DEAE-cellulose-prepared 7S gamma globulin fractions of anti-A4 and anti-A5 were conjugated with fluorescein isothiocyanate or lissamine rhodamine B and purified on Sephadex G-25. The anti-A5 conjugates of contrasting color stained the cytoplasm of plasma cells and lymphoid cells of lymph nodes from the A5 homozygotes and A4-A5 heterozygotes but did not stain the cells of the A4 homozygote. The anti-A4 conjugates were similarly specific. Immunofluorescent techniques were used to investigate localization of the two allotypes in the heterozygote rabbit. The same follicle in adjacent sections stained with both the anti-A4 and the anti-A5 conjugates. Double staining of a single section with

anti-A4 and anti-A5 in either order showed virtually no cells possessing one allotype without the other in its cytoplasm. The results obtained support the hypothesis that gamma globulins of both types are produced in the same cell. Complete article.

6588

Crabbe, P.A.; Carbonara, A.O.; Heremans, J.F. 1965. The normal human intestinal mucosa as a major source of plasma cells containing gamma-A immunoglobulin. Lab. Invest. 14:235-248.

Biopsy specimens from the duodenal and jejunal mucosa were obtained from eight normal adult subjects. Cryostat sections of the specimens were studied by fluorescein-labeled antisera specifically reacting with the three types of immunoglobulins, gamma-G (7S gamma), gamma-A (gamma-1A), and gamma-M (19S gamma). A large predominance of gamma-A-containing plasma cells in the stroma of the intestinal mucosa was observed. Quantitative data indicated the mean population density of gamma-A cells to be 181,000 per cu mm of interstitial tissue, against 18,000 and 30,000 cells per cu mm for the gamma-G and gamma-M cells, respectively. There was no statistically significant difference between the sizes of nucleocytoplasmic ratios of the cells containing each type of immunoglobulin.

6589

Forman, W.B. 1964 Cellular site of fibrinogen synthesis. New Physician 13:70-74.

The hepatic parenchymal cell is the source of fibrinogen production, according to data obtained with the fluorescent antibody technique. With both normal and abnormal stimulation of fibrinogen synthesis, hepatic parenchymal cells responded. In normal dogs it is thought that fibrinogen is present in small amounts in most parenchymal cells, with only a few cells containing sufficient stored fibrinogen to stand out from natural autofluorescence. Female dogs in the last month of their pregnancy have a naturally stimulated fibrinogen synthesis and many parenchymal cells are vividly tagged by the fluorescent anti-fibrinogen. Fibrinogen production was promoted in several dogs by defibrination, using thrombin. Activation of the fibrinolytic system occurred in late pregnancy and following defibrination with thrombin. The relationship of the coagulation and lytic system was considered with respect to normal physiology and the roles assumed in thrombotic disease.

6590

Forman, W.B.; Barnhart, M.I. 1964. Cellular site for fibrinogen synthesis. J. Amer. Med. Ass. 187:128-132.

The hepatic parenchymal cell is the source of fibrinogen production according to data obtained with the fluorescent antibody technique. With both normal and abnormal stimulation of fibrinogen synthesis only hepatic parenchymal cells responded. Spleen, lymph node, bone marrow, and peripheral blood also were examined, with no specific fluorescence noted. Fibrinogen production was promoted in several dogs by defibrination with thrombin. Cellular fluorescence was seen 5.5 hours after defibrination peak and, in the next hour, the circulating fibrinogen level was restored to normal. A rate study showed that fibrinogen was synthesized more rapidly than prothrombin. Activation of the fibrinolytic system occurred following defibrination with thrombin. The relationship of the coagulation and lytic systems was considered with respect to normal physiology and the roles assumed in thrombotic disease.

6591

Geiler, G. 1965. Studies on the immunohistochemical demonstration of fibrin on biopsy and biopsy material. Histochemie 5:5:361-365. In German.

A specific demonstration of fibrin was made by direct FA. Tests were carried out on material obtained by biopsy and autopsy. Antigenicity of fibrin is maintained over long periods. The method is therefore especially suitable for the demonstration of fibrin in postmortem material.

6592

Gokcen, M.; Yunis, E. 1963. Fibrinogen as a part of platelet structure. Nature 200:590-591.

Direct FA was used to supply evidence that fibrinogen is part of the platelet structure. Enzyme treatment of the platelets helped establish this. Fibrinogen was present in the cytoplasm of the megakaryocytes, suggesting the site of production.

6593

Hamashima, Y.; Harter, J.G.; Coons, A.H. 1964. The localization of albumin and fibrinogen in human liver cells. J. Cell Biol. 20:271-279.

Human liver sections were stained with antihuman serum albumin and/or antihuman fibrin monomer fluorescent conjugates. Approximately 10 per cent of the hepatic cells stained specifically for human serum albumin, 1 per cent for fibrinogen, and 0.1 per cent for both. Approximately 18 per cent of the Kupffer cells stained specifically for human serum albumin and 33 per cent for fibrinogen. Staining of both cell types was mainly cytoplasmic, although albumin was found in the nuclei of some parenchymal cells, depending on the method of fixation. Cytoplasmic granules staining specifically for fibrinogen could be seen just inside the cell membrane facing the bile caniculi in many more parenchymal cells than the 1 per cent showing diffuse cytoplasmic staining. The technical difficulties involved in preparing fluorescent conjugates against these antigens and in the fixation of these antigens for immunofluorescent staining are discussed.

6594

Horowitz, R.E.; Burrows, L.; Paronetto, F.; Wildstein, W. 1963. Immunocytochemical observations on canine kidney monografts. Federation Proc. 22:677:274.

Thirty-three primary kidney transplants were performed in dogs: 26 homografts, 5 autografts, and 2 isografts. Implanted kidneys were removed at intervals from 12 hours to 7 days. At 12 hours all kidneys showed perivascular edema; autografts and isografts showed no further abnormalities, but homografts showed progressive perivascular infiltration by plasma cells, lymphocytes, and histiocytes, and at 72 hours vacuolization of the media of blood vessels. Coincident with media vacuolization endothelial proliferation with subsequent thrombosis and parenchymal necrosis was seen. Cryostat sections of the kidneys were treated with fluoresceinated rabbit anti-dog gamma globulin, alpha-2 globulin, albumin, and fibrinogen. After 24 hours many gamma globulin-containing cells were seen and at 72 hours droplets of gamma globulin appeared in the media of blood vessels, corresponding in location to the vacuoles seen by light microscopy. Gamma globulin fluorescence could be abolished by pretreatment with pH 3.2 buffer. Alpha-2 globulin was seen in similar foci as gamma, but less. No albumin or fibrinogen was seen in vessel walls, and autografts and isografts exhibited no vascular globulin. Antigen-antibody complexes, localized or formed in the walls of blood vessels, may stimulate endothelial proliferation resulting in thrombosis and necrosis, i.e., rejection. Complete article.

6595

Kaeberle, M.L.; Segre, D. 1964. Intestinal absorption of homologous and heterologous serum globulins by the newborn pig. Amer. J. Vet. Res. 25:1096-1102.

FITC-labeled swine gamma globulin or sulfanilic-acid-azo-swine globulin was taken up by epithelial cells of newborn pigs throughout the length of the small intestine within 2.5 hours of oral administration. Globules of the material were massed in the supranuclear cytoplasm, the greatest quantities being in the epithelial cells of the ileum. Porcine, ovine, and equine globulin concentrates that contained diphtheria and tetanus antibodies were administered to colostrum-deprived pigs by oral or intraperitoneal routes within 12 hours of birth. Orally administered equine antibodies were absorbed as readily as those of porcine origin, but antibodies of ovine origin were absorbed only one-half as efficiently. Tetanus antibodies of ovine and porcine origin were absorbed more readily than the corresponding diphtheria antibodies. Diphtheria antibodies of equine origin were taken up more efficiently than the tetanus antibodies.

6596

Kopp, W.L. 1963. Demonstration of serum proteins on washed leukocytes by means of fluorescent antibodies. J. Lab. Clin. Med. 62:18-23.

Washed leukocytes of normal and atopic persons were incubated with fluorescent rabbit antihuman globulins. After thorough washing of the cells, fluorescent globules were seen on the surface of leukocytes of all donors. Leukocytes incubated with fluorescent normal rabbit globulins did not fluoresce. These findings indicate that serum proteins adhere to thoroughly washed human leukocytes from both normal and atopic subjects.

6597

Kouvalainen, K. 1964. Significance of Hassall's corpuscles in the light of their morphological and histochemical appearance. Ann. Med. Exp. Biol. Fenn. 42.177-184.

When studying the human thymus histochemically it was observed that most of the Hassall's corpuscles contain 5-nucleotidase, which was not seen in other parts of the thymus. By using the fluorescent antibody method, cells containing gamma globulin were seen in the thymus and also in some Hassall's corpuscles. Based on the results mentioned and on the general morphology of Hassall's bodies, the possible immunological significance of these structures has been discussed. It is suggested that Hassall's corpuscles eliminate forbidden clones, and/or they produce informative fragments of deoxyribonucleic acid for the lymphoid cells.

6598

Leonard, E.J.; Haydu, S. 1965. Immunofluorescent localization of cardioglobulin. Federation Proc. 24:282:177.

Cardioglobulin (CG) is a mammalian plasma protein system, identifiable by its inotropic action on frog heart, to which it binds firmly to form CG frog heart. If CG were cardiotonic for mammals, it might be localized on heart, and in turn bind antibodies to CG that could be traced by immunofluorescence. Rat CG was injected into guinea pigs, which made antibodies that inhibited CG inotropic action on frog heart; by immunofluorescence these were localized to muscle surface of CG from heart, not to heart without CG. Thus, functionally and histologically the antiserum had an anti-CG. Although the antiserum contained several antibodies, as tested immunoelectrophoretically against rat serum, passage through a series of CG from hearts adsorbed out primarily one antibody. When antiserum was layered onto rat tissue slices no specific fluorescence was found on brain, smooth muscle, or liver, but occurred on heart cell surfaces as well as skeletal muscle, kidney glomeruli, and choroid plexus, in the basement membrane region of kidney tubules, and on specific sites in other tissues now under study. Since fluorescence was absent or markedly reduced with antiserum adsorbed by CG frog hearts, the observed localization should be due to anti-CG. Unless there is tissue antigen cross-reactive with anti-CG, results suggest CG may have functional significance for many cell types. Complete article.

6599

Lundberg, C.; Boesman, M.; Fireman, P.; Gitlin, D. 1965. Cellular site of gamma-1A globulin synthesis. Federation Proc. 24:2072:502.

Lymph nodes and spleens of normal children and lymph nodes of children with ataxia telangiectasia who had little or no detectable plasma gamma-1A globulin were examined by the fluorescent antibody method for cells containing gamma-1A and 7S gamma-2 globulins. Goat antiserum specific for human gamma-1A globulin was labeled with fluorescein and rabbit antiserum specific for 7S gamma-2 globulin was labeled with rhodamine B. Cells containing gamma-1A globulin were found in the medullary areas of normal lymph nodes and in the red pulp of normal spleen: the cells possessed eccentrically placed nuclei, were larger than lymphocytes, and had a nuclear to cytoplasmic ratio of approximately 1:1. Although these cells could be found in lymph nodes of patients with ataxia telangiectasia they were far fewer in number than in normal nodes; the numbers of cells containing 7S gamma-2 globulin in nodes of ataxia telangiectasia were similar to those found in normal nodes. In sections of normal nodes obtained for both gamma-1A globulin and 7S gamma-2 globulin,

a given cell appeared to contain predominantly only one of these gamma globulins, suggesting that at least most of the cells synthesizing gamma-1A globulins may be distinct from those synthesizing 7S gamma-2 globulins. Complete article.

6600

Mancini, R.E.; Vilar, O.; Alvarez, B.; Seiguer, A.C. 1965. Extravascular and intratubular diffusion of labeled serum proteins in the rat testis. J. Histochem. Cytochem. 13:376-385.

Rat whole serum, albumin, globulins, and fibrinogen were labeled with fluorescent dyes (RB 200, CI No. 45100 and FITC). Albumin was also tagged with radioactive iodine (I-131) and tritium (H-3). Heterologous albumin was also similarly labeled. The proteins were intravenously injected in prepubertal, pubertal, and adult rats and their decay in the circulation and histological distribution in the testis and epididymis studied. FA was also used for tracing. With the exception of fibrinogen, labeled serum proteins rapidly appeared in the lumina of vessels, diffused extravascularly in the intertubular spaces, and between the germinal cells and in the lumina of seminiferous tubules. Labeled material was present in the lumen of epididymal canaliculi but not in the ductus deferens. This extravascular and intratubular diffusion was parallel to the fast component of the time decline curve of labeled homologous serum proteins in the circulation. There was no great difference between young and adult rats in the extravascular diffusion process, but intratubular passage was higher in pubertal and adult animals. FA showed localization of proteins similiar to the direct protein fluorescent tracing.

6601

Mancini, R.E.; Vilar, O.; Heinrich, J.J.; Davidson, O.W.; Alvarez, B. 1963. Transference of circulating labeled serum proteins to the follicle of the rat ovary. J. Histochem. Cytochem. 11:80-88.

Rat whole serum, albumin, globulins, and fibrinogen fractions were labeled with sulforhodamine B (CI No. 45100) and FITC. Albumin was also tagged with radioactive iodine (I-131). Labeled serum fractions were intravenously injected in adult female albino rats and their decay in the circulation and histological distribution in the ovaries were studied. Labeled fluorescent antibodies against rat serum fractions were also applied to the ovary sections of intact rats as well as of rats previously injected with unlabeled serum fractions. Labeled whole serum, albumin, and globulins decay in the circulation with a half-life value of 2.8 days for the first, 2.6 days for the second, and 3.1 days for the last. Parallel with the fast component of this time decline curve, serum fractions diffuse extravascularly in the ovary and appear in the zona pellucida and cytoplasm of the oocyte in the growing follicles, via thecal capillaries, intercellular spaces of granulosa layer, and antrum. Immunohistological techniques suggest that endogenous circulating serum fractions may be transmitted to the follicles following the same route.

6602

Mellors, R.C ; Korngold, L. 1963. The cellular origin of human immunoglobulins, gamma-2, gamma-1M, gamma-1A. J. Exp. Med. 118:387-397.

A study was made of the cellular origin of human immunoglobulins. Two closely related families of cells form immunoglobulins in human lymphoid tissue: germinal (reticular) centers and plasma cells. Thus their cellular origin in addition to their known antigenic relationships further justifies placing the immunoglobulins in one family of proteins. Immunoglobulins were also formed to a small extent in primitive reticular cells that resembled those of germinal centers but were separated from them. In general only one type of immunoglobulin was present in an individual cell or germinal center, but gamma-2 and gamma-1M globulins were identified on occasion in the same plasma cell and germinal center by FA, using contrasting labels. A peculiarity of the fetal thymus gland was the presence of immunoglobulin in a small number of cells of primitive reticular appearance and in the Hassall corpuscles.

6603

Moore, R.D.; Schoenberg, M.D. 1964. The response of the histiocytes and macrophages in the lungs of rabbits injected with Freund's adjuvant. Brit. J. Exp. Pathol. 45:488-497.

The cellular response in the lungs of rabbits given complete Freund's adjuvant intravenously was considered in relation to the appearance and kind of circulating antibody, the presence of antigen and antibody in the cells, and the development of tissue sensitivity to the mycobacteria. The proliferation of histiocytes in the alveolar walls, the accumulation of macrophages in the alveolar spaces, the appearance of polymorphonuclear leukocytes, lymphocytes and plasma cells, and the formation of granulomata in the lungs occurred before any specific immunological reaction was recognized. The large mononuclear cells of the granulomata differed from the macrophages and histiocytes both structurally and functionally. Circulating antibody and skin sensitivity were not detected until the cellular reaction was well established. The presence of plasma cells containing gamma globulin at a time when 7S antibody appears in the circulation is described.

6604

Murakami, M. 1964. Mechanism of lipid deposition on arterial wall. Jap. Heart J. 5:397-398.

Although the encrustation theory is still acceptable for the pathogenesis of lipid in the arterial wall, one can add additional important mechanisms. Fibrin and beta-lipoprotein are found in association in arterial walls.

6605

Murakami, M.; Sekimoto, H.; Tatsuguchi, M.; Yasuda, Y.; Masuda, S.; Matsumoto, K.; Shinagawa, T.; Genda, A.; Ikeshima, T.; Ozaki, H.; Yamada, T.; Murakami, M. 1964. Studies on the role of fibrinogen on lipid deposition into the aorta. Jap. Heart J. 5:108-114.

The purpose of this study was to determine whether the presence of fibrinogen has an effect on the deposition of beta-lipoprotein into the aorta. The results obtained with the fluorescent antibody technique indicated that accumulations of beta-lipoprotein in the intima were associated with the presence of fibrin. It is suggested that fibrinogen enhances the deposition of lipid complexes from abnormally lipaemic sera.

6606

Ohta, G.; Tanishima, K.; Doishita, K.; Uno, Y. 1964. Endothelial plasma thin layer. Acta Hematol. Jap. 27:482-488.

Using FA, it was demonstrated that the plasma thin layer, consisting of fibrin or fibrinogen and/or globulin, covered the inner surface of the vascular endothelium from heparinized rats treated with bovine thrombi, EACA, or trypan blue singly or in combination. The layer tended to be more frequently in the small arteries than in the veins of the kidney and was present equally in both vessels of the lungs. The plasma thin layer was on the aortic endothelium of the orifice of intercostal arteries from the rats so treated. This was rare in any type of vessels of normal rats. It seems likely, therefore, that the endothelial plasma thin layer is composed of fibrin, platelets, and globulin, possibly as a result of an intravascular coagulation operating on the inner surface of the endothelium. Then the occurrence, even though rare, of the endothelial plasma layer in the intima of the normal rat vessels suggests strongly that a microcoagulation might occur spontaneously as a physiological phenomenon.

6607

Oystese, B.; Torgersen, O. 1964. Excretion of proteins from the human gastric mucosa: An immunohistochemical study. Acta Pathol. Microbiol. Scand. 60:93-98.

In resected and biopsy material from human stomachs large amounts of excreted serum proteins (albumin and gamma globulin) were demonstrated in the mucosa substance outlining the epithelial surface of human gastric mucosa. These proteins were also located in the entire lumina of the crypts, as well as in some atypical glands in cancer tissue. By the method used, the proteins could not be demonstrated with certainty in the cytoplasm of the epithelial cells.

6608

Pernis, B.; Chiappino, G. 1964. Identification in human lymphoid tissues of cells that produce Group 1 or Group 2 gamma globulins. Immunology 7:500-506.

The cells that produce Group 1 and Group 2 gamma globulins have been localized in human lymphoid tissues. This has been done with antisera specific for Group 1 or Group 2 gamma globulins prepared by immunizing rabbits with purified Bence Jones proteins of the corresponding group and subsequently conjugating with different fluorochromes. The immunofluorescence observations have shown that in the red pulp of the spleen of adult humans two populations of plasma cells, present in approximately equal numbers, can be differentiated on the basis of the type of gamma globulin produced. The cells in the germinal centers of lymphoid follicles in the spleen and lymph nodes appear, instead, to contain both Group 1 and Group 2 gamma globulins.

6609

Post, R.S. 1963. Formation of lipid-protein complexes in renal epithelial cells in the course of protein reabsorption. J. Lab. Clin. Med. 62:1001-1002.

Serum proteins penetrate normal glomerular structures separating capillary lumen and Bowman's space at a variable rate. Protein loading in the animal and human results in an increased loss of protein from the glomerulus. Morphologic changes occur that suggest intracellular localization of protein in a system of ducts and vacuolar structures that, as determined by electron microscopy, are membrane-lined. The author has been able to demonstrate, with FA, bovine serum albumin in discrete structures within epithelial cells of the proximal tubules and glomeruli of rats within 24 hours of injection of the protein. In addition, histochemical studies and observed variation in fluorescence intensity

within the glomerular cytoplasmic structures correlate to suggest biochemical alteration of the contained protein with formation of lipid-protein complexes. With the same technique, rat globulin was demonstrated after bovine albumin loading within both types of epithelial cells in amounts greater than normal. These latter observations cast some doubt on the specificity of globulin localization in the glomerulus. A special tissue preparation technique is described.

6610

Rice, W.G. 1963. Demonstration of fibrinogen in the Kupffer cells and endothelium by immunofluorescence. Amer. J. Pathol. 43:2a.

An antiserum to washed, human, freshly clotted fibrin was prepared in rabbits. After absorption with human serum a specific precipitin having the mobility of fibrinogen was demonstrable by immunoelectrophoresis against human plasma. This antiserum was applied to frozen sections of washed human tissues and the site of specific immunofluorescence identified. Negative controls signified the specificity of the fluorescence. The fluorescence was concentrated in the Kupffer cells outlining the hepatic sinusoids. Some scattered fluorescence was also found in endothelial cells of renal capillaries and glomeruli. Fluorescence was not present in the splenic sinusoids. Photographic evidence indicated that this was probably not free fibrin, as previously suggested, but was contained in structural units with nuclei conforming to the endothelial cells. It was concluded that a substance having the immunologic specificity of fibrinogen was concentrated in sinusoidal endothelial cells, especially Kupffer cells. Complete article.

6611

Riddle, J.M.; Barnhart, M.I. 1964. Ultrastructural study of fibrin dissolution via emigrated polymorphonuclear neutrophils. Amer. J. Pathol. 45:805-823.

The cytocomposition of an inflammatory exudate mediated by fibrin was almost exclusively polymorphonuclear neutrophils and eosinophils. The percentage of each present was clearly time dependent. The distribution of fibrin throughout the inflammatory process is described. Ultrastructural studies and FA staining assisted in location and identification of fibrin.

6612

Schoenberg, M.D.; Rupp, J.C.; Moore, R.D. 1964. The cellular response of the spleen and its relationship to the circulating 19S and 7S antibody in the antigenically stimulated rabbit. Brit. J. Exp. Pathol. 45:111-119.

From these results it can be tentatively concluded that in the spleen the plasma cell is associated with the production of 7S immune globulin and a large basophilic cell is associated with the production of 19S immune globulin. It is unlikely that these latter cells give rise to the mature plasma cell. This does not preclude the plasma cell from arising from lymphocytes, but a sequential change from a large cell containing antibody to a mature plasma cell containing antibody has not been established.

6613

Steinberg, A.G. 1964. Population, immunogenetic, and biochemical studies on the Gm(b) factors of human gamma globulin. Symp. Quart. Biol. 29:449-454.

The data have shown that the genetically determined gamma globulin antigens Gm(a), Gm(b), Inv(a), and Inv(b) are at least partially dependent upon the quaternary structure of the molecule and that the Gm(b-w) antigen is entirely dependent on this structure. They have shown also that the specificity for Gm(b-w) as for the other Gm factors, resides in the H chain. Immunofluorescent studies, although very preliminary, tend to confirm that a given cell produces Gm(a) or Gm(b) or neither, but not both.

6614

van Furth, R.; Schuit, H.R.E.; Hijmans, W. 1965. The immunological development of the human fetus. J. Exp. Med. 122:1173-1188.

The immunogenesis of the human fetus has been investigated by means of the formation of immunoglobulins in vitro, immunofluorescence, morphological studies, and analysis of the immunoglobulins in the serum. Twenty fetuses that were born alive were studied. Fetal ages ranged from 13 to 31 weeks. The results of the spleen cultures demonstrated the synthesis of 7S gamma-2 globulin (IgG) and 18S gamma-1 macroglobulin (IgM), which starts at about the 20th week of gestation. In the serum, IgM could be detected at about the same period. The immunofluorescent staining of the spleen tissue showed that medium-sized and large lymphoid cells as well as plasma cells (even with Russell bodies) were positive for either IgG or IgM. The peripheral blood also contained a small number of medium-sized IgG- and IgM-positive cells. Both the spleen and the peripheral blood showed a considerable number of fluorescent

small lymphocytes that contained IgM exclusively. The relatively high ratio of IgM to IgG production prenatally, compared with the postnatal situation, agrees with a predominantly primary antibody response in fetal life. In general, the fetal thymus did not synthesize immunoglobulins.

6615

Wilken, H. 1963. Immunohistological examinations on the placenta with fluorescein-labeled antisera. Zentralbl. Gynakol. 85:1193-1202. In German.

The placentas of healthy and diseased pregnant women were examined using FA. The gamma globulins of the antiglobulin sera marked with fluorescein in the placental tissue show a characteristic localization. They are predominantly found in the decidua graviditatis, the villus epithelium and villus stroma, the amnion epithelium, and in the adventitia and media of the fetal placental vessels. The origin of the gamma globulins is discussed.

B. PATHOLOGY

6616

Becker, B.; Unger, H.; Coleman, S.L.; Keates, E.U. 1963. Plasma cells and gamma globulin in trabecular meshwork of eyes with primary open-angle glaucoma. Arch. Ophthalmol. 70:38-41.

Fifty-two trephine buttons from eyes with primary open-angle glaucoma were studied for staining with fluorescein-labeled antihuman gamma globulin. Seventy-five per cent of these buttons demonstrated fluorescent staining of the trabecular meshwork, suggesting the presence of gamma globulin. Seventy-five per cent of 28 surgical or autopsy eyes with primary open-angle glaucoma demonstrated similar staining. Only 14 per cent of 65 routine autopsy eyes showed this staining pattern. Sections from 23 of 28 eyes with chronic simple open-angle glaucoma and 20 trephine buttons from these eyes revealed the presence of plasma cells. Similar cells were present in only 13 of 65 routine autopsy eyes.

6617

Brzosko, W.; Nowoslawski, A. 1963. Immunohistochemical studies on Pneumocystis pneumonia. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 11:563-564.

Immunoelectrophoretically monospecific, FITC labeled globulin fractions (albumin, gamma globulin, and fibrinogen) were used. Large quantities of gamma globulin exudate were found closely bound to Pneumocystis carinii conglomerates in the bronchiolar and alveolar lumina; prolonged washing of sections in a 0.02 M citrate buffer solution at pH 3.3 resulted in almost complete removal of intraalveolar gamma globulin from the P. carinii conglomerates. Small amounts of albumin were detected in the alveolar exudate, but unlike the gamma globulin, it was quickly washed out with physiological saline. Sections of lung stained for fibrin and/or fibrinogen revealed specific fluorescence in the vascular lumina only; no fibrin and/or fibrinogen was demonstrated in the intra-alveolar exudate. There was a marked activation of germinal centers in all of the peribronchial and hilar lymph nodes with the presence of many mature and Russell-body plasma cells. BA-46-13333.

6618

Burkholder, P.M. 1965. Immunohistopathologic study of localized plasma proteins and fixation of guinea pig complement in renal lesions of diabetic glomerulosclerosis. Diabetes 14:755-770.

Results of immunohistopathologic study of kidneys from six patients with diabetic glomerulosclerosis are presented and discussed. Gamma globulin localized in hypereosinophilic glomerular exudates, hyaline arterioles, and coarsely fibrillar non-'cannon ball' type glomerular nodules may represent antibody globulin in immune complexes. Gamma globulin localized diffusely in a delicate 'membranous' pattern in glomerular capillary walls, in 'cannon ball' type nodules, in globlets within tubular epithelial cells, and in tubular cast, does not fix heterologous complement. Therefore, it is different from the immunoglobulin in the latter sites. Evidence has also been presented that indicates that precipitation of fibrin in glomerular capillary walls, possibly in certain instances at sites of localized antigen-antibody complexes, and in glomerular capsules may play a significant role in the development of certain glomerular nodules and of thickened glomerular capsules.

6619

Burstein, R.; Berns, A.W.; Frankel, S.; Blumenthal, H.T. 1965. The occurrence of an immunopathologic vascular lesion in normal and abnormal pregnancies. Amer. J. Obstet. Gynecol. 92:78-86.

Evidence has been presented supporting the concept that an immune reaction occurs during pregnancy. Such evidence consists of the occurrence of a proliferative vascular lesion on the maternal, but not on the fetal, side of the placenta, which increases in frequency during the first trimester, appears to fall off subsequently, only to rise again at term, although not to the initial level of frequency and intensity. Such lesions bind antihuman globulin and complement, showing more intense binding of the latter than of the former. It is also supported by the occurrence of nodular collections of lymphocytes, which is most intense in the term uterus. Evidence is discussed indicating that there is a partial suppression of this immune reaction in normal pregnancy, probably by the maternal hormones, thus permitting the fetus to develop to term.

6620

Burtin, P.; Buffe, D. 1964. Immunofluorescence studies of human plasmocytes of gamma and beta-2A myelomas. Pathol. Biol. 12:5-13. In French.

Bone marrow has been withdrawn from 32 patients suffering from multiple myeloma. The slides made after washing have been stained by fluorescent anti - gamma globulins and anti - beta-2A globulin successively. These antibodies were marked with fluorescein isothiocyanate or sulphorhodamine. In at least 50 per cent of the cases no cellular fluorescein was seen. Eighteen slides gave positive images, sometimes binucleated or abnormal plasmocytes of variable size and age became fluorescent. Often, but not always, these plasmocytes had a granular structure. The fluorescence was cytoplasmic, sometimes with apparently intranuclear granularities. Plasmocytes were shown to contain gamma globulins, in cases of gamma myeloma and sometimes of beta-2A myeloma, or to contain beta-2A globulin, especially in cases of beta-2A myeloma, but never both. Cells can thus synthesize only one immunoglobulin.

6621

Choi, J.H.; More, R.H.; Wylie, J.C.; Haust, M.D. 1965. A study of an alum-albumin granuloma by the fluorescent antibody technique. Lab. Invest. 14:568.

The fate of albumin in an alum-induced granuloma was studied with FITC-conjugated anti-human serum albumin. The antiserum contained antibodies directed against transferrin, alpha-1 lipoprotein and alpha-1 glycoprotein in addition to albumin. It was possible to remove anti-transferrin and anti-alpha-1 lipoprotein by adsorption with human serum fractions. An alum-precipitated solution of HSA was injected subcutaneously and intramuscularly into rats. Tissues from injection sites were removed on days 1, 3, 5, 7, 10, and 14 and bisected. One-half of the tissues were rapidly frozen and FA stained. Appropriate controls were employed. The other half of the tissues were fixed in formalin, embedded in paraffin, and section-stained with hemalum-phloxine-safran. A granuloma formed at the site of injection. Initially albumin was noted in the extracellular tissue, but soon appeared within macrophages, reaching a maximum concentration within the cells on the 7th day. Thereafter the intracellular albumin slowly declined. Complete article.

6622

Cohen, S.; Solomon, A.; Paronetto, F.; Popper, H. 1963. Subacute hepatitis in Waldenstrom's macroglobulinemia. Amer. J. Med. 34:256-263.

A patient with primary macroglobulinemia, with characteristic organ manifestations, had subacute fatal hepatitis, apparently homologous serum jaundice. Macroglobulins were demonstrated by FA in plasma cells and in basophilic reticuloendothelial cells, the latter sometimes occurring as littoral cells of sinusoids of liver and spleen. Very few of these hepatic and splenic cells contained 7S gamma globulin. The macroglobulin distribution in the liver corresponded to the distribution of 7S globulins in similar cases of subacute hepatitis in the absence of macroglobulinemia. This was interpreted to indicate that the macroglobulin-producing cells in the liver were reactive rather than neoplastic, immunologically competent cells. Casts in the distal convoluted renal tubules reacted with antibody to 19S macroglobulins but were probably cross-reacting 2S globulins.

6623

Gajl-Peczalska, K. 1964. Plasma protein composition of hyaline membrane in the newborn as studied by immunofluorescence. Arch. Dis. Childhood 39:226-231.

The lungs of six newborns with well-defined hyaline membrane disease were studied by immunofluorescence. It was found that the hyaline membranes were composed of fibrin, gamma globulin, and albumin, which also focally impregnated the alveolar septa. Moreover, cell nuclei were found within the membranes by the fluorescent antibody procedure. The hyaline membrane may be formed in the plasma-clotting process resulting from capillary wall damage and increased capillary permeability.

6624

Gajl-Peczalska, K. 1964. The analysis of the hyaline membranes in newborn infants by means of immunofluorescence. Arch. Dis. Childhood 39:301.

The lungs of six newborn infants with well-defined hyaline membrane disease were studied by FA. The hyaline membranes were composed of fibrin, gamma globulin, and albumin, which also focally impregnated the alveolar septa. Cell nuclei were found within the membranes by means of fluorescent antibody procedure. It was concluded that the hyaline membrane might be formed in the plasma-clotting process resulting from capillary wall damage and increase of capillary permeability. Complete article.

6625

Gleich, G.J.; Condemi, J.J.; Vaughan, J.H. 1965. Dysgammaglobulinemia in the presence of plasma cells. New Engl. J. Med. 272:331-340.

An 8-year-old girl with a history of repeated cervical abscesses was found to have a marked elevation of the gamma-1M globulin concentration and apparent absence of the gamma-2 and gamma-1A globulins. The urinary microgammaglobulin, gamma-uL, was present. After immunization with a variety of antigens, gamma-1M antibodies were produced. Even on prolonged immunization over an 8-month interval, the antibody to typhoid H antigen remained gamma-1M. Normal numbers of mature plasma cells were seen in bone-marrow aspirates and in a lymph-node biopsy. By immunofluorescent staining, gamma-1M was demonstrated in the plasma cells of the bone marrow.

6626

Haust, M.D.; Wyllie, J.C. 1963. Demonstration of fibrin in the human white arteriosclerotic plaque by the fluorescent antibody technique. Amer. J. Pathol. 43:8a.

Previous studies using conventional stains indicated that fibrin contributed significantly to the early development of certain arteriosclerotic lesions. Fibrin was not consistently demonstrated in their later stages. FA was used to demonstrate fibrin in a moderately advanced arteriosclerotic lesion, the white plaque. Fifty white plaques were obtained from human aortas within 6 to 8 hours postmortem. Serial frozen sections were stained with the Fettrot stain for neutral fat and for fibrin by FA. Formalin fixed sections were stained with hemalum-phloxine-saffron and Mallory's phosphotungstic acid-hematoxylin (PTAH). Fibrin was demonstrated in 76 per cent of all plaques. Moderate amounts were found in plaques of young connective tissue containing areas of insudation. A few plaques had atheromatous 'cores' that were rich in fibrin. The remaining 24 per cent contained little or no fibrin. Plaques consisted of mature connective tissue. When present, fibrin was found in small deposits usually deeply situated in the plaque. By PTAH, fibrin was demonstrated in only 40 per cent of all plaques.

6627

Haust, M.D.; Wyllie, J.C.; More, R.H. 1964. Atherogenesis and plasma constituents: I. Demonstration of fibrin in the white plaque by the fluorescent antibody technique. Amer. J. Pathol. 44:255-267.

Fifty white atherosclerotic plaques in human aortas were examined for the presence of fibrin by the fluorescent antibody technique. Fibrin was demonstrated at all stages of development in this form of arteriosclerotic lesion. The largest quantities of fibrin were found in the young connective tissue plaques, but small amounts were detected even in mature lesions. The presence of fibrin indicates that plasma proteins are being deposited in or on the vessel wall during the process of arteriosclerosis. Fibrin serves as a convenient marker of such an event. Plasma proteins may be deposited in the arterial intima by incorporation of mural thrombi, infiltration of plasma, or capillary hemorrhages in the vicinity of developing plaques. Less well-defined are disturbances in the normal transport of plasma constituents across the arterial wall from the lumen to the vasa vasorum. Presumably any interference with this process could also result in the deposition of plasma proteins within the intima. The organization of these deposits contributes significantly to the formation of white arteriosclerotic plaques.

6628

Horowitz, R.E.; Stuyvesant, V.W. 1964. Fibrinogen in the amyloidosis of familial Mediterranean fever. Lab. Invest. 13:955.

Amyloid may represent precipitated or locally formed antigen-antibody complex. Gamma globulin has been identified in amyloid deposits by FA. However, ferritin-labeled antibody electron microscopic studies have shown that gamma globulin is not an intrinsic part of the amyloid fibril. Furthermore, many patients with amyloidosis have a concomitant or preceding hypergammaglobulinemia. Gamma globulin in amyloid deposits may represent passive trapping of a protein. To test this hypothesis, amyloid deposits in a patient with hyperfibrinogenemia were studied by FA. The kidneys showed severe glomerular amyloidosis. Frozen sections treated with fluorescent anti-human fibrinogen showed positive glomerular fluorescence, indicating the presence of fibrinogen in the glomerular amyloid. Such fluorescence could be diminished by pretreatment with antiserum but not by pretreatment with acid buffers. Staining with anti-human gamma globulin also resulted in fluorescence, but use of anti-rabbit or anti-guinea pig sera did not. Thus the amyloid deposits contain fibrinogen. Fibrinogen is not part of an antigen-antibody complex but probably represents nonspecific deposition of a plasma protein present in high concentration.

6629

Horowitz, R.E.; Stuyvesant, V.W.; Wigmore, W.; Tatter, D. 1965. Fibrinogen as a component of amyloid. Arch. Pathol. 79:238-244.

Immunofluorescent studies of the amyloid deposits in a patient with familial Mediterranean fever with hyperfibrinogenemia show that the amyloid contains a preponderance of fibrinogen and only small amounts of gamma globulin. Amyloid deposits in patients with hypergammaglobulinemia contain mainly gamma globulin but also small amounts of fibrinogen. It is suggested that amyloid represents merely a deposition of any plasma protein that is present in high concentration, and that neither gamma globulin nor antigen-antibody complexes are necessary components of amyloid.

6630

Kantor, F.S. 1965. Fibrinogen precipitation by streptococcal M protein: II. Renal lesions induced by intravenous injection of M protein into mice and rats. J. Exp. Med. 121:861-872.

Intravenous injection of Type 1 streptococcal M protein into mice and rats produced lesions confined to renal glomeruli. Thrombi of eosinophilic amorphous material, seen to occlude glomerular capillaries, were shown to contain M protein and fibrinogen. Gradual regression of the morphological lesions was observed during the 3 weeks following injection. Initial abnormal proteinuria and azotemia returned to control levels by the end of the 1st week; a second rise in urinary protein excretion and urea retention was demonstrated in some rats coincident with appearance of anti-M antibodies. The mechanism of renal localization of streptococcal M protein by means of a complex with fibrinogen was suggested, which may comprise an initial phase in the pathogenesis of acute poststreptococcal glomerulonephritis.

6631

Kao, V.C.Y.; Wissler, R.W. 1965. A study of the immunohistochemical localization of serum lipoproteins and other plasma proteins in human atherosclerotic lesions. Exp. Mol. Pathol. 4:465-479.

Multiple unfixed 4u sections from various areas of aortas from 28 humans ranging in age from premature infants to one 94-year-old subject were studied microscopically by using fluorescent-labeled rabbit antibodies to immunoelectrophoretically 'pure' human plasma fractions, including LD lipoproteins and HD lipoproteins, albumin, gamma globulin, and fibrinogen. Some tissues were immersed in fixatives prior to staining. The fixation methods tested were not suitable for this type of investigation. The severity of the atherosclerotic lesions sampled varied from no gross

evidence of disease, through fatty streak, fatty plaque with fibrous cap to complicated lesions. Adjacent cryostat sections from each aortic area were stained with Oil red O as well as Mallory picrofuchsin. Suitable control sections were also studied. Fluorescent anti-LD lipoproteins consistently stained diseased areas of aortic intima where lipid was revealed by Oil red O stain. The fluorescent anti-HD lipoprotein stained the diseased areas very faintly or not at all. The fluorescent anti-fibrinogen stained focal areas of intima that also contained lipoproteins. The fluorescent anti-albumin and anti-gamma globulin stained none of the lesions.

6632

Koffler, D.; Friedman, A.H. 1964. Localization of immunoglobulins in chronic thyroiditis. Lab. Invest. 13:239-245.

Gamma globulin was localized in colloid in six cases of chronic thyroiditis but rarely in epithelial cytoplasm. Binding in vivo of a component of complement was demonstrated in corresponding areas of colloid. Complement was fixed in vitro to colloid and in one case of epithelial cells. Both gamma globulin and complement could be eluted by acid buffer. Serum proteins associated with exudation were not found in colloid or epithelium. The localization of gamma globulin and complement in colloid may represent cytotoxic antigen-antibody complexes of pathogenetic significance in human thyroiditis.

6633

Kopf, A.W.; Morrill, S.D.; Silberberg, I. 1965. Broad spectrum of leukoderma acquisitum centrifugum. Arch. Dermatol. 92:14-35.

A series of diverse neuroectodermally derived tumors associated with halos of leukoderma is presented. Clinically these lesions have in common a centrally placed, usually pigmented tumor encircled by a zone of hypopigmentation. The histological findings include reduction or absence of epidermal melanin, but persistence of amelanotic melanocytes in the leukodermic halo; a variety of tumors including nevuscell nevus, neuroid nevus, blue nevus, neurofibroma, and malignant melanoma; variable numbers of small dark cells whose nature is unclear but that probably represent in part small nevus cells and in part lymphoid cells; and damage to some tumor cells that presumably could be the cause of their destruction. Also presented are histochemical demonstrations of tyrosinase activity and immunohistochemical studies for presence of gamma globulin in the tumors. Using the fluorescent antibody technique, it was not possible to show gamma globulins in sera from the patients directed against their tumors.

6634

Lange, K.; Wachstein, M.; Wasserman, E.; Alptekin, F.; Slobody, L.B. 1963. The congenital nephrotic syndrome: An immune reaction? Amer. J. Dis. Children 105:338-345.

The interpretation of the congenital nephrotic syndrome as a tubular malformation does not explain the presence of erythrocytes, granular casts, and a severe proteinuria in these newborns. In a newborn infant with a congenital nephrotic syndrome the presence of gamma globulin, complement components C1 and C3, and undivided complement could be shown on many but not all glomerular loops by immunofluorescent methods. This indicates a tissue-destroying, complement-binding antigen-antibody reaction. The finding of many albumin reabsorption droplets in the tubular epithelium refutes the explanation that the proteinuria is due to a lack of protein reabsorption by the malformed tubuli. The possible origin of the antibody against the fetus kidney in the mother and the possible reasons for its formation are discussed.

6635

Marmont, A.; Chiappino, G.; Damasio, E. 1963. Demonstration by immunofluorescence microscopy of macroglobulinopoietic cell elements in Waldenstrom's disease. Schweiz. Med. Wochensch. 93:1445-1448. In French.

The exact nature of the cells concerned in the production of beta-2 macroglobulin in Waldenstrom's disease is still under discussion. Despite the introduction of immunofluorescence microscopy, investigators are still divided as to whether the plasmocytoid or lymphoid series is involved. In a case of Waldenstrom's macroglobulinemia in which a macroglobulin with rheumatoid-like properties in very high titers could be demonstrated, anaplastic lymphoid cells were found in the bone marrow and spleen aspirates. By means of anti-beta-2 macroglobulin and anti-gamma globulin sera and globulin fractions, conjugated with FITC and rhodamine B isothiocyanate respectively, it was demonstrated that the beta macroglobulin was contained, and presumably synthesized, chiefly in the young lymphoid cells, and sometimes also in plasmocytoid cells.

6636

Mazzei, D.; Del Giacco, G.S.; Curletto, R. 1964. Demonstration by immunofluorescence of beta-2M globulin in lymphoid-type reticular bone marrow cells in a case of Waldenstrom's macroglobulinemia. Hematologica 49:573-577. In Italian.

A Waldenstrom macroglobulinemia bone marrow was studied by the immunofluorescent technique. It appears that beta-2M macroglobulin is produced by lymphoid reticular cells.

6637

McCormick, J.N. 1963. An immunofluorescence study of rheumatoid factor. Ann. Rheum. Dis. 22:1-10.

Fluorescent aggregated human gamma globulin and normal or immune rabbit globulins have been used to trace the distribution of rheumatoid factor in rheumatoid lymph nodes and synovium. Rheumatoid factor was detected by each class of reagent in plasma cells, in lymph nodes and synovium, and in the intrinsic cells of reactive follicles. The labeled rabbit globulin reagents also reacted with rheumatoid factor at various extracellular connective tissue sites. Mixed-staining experiments with contrastingly labeled aggregated human gamma globulin and the rabbit globulins showed that some plasma cells and intrinsic cells reacted with either the aggregate or the rabbit globulins and that others reacted with both. Various combinations of each fluorescence reaction were found in individual cells and germinal centers. Mixed-staining procedures with contrastingly labeled anti-7S and a specific anti-19S antisera indicated that 7S gamma globulin was associated with macroglobulin at similar sites. Postulations on the source and action of immune globulins are made.

6638

McFarlin, D.E.; Strober, W.; Wochner, R.D.; Waldmann, T.A. 1965. Immunoglobulin A production in ataxia telangiectasia. Science 150:1175-1177.

Serum from five patients with ataxia telangiectasia contained no detectable immunoglobulin A (IgA). However, there was evidence by FA of IgA in the bone marrow of the three patients so examined, suggesting that the defect in IgA production was not complete. IgA was in the saliva of all five patients and in the parotid gland of the one patient studied. This is further evidence of IgA synthesis by the salivary glands.

6639

Melin, H. 1964. An atrophic circumscribed skin lesion in the lower extremities of diabetics. Acta Med. Scand. Suppl. 176:1-75.

A survey is presented of the so-called long-term diabetic vascular changes and of skin lesions associated with diabetes. A previously unknown skin lesion in diabetes is decribed. It consists of small, rounded, brownish, atrophic and circumscribed lesions localized in the lower extremities. A fluorescence-microscopic examination of skin from diabetics with atrophic lesions with antihuman gamma globulin revealed fluorescence, partly in the capillary walls immediately under the epidermis and partly in the basal cell layer of the epidermis. The latter fluorescence was similar to that demonstrated by former investigators in skin from patients with discoid and systemic lupus erythematosus. As in systemic lupus erythematosus, this fluorescence was not only observed in the skin lesions, but also in apparently intact skin. The possibility of the atrophic skin lesions in diabetes being due to changed immunological conditions is discussed.

6640

Morris, R.H.; Vassalli, P.; Beller, F.K.; McCluskey, R.T. 1964. Immunofluorescent studies of renal biopsies in the diagnosis of toxemia of pregnancy. Obstet. Gynecol. 24:32-46.

A continuing immunofluorescent study of renal biopsies from patients with toxemia of pregnancy, with reference to its diagnostic value, is reported. Fifty biopsies were performed in 46 patients. In 18 there was clear-cut clinical evidence of toxemia, and in all of these, bright, diffuse staining with antifibrinogen serum was seen in glomeruli. In 11 patients with no evidence of toxemia, such glomerular staining was absent. Among a group of 17 patients, with some of the clinical features of toxemia, positive staining was found in seven. It is believed that these seven patients had toxemia. These findings are interpreted as being the result of slow intravascular clotting. Coagulation and fibrinolytic studies performed in the majority of these patients revealed no differences between normal pregnancy and toxemia, with the exception of a tendency toward lower platelet counts in toxemia.

6641

Nowoslawski, A. 1963. Immunohistochemical studies on the presence and distribution of plasma proteins in early atherosclerotic lesions in coronary arteries. Rozpr. Wydz. Nauk Med. 8:137-172. In Polish.

FA was used to demonstrate the presence of tissue-bound plasma proteins in early atherosclerotic lesions. Plasma fractions and their relation to fatty streaks were principally studied. Results indicated an insudation rather than an incrustation process for presence of plasma proteins in the lesions. The low concentration of gamma globulin mitigates against an immunological basis for the lesion.

6642

Nowoslawski, A.; Brzosko, W. 1965. Immunohistochemical analysis of amyloid. Patol. Pol. 16:205-214. In Polish.

Immunofluorescent analysis of human secondary amyloidosis has shown the presence of bound globulins, fibrin, and/or fibrinogen and albumin. The immunofluorescent complement fixation test and labeled rheumatoid factor staining gave positive results that may be considered circumstantial evidence of immune genesis of amyloid.

6643

Paronetto, F.; Schaffner, F.; Popper, H. 1964. Immunocytochemical and serologic observations in primary biliary cirrhosis. New Engl. J. Med. 271:123-128.

In the areas of chronic nonsuppurative cholangitis of primary biliary cirrhosis, basophilic hepatic mesenchymal cells contained, and probably formed, gamma-1M globulin (19S macroglobulin) as shown by immunofluorescence. These cells were much more numerous than those containing gamma-2 globulin (7S gamma globulin), which are the mesenchymal cells seen in other forms of chronic active liver disease. Rheumatoid factor was not demonstrated in the hepatic mesenchymal cells. The serum of patients with primary biliary cirrhosis frequently demonstrates elevated levels of gamma-1M globulin, rheumatoid factor, and substances binding to nuclear material sensitive to deoxyribonuclease or periodate treatment. Patients with primary biliary cirrhosis seem to have an alteration of the immune mechanism, suggesting a peculiar reactivity rather than a specific etiology in this disorder.

6644

Paronetto, F.; Koffler, D. 1965. Immunofluorescent localization of immunoglobulins, complement, and fibrinogen in human diseases: I. Systemic lupus erythematosus. J. Clin. Invest. 44:1657-1664.

FA studies on tissues of patients with systemic lupus erythematosus (SLE) revealed gamma-2 and gamma-1M immunoglobulins, complement, and fibrinogen localized in renal glomeruli and vessels of kidney, spleen, heart, and liver. Alpha-2 macroglobulin, albumin, and gamma-1A globulin were absent from glomeruli, but the latter was visualized in tubular epithelium. Gamma-1M and gamma-2 globulins were eluted by acid buffers. Nuclear localization of all immunoglobulins was seen in the renal tubular epithelium in only three of the 16 patients investigated. These gamma-2 and gamma-1M globulins may be antibody components of immune complexes localized in the vascular and glomerular lesions of SLE. Fibrinogen deposition may contribute to renal glomerular damage.

6645

Pernis, B.; Ballabio, C.B.; Chiappino, G. 1963. Presence of the rheumatoid factor in vascular lesions in malignant rheumatoid arthritis: Study with fluorescent antibodies. Reumatismo 15:187-199. In Italian.

Treatment of histological sections with fluorescent antibody against human gamma globulin demonstrates an elective distribution of gamma globulins along the arteriolar walls. This distribution is constant and uniform.

6646

Porter, D.D.; Dixon, F.J.; Larsen, A.E. 1965. Metabolism and function of gamma globulin in Aleutian disease of mink. J. Exp. Med. 121:889-900.

Aleutian disease - affected mink, which are markedly hypergammaglobulinemic, show a decreased half-life of the serum gamma globulin indicating that the hyperglobulinemia is due to increased production. No evidence was obtained that the gamma globulin was antibody to the infectious agent, to autologous or isologous tissues, or to antigens the animal was responding to prior to development of the disease. The increased gamma globulin was found to be of 6.4S, and gamma globulin containing protein-protein complexes of 9S to 17S and 22S to 25S classes were observed in sera of affected mink. The findings are most consistent with the Aleutian disease virus acting as a direct and somewhat selective stimulus to plasma cell proliferation. There is no evidence that the arterial and glomerular lesions of Aleutian disease have an immunologic pathogenesis. It seems possible that these vascular changes may be directly caused by the viral agent, or may be the result of the increased gamma globulin levels.

6647

Rawson, A.J.; Abelson, N.M.; Hollander, J.L. 1965. Studies on the pathogenesis of rheumatoid joint inflammation: II. Intracytoplasmic particulate complexes in rheumatoid synovial fluids. Ann. Intern. Med. 62:281-284.

An immunofluorescent technique for identification of particulate matter in the cytoplasm of synovial fluid leukocytes has been developed. This employs antisera to 7S gamma globulin and 19S macroglobulin. Joint fluids from 55 arthritic patients have been examined by this technique. Particles containing determinants of both 7S and 19S globulin were identified in cells of 75 per cent of 29 rheumatoid arthritic fluids, 88 per cent of fluids from rheumatoid variants, two of five gouty fluids, but in no fluids from septic arthritis, osteoarthritis, or pseudogout.

6648

Riddle, J.M.; Barnhart, M.I. 1965. The eosinophil as a source for profibrinolysin in acute inflammation. Blood 25:776-794.

The emigration sequence and cytology of acute inflammation produced by a variety of stimulants was studied in dogs by a modified skin window procedure. Fibrinogen, fibrin, or proteolytic enzymes that promote fibrin formation selectively attracted eosinophils into the inflammatory site. The fluorescent antibody technique was employed to assess the level of cellular profibrinolysin. Only inflammatory eosinophils and bone marrow eosinophils were marked with rhodamine antiprofibrinolysin. Staining was confined to intra- and extra-cellular eosinophil granules and coalesced masses. The intensity of fluorescence varied somewhat and perhaps reflected profibrinolysin release into the inflammatory exudate. Exudative eosinophils, like all members of the bone marrow eosinophilic series, contain profibrinolysin localized in the specific granules. Eosinophils that migrate and collect at an inflammatory site clearly transport profibrinolysin to an area of fibrin deposition. Granule release of profibrinolysin provides one mechanism for fibrinolysis that probably facilitates wound repair.

6649

Riddle, J.M.; Bluhm, G.B.; Barnhart, M.I. 1965. Fibrin: Its role in the development and perpetuation of rheumatoid synovitis. Arth. Rheum. 8:463.

Fibrin, exudative neutrophils, and rheumatoid factor comprise a triad frequently present in rheumatoid synovitis. Synovial fluids containing these three constituents exhibited numerous inclusion-bearing neutrophils (R.A. cells). Neutrophil granules interacted with the inclusion phagosomes to form lysophagosomes. By using immunofluorescence we found

that these lysophagosomes contain fibrin alone, fibrin combined with rheumatoid factor, and altered gamma globulin - rheumatoid factor complexes. Each of these three groups of particulate materials was segregated into separate lysophagosomes within a single neutrophil. Hollander previously identified complexes of gamma globulin - rheumatoid factor as one of the inclusions within the R.A. cell. Our data establish that all three substances participate in inclusion formation. Despite the presence of these diversified substances, the inclusions appeared identical when studied by light microscopy. Fibrin and fibrin combined with rheumatoid factor are phagocytized by neutrophils. Lysophagosome formation results with the subsequent release of lysosomal (neutrophil granule) enzymes. These enzymes convert extravascular fibrinogen to fibrin, which may persist as a single entity or interact with available rheumatoid factor. More neutrophil emigration ensues and the inflammation is sustained. Thus, fibrin initiates and recycles the acute inflammatory process of rheumatoid arthritis. Tissue damage follows as a consequence of fibrin phagocytosis, lysophagosomal formation, and enzyme release.

6650

Rukosuev, V.S. 1965. Immunomorphological identification of fibrin in amylcid masses. Arkh. Patol. 27:32-35. In Russian.

Distribution of fibrin in the spleen was studied in autopsy material by the fluorescent antibody method. Fibrin was revealed in the amyloid masses in secondary amyloidosis and in the pulp of control spleens. Such fibrin distribution is considered normal.

6651

Sakaguchi, H.; Dachs, S.; Grishman, E.; Paronetto, F.; Salmon, M.; Churg, J 1965. Hepatic glomerulosclerosis: An electron microscopic study of renal biopsies in liver diseases. Lab. Invest. 14:533-545.

Twenty-four renal biopsies of patients with various types of liver disease were examined by light and electron microscopy, and in five cases with fluorescence microscopy. Light microscopy showed mild changes; electron microscopy revealed definite changes. The changes were milder in acute liver disease than in chronic liver disease but were found in all patients and were similar regardless of the nature of liver disease. FA demonstrated gamma globulin distributed along the basement membrane. The term hepatic glomerulosclerosis in preference to cirrhotic glomerulosclerosis is suggested for the glomerular lesions in patients with liver disease.

6652

Santos-Buch, C.A.; Campbell, W.G., Jr. 1964. Immunofluorescent study of necrotizing arteritis induced in rabbits. Federation Proc. 23:814:236.

Necrotizing arteritis and hypertension were induced in rabbits by unilateral silk-and-turpentine perinephritis followed in 7 days by contralateral nephrectomy. Early arterial lesions were seen in each of the nine rabbits of Group I sacrificed from 5 to 8 days following nephrectomy; healing lesions were found in each of the five rabbits of Group II sacrificed 15 days after nephrectomy. Quick-frozen blocks of diaphragm and ileum were serially sectioned and studied by immunofluorescent technique, using fluorescein-conjugated guinea pig antibodies prepared against rabbit washed platelets, purified fibrin, and gamma globulin. In Group I, intense, immunologically specific localization of each of the conjugates was seen in the intima and inner portion of the media of the early arterial lesions and little or no appreciable uptake of guinea pig complement was demonstrated by the method of Klein and Burkholder. In Group II, healing lesions showed an intense concentration of anti-rabbit-fibrin and anti-rabbit-platelet conjugates. In contrast, these healing lesions demonstrated a less intense reaction with anti-rabbit gamma globulin and no uptake of guinea pig complement. Complete article.

6653

Scott, D.G.; Rowell, N.R. 1965. Preliminary investigations of arteritic lesions using fluorescent antibody techniques. Brit. J. Dermatol. 77:211-220.

Frozen sections prepared from 27 biopsy specimens obtained from 25 cases of reticulate, nodular, necrotizing, or erythematous lesions of the skin were stained with fluorochrome-labeled antihuman globulin conjugate and, later, with hematoxylin and eosin. A histological study of paraffin sections prepared from the original biopsy specimens was also made. Major histopathological changes were found in 17 biopsies and globulin was demonstrated in 14 of these. Globulin was found in only three of ten lesions showing minor histopathological change (perivascular cuffing with lymphocytes). The presence of globulin tended to reflect the distribution of the histological changes, not their severity. No consistent relationship between the clinical appearances and the presence or distribution of globulin was demonstrated. Globulin may be concerned either in the progression or regression of lesions rather than in their initiation, in which the perivascular lymphocytes may be involved.

6654

Spear, G.S.; Kihara, I. 1964. The glomerulus in cyanotic congenital heart disease: An immunofluorescent study. Bull. Johns Hopkins Hosp. 115:481-493.

In two cases of congenital heart disease, material was present in the glomerulus that reacted with fluorescein-labeled rabbit antihuman Fraction II antiserum. In one case the material was identified as gamma globulin. The material appeared to be in the mesangium. The exact fluorescent pattern recorded here does not appear to have been previously reported.

6655

Talal, N.; Bunim, J.J. 1964. The development of malignant lymphoma in the course of Sjogren's syndrome. Amer. J. Med. 36:529-540.

Of 58 patients with Sjogren's syndrome reticulum cell sarcomas developed in three, and lesions resembling Waldenstrom's macroglobulinemia in a fourth. These patients have certain clinical and laboratory features in common which distinguish them from patients with the usual benign cases of Sjogren's syndrome. The gamma globulin abnormalities have been investigated by immunoelectrophoretic analysis, ultracentrifugation, and fluorescent antibody techniques. The relationship between connective tissue diseases, gamma globulin abnormalities, thymomas, and malignant lymphomas is discussed. The hypothesis is presented that in Sjogren's syndrome the chronic state of immunologic hyperactivity and the proliferation of immunologically competent cells producing abnormal tissue antibodies predispose to the relatively frequent development of malignant lymphoma.

6656

Tomasi, T.B., Jr.; Tan, E.M.; Solomon, A.; Prendergast, R.A. 1965. Characteristics of an immune system common to certain external secretions. J. Exp. Med. 121:101-124.

The gamma-1A present in saliva and colostrum exists largely in the form of higher polymers, the major component of which has a sedimentation coefficient of 11S. The 11S gamma-1A in these fluids differs from the polymers found in normal and myeloma sera both immunologically and by the fact that their sedimentation coefficients are unaffected by disulfide bond reduction in the absence of urea. However, like other gamma globulins, the 11S gamma-1A molecules consist of multiple polypeptide chains linked by disulfide bonds. Local synthesis of gamma-1A in the salivary gland has been shown by fluorescent and autoradiographic studies, although the fraction of the total salivary gamma-1A that is derived from local production is uncertain. No evidence was obtained

of transport of intravenously administered radio-labeled 7S gamma-1A from serum to saliva. Immunological specificity has been demonstrated in the salivary and colostral gamma-1A. Antibody activity has been demonstrated in saliva and colostrum and has been shown to be of the gamma-1A type. In both of these fluids activity is associated primarily with gamma-1A polymers of 11S and 18S sizes. There appears to be an immunological system that is characteristic of certain external secretions. Its properties, including the local production of a distinctive type of antibody, separate it from the systemic system responsible for the production of circulating antibody.

6657

Vassalli, P.; McCluskey, R.T. 1964. The pathogenic role of fibrin deposition in immunologically induced glomerulonephritis. Ann. N.Y. Acad. Sci. 116:1052-1062.

Use was made of a sheep antirabbit kidney serum that consistently produced severe glomerulonephritis in rabbits, frequently leading to complete glomerular obliteration in 2 or 3 weeks. Histologic and immunofluorescent observations demonstrated that fibrin was deposited within glomerular tufts, in cells, in an area of focal necrosis, or in the form of wire loops. Fibrin was also seen in Bowman's space, often in association with crescents. In the course of 2 to 3 weeks, the fibrin generally disappeared and during this time progressive glomerular obliteration with accumulation of hyalin was often seen. To explore the role of fibrin further, groups of rabbits were treated with Coumadin, following the administration of the nephrotoxic sheep serum. The treatment resulted in marked diminution or complete suppression of the lesions. Fibrin deposition within glomeruli was prevented, endothelial proliferation was markedly diminished, and crescent formation was entirely eliminated. The glomerular obliteration observed in untreated rabbits was not seen in Coumadin-treated animals. The suppressive effect of Coumadin did not appear to result from interference with immune mechanisms.

6658

Vassalli, P.; McCluskey, R.T. 1964. The pathogenic role of the coagulation process in rabbit Masugi nephritis. Amer. J. Pathol. 45:653-677.

The pathogenic role of the coagulation mechanism in a form of Masugi nephritis produced in rabbits by sheep nephrotoxic serum was studied. Histologic observations showed a severe proliferative glomerulonephritis with frequent fibrin or fibrinoid deposits in glomeruli, crescent formation, and progressive diffuse glomerular sclerosis. Immunofluorescent studies with antifibrinogen serum showed positive staining within fibrin deposits and diffusely within proliferating intracapillary cells. When rabbits were given injections of rabbit antisheep gamma globulin antibodies shortly after the administration

of sheep nephrotoxic serum, massive intraglomerular fibrin deposition occurred rapidly. Treatment with warfarin resulted in prevention of fibrin and fibrinoid deposits, diminution or suppression of intracapillary cell swelling and proliferation, and prevention of crescent formation and glomerular sclerosis. FA revealed comparable amounts of rabbit gamma globulin along the glomerular basement membranes in treated and untreated rabbits. Results of this immune reaction are discussed.

6659

Vassalli, P.; Morris, R.H.; McCluskey, R.T. 1963. The pathogenic role of fibrin deposition in the glomerular lesions of toxemia of pregnancy. J. Exp. Med. 118:467-478.

In an immunofluorescent study of renal biopsies from patients with toxemia of pregnancy, the glomeruli consistently showed bright staining for fibrin within endothelial cells, as well as occasional deposits along the basement membrane. Gamma globulin was only occasionally demonstrable, generally in the form of irregular deposits along the basement membrane. Beta-1C was absent, and albumin was not seen in glomeruli except in the form of droplets within epithelial cells. In biopsies from pregnant patients without toxemia, only equivocal staining for fibrin was seen. The accumulation of fibrin in glomeruli reflects a prolonged state of intravascular clotting in toxemia and shows that the arrest in glomeruli of some form of circulating fibrin constitutes the basic pathogenic mechanism of the glomerular damage in this disease.

6660

Vigliani, E.C.; Pernis, B. 1963 Immunological aspects of silicosis. Bibl. Tuberc. 17:230-279

Silicosis appears to be a disease with an important immunological component. This is supported by the abundant deposition of gamma globulins in the silicotic nodules, that has been demonstrated with various methods including FA, by the concentration of plasma cells and their precursors at the sites of the silicotic reactions, and by many serological alterations observed both in man and in experimental animals. The main immunological abnormality in silicosis consists in a localized and prolonged stimulation of the immune system that appears to be hyperreactive toward a variety of antigens Quartz particles induce this stimulation as a consequence of the continued destruction of the macrophages that phagocytose them.

6661

Wyllie, J.C.; Haust, M.D. 1963. Demonstration of fibrin in early fatty lesions of human arteriosclerosis by fluorescent antibody method. Federation Proc. 22:545:251.

This study forms part of an investigation of the role of blood constituents in arteriosclerosis. An antiserum was prepared against Fraction I of human plasma and adsorbed with human serum to render it specific for fibrinogen. Characterization by the Ouchterlony technique showed a single precipitin arc with human plasma and none with human serum. The arc was identified as the fibrinogen-antifibrinogen complex by immunoelectrophoresis. The gamma globulin fraction, conjugated with fluorescein isothiocyanate, stained fibrin specifically in human blood clots and mural thrombi. Fifty aortic fatty lesions were bisected, one half was frozen and sections of it stained with the conjugate. Serial sections were stained with Fettrot stain for neutral fat. For comparison, the other half was fixed in formalin and processed conventionally for HPS and PTAH stains. Fibrin was readily identified in most of the fatty lesions as scattered linear deposits associated with splitting and fragmentation of elastica and small lipid accumulations. By comparison, the PTAH stain failed to demonstrate fibrin in these lesions. Complete article.

6662

Wyllie, J.C.; More, R.H.; Haust, M.D. 1964. Demonstration of fibrin in yellow aortic streaks by the fluorescent antibody technique. J. Pathol. Bacteriol. 88.335-338.

Fifty fatty streaks and dots were obtained from human aortae and stained for fibrin with a fluorescein-conjugated antibody to human fibrinogen. Fibrin was identified in significant quantities in all of the lesions examined. It was also invariably associated with small isolated lipid deposits in the intima. Fibrin thus appears to be an important constituent of the early fatty lesions. Its presence indicates that plasma proteins as well as lipids are involved in the progression of atherosclerosis.

IV. BASIC IMMUNOLOGY

A. ANTIBODY IN SITU

6663

Balfour, B.M.; Cooper, E.H.; Alpen, E.L. 1965. Morphological and kinetic studies on antibody-producing cells in rat lymph nodes. Immunology 8:230-244.

The secondary response of regional lymph nodes to immunization with diphtheria toxoid adsorbed to aluminum phosphate has been studied with a combined immunofluorescent and autoradiographic technique. No difference was detected in the kinetic pattern of the specific antibody-containing cells compared with the general population of those containing gamma globulin. The morphology of the replicating antibody-containing cells and their progeny is described. A population of large basophilic, DNA-synthesizing cells that did not contain gamma globulin was found in the nodes. Rates of cell division were examined in rats immunized with human serum albumin. The blast cells were found to have a rapid turnover and their progeny, the plasma cells, a short life span.

6664

Cohen, E.P. 1964. Kinetics of antibody-forming cell proliferation after antigen. Federation Proc. 23:1462:344.

There is increasing evidence that precursors of antibody-forming cells divide after exposure to antigen. This study relates to the time of onset and duration of precursor cell division after antigen injection. The double label technique as described by Baney et al, was used: the first part is the identification of antibody-forming cells by specific fluorescein stain, the second is relocalization of the fluorescent cell after autoradiography. Primarily, immunized mice were injected intravenously with BBG adsorbed onto bentonite. At varying times thereafter, their spleens in suspension were incubated in vitro in a culture medium containing tritium-labeled thymidine. After incubation, the washed cells were injected intravenously into isologous X-irradiated recipients. After a suitable period, the recipient spleens were examined for antibody-forming cells. The results indicate that precursors of antibody-forming cells begin to divide approximately 3 hours after antigen injection, and that cell division is complete between 24 and 48 hours. Suggestive evidence that antibody-forming cells divide asymmetrically is found in the observation that antibody-forming cells in cluster are unequally labeled. Complete article.

6665

Cohen, E.P.; Talmage, D.W. 1965. Onset and duration of DNA synthesis in antibody-forming cells after antigen. J. Exp. Med. 121:125-132.

Within 5 hours after the intravenous injection of particulate antigen into the primarily immunized mouse, precursors of antibody-forming cells began DNA synthesis, as shown by the incorporation of tritium-labeled thymidine. DNA synthesis continued for at least 24 hours and essentially stopped by 48 hours. No DNA synthesis in antibody-forming precursor cells occurred before the injection of antigen.

6666

Cohen, M.J.; Cohen, E.P. 1964. Proliferation of transferred spleen cells after antigenic challenge. Nature 203:418-419.

A combined FA-autoradiography method was used. Cells containing antibody were determined by FA and mitosis by autoradiography. Mitosis of donor antibody-producing cells occurs after injection of antigen into the recipient as well as the intact animal.

6667

Curtain, C.C.; Baumgarten, A. 1965. Immunocytochemical localization of a 19S gamma-1 macroglobulin cold haemagglutinin. Australian J. Exp. Biol. Med. Sci. 43:157-162.

A gamma-1 macroglobulin with cold hemagglutinating activity of the anti-I type was localized by FA in the bone marrow plasma cells and their precursors in a patient suffering from lymphoma. No localization occurred in the 'naked' lymphocytes whose presence in the marrow gave rise to a diagnosis of lymphoma. The plasma cells containing the macroglobulin appeared to be morphologically identical to those containing normal 7S gamma-2 globulin, and in no case was the presence of both proteins observed in the same cell.

6668

Dixon, F.J.; McConahey, P.J. 1963. Enhancement of antibody formation by whole-body X-radiation. J. Exp. Med. 117:833-848.

Enhancement and acceleration of the antibody response can be achieved in some situations with large, cytotoxic doses of whole-body X-radiation given after antigenic stimulation. The timing of radiation is critical and varies with the kind and physical form of the antigen. The mechanism is different from enhancement by endotoxin or colchicine. FA was used to demonstrate anti-bovine gamma globulin in plasma cells of the spleens and lymph nodes of antigen and X-ray-treated animals.

6669

Evans, E.E.; Bryant, R.E.; Kent, S.P.; Moyer, M. 1965. Immunological memory in amphibians. Federation Proc. 24:2081:504.

In a comparative study of antibody responses of lower vertebrates, immunological memory was examined in amphibians, Bufo marinus. Primary, secondary, and tertiary responses were induced with bovine serum albumin (BSA), salmonella H (STH), and T2 coliphage. Antigens were injected into the dorsal lymph spaces of animals kept at 25 and 35 C. Antibodies to BSA were measured by precipitation and immunofluorescent staining. Secondary and or tertiary responses to BSA differed from primary in more rapid disappearance of antigen, shorter latent (induction) period, and higher level of antibody. Secondary STH generally did not produce agglutinin exceeding the maximum primary titer. In some animals, secondary T2 produced levels of neutralizing antibody exceeding the maximum primary response, but in others there was no secondary rise. In studying responses to BSA, antibody-forming cells were observed in tissue sections of spleen, liver, kidney, and lymph node stained by an immunofluorescent method; reduction in the latent period was noted in anamnestic groups. These amphibians, thus, can exhibit immunological memory, but the nature of the response varies with the antigen and with the individual. Complete article.

6670

Eveland, W.C. 1964. Use of a fluorescein-labeled sonically disrupted bacterial antigen to demonstrate antibody-producing cells. J. Bacteriol. 88:1476-1481.

Cells obtained by primary tissue culture of the spleens of chickens immunized with sonically disrupted Escherichia coli 0111 organisms were stained with a fluorescein-labeled homologous antigen by use of direct immunofluorescent methods. Brilliant staining of the cytoplasm in cells from immunized birds appeared to be diffuse in certain cells and rather globular in others. In contrast, cells from nonimmunized birds showed no staining at all. The cells involved in the specific reaction appeared to be those of the lymphocyte-monocyte-plasma cell types, as shown when stained by the May-Grunwald-Giemsa method Preparations stained by the methyl green-pyronin technique revealed an increase in the pyroninophilic cells in the preparations from the immunized birds, thus demonstrating increased amounts of ribonucleic acid in these cells, which in turn is consistent with the presence of antibody globulin. Specificity of the reaction was confirmed also by staining antibody-coated E. coli 0111 organisms with the conjugate, precipitin reaction with specific antibody, and specific agglutination with circulating antibody from the immunized birds.

6671

Fischer, K.; Dorszewski, E. 1965. A contribution to the immunofluorescence technique for demonstration of cellular fixed antibodies. Monatsschr. Kinderheilk. 113:175-176. In German.

We were able to observe that in a few patients, after long steroid medication, the antihuman globulin - fluorescent test became negative using human leukocytes, whereas the fluorescent test up to now has always remained positive with chicken erythrocytes. On the other hand, with the use of human leukocytes we frequently saw nonspecific reactions.

6672

Han, S.S.; Johnson, A.G.; Han, I.H. 1965. The antibody response in the rat: 1. A histometric study of the spleen following a single injection of bovine gamma globulin with and without endotoxin. J. Infect. Dis. 115:149-158.

Changes in the area of the lymphocytic and reticular zones of rat spleens were measured as a function of time following injection of bovine gamma globulin and this antigen plus endotoxin. Antibody titers were considerably enhanced in the latter rats. A biphasic response was observed in the areas of both zones in both groups of rats. In rats receiving only antigen, an initial abrupt decrease in both zones occurred within a period of 24 hours. This was followed by an increase to normal levels of the lymphocytic zone, which was maintained. The area of the reticular zone increased to near-normal levels, subsequently decreased again, and returned to normalcy. Parallel changes occurred in rats stimulated with endotoxin plus bovine gamma globulin, but the return to normal or near-normal levels of both zones occurred on the 6th to 7th day after antigen This was 5 to 6 days earlier than in rats receiving only the antigen. A possible role the changing pattern of splenic white pulp could play in antibody formation was discussed. Antibody-containing cells were located by indirect FA.

6673

Hiramoto, R.; Bernecky, J.; Jurand, J.; Hamlin, M. 1964. The use of paired fluorescent labeled antibodies. J. Histochem. Cytochem. 12:14-15.

Tetramethylrhodamine, lissamine rhodamine, 1-dimethylaminonaphthalene-5-sulfonyl chloride (DSC), and fluorescein were used to determine their suitability for paired labeled fluorescent antibody studies. The possibilities of quenching fluorescent labels with change of pH was also investigated. Horse anti-rabbit globulin was labeled with the various dyes and served as the reagent to detect rabbit antibodies. The fluorescent reagents contained 5.6, 7.9, and 7.0 residue per mole protein respectively.

Sensitized rat kidney served as the tissue for study. This was prepared by injecting rats intravenously with rabbit anti-rat kidney globulin. Two labels used simultaneously on a single kidney section showed yellow fluorescence when both the red (rhodamine) and the green (fluorescein) reacted at the same antigenic sites. Quenching the fluorescence for fluorescein could be accomplished by dropping the pH of the medium to 3. Partial reactivation of fluorescence occured when the medium was made alkaline. Fluorescein fluoresced well from pH 11 to 7. Appreciable quenching occurred at pH 5 and 4. From pH 4 complete reactivation occurred when the medium was made alkaline. Tetramethylrhodamine fluoresced well from pH 11 to 4; some loss of fluorescence was seen at pH 3. Lissamine rhodamine fluoresced best at pH 7 and 6. DSC was a poor label. At pH 2 all showed irreversible quenching. Complete article.

6674

Hiramoto, R.N.; Hamlin, M. 1964. Detection of two antibodies in single plasma cells by the paired fluorescence techniques. Federation Proc. 23:1456:343.

Cohn Fraction II human gamma globulin was column-purified and employed to immunize guinea pigs. The globulin fraction as well as papain digests were assayed against individual guinea pig hyperimmune serum by immunoelectrophoresis and Ouchterlony plates. Animals that showed one precipitin band against the whole gamma globulin fraction but two antigenically distinct bands against the digests were selected for study to determine whether these two distinct antibodies to the gamma globulin Fragments, I and II, were being synthesized within single cells. Rabbit antihuman gamma globulin sera were labeled with either aminotetramethylrhodamine or fluorescein isothiocyanate. Plasma cells containing two antibodies were detected by the indirect staining method. Frozen sections of spleens were treated with isolated Fragment I, then stained with aminotetramethylrhodamine conjugate capable of reacting with this fragment. The same section was treated with Fragment II and stained with fluorescein conjugate specific for this fragment. Observations showed orange, green, and yellow fluorescence, indicating that some plasma cells can synthesize one and others may synthesize two distinct types of antibodies. Gradation of staining indicated greater content of one type of antibody over the other. Complete article.

6675

Hiramoto, R.N.; Hamlin, M. 1965. Detection of two antibodies in single plasma cells by the paired fluorescence technique. J. Immunol. 95:214-224.

The concept that a single antibody-forming cell may have the capacity to synthesize more than one antibody simultaneously was tested. This problem was approached by using a single antigen on which two determinant groups could be detected. Guinea pigs were hyperimmunized with a column-purified human gamma globulin preparation. Animals showing antibodies that reacted with a single band to the whole antigen but with two bands to the papain-digested antigen by immunoelectrophoresis were sacrificed and the spleen sections studied to see if any single cell was responding with two antibodies to both fragments of the whole antigen. A multiple antibody response in individual cells was detected by the sensitive indirect paired fluorescence technique. From a total of 482 antibody-containing cells counted, 30 per cent had antibodies directed toward the CP-I fragment and 24 per cent to the CP-II fragment. It appeared that about 45 per cent of the cells contained antibodies directed to both the CP-I and CP-II fragments. The gradation of colors caused by the combination of tetramethylrhodamine and fluorescein conjugates in the multiple antibody-producing cells and the selective quenching studies of fluorescence at pH 4.0 indicate that different cells contained different amounts of one type of antibody over the other.

6676

Hiramoto, R.; Hamlin, M. 1965. Multiple antibodies in single cells. Federation Proc. 24:1399-381.

The capacity of a single immunocyte to synthesize more than one antibody simultaneously has been a subject of prime interest. An approach to this problem was made by using a single antigen, DEAE-purified human gamma globulin. Sera from guinea pigs made hyperimmune with this antigen were assayed immunoelectrophoretically. Animals showing antibodies that react with production of a single band to the whole antigen but with two bands to the digested antigen were tested at the cellular level by the indirect paired fluorescence method to see if any single cell is responding with double antibodies to both fragments of the globulin antigen. Indeed, a large proportion of cells in response to the stimulus of a single antigen could produce two types of antibodies. From a total of 482 antibody-containing cells counted, 30 per cent had antibodies directed toward the F fragment and 24 per cent to the S fragment. It appeared that about 45 per cent of the cells contained antibodies directed to both the F and S fragments. The gradation of colors caused by the combination of tetramethylrhodamine and fluorescein conjugates in the multiple-antibody-producing cells and the selective quenching studies of fluorescence at pH 4.0 indicate that different cells contained different amounts of one type of antibody over the other. Complete article.

6677

Hirschhorn, K.; Kolodny, R.; Hashem, N. Bach, F. 1963. Mechanism of in vitro mitosis and immune response of human peripheral blood lymphocytes. Arth. Rheum. 6:276.

We have shown that when the red cell agglutination activity of phytohemagglutinin is removed by adsorption, the supernatant retains mitogenic and leukoagglutinating activity. With the use of radioactive amino acids incorporated into the medium, we have demonstrated that by 4 hours the stimulated cells produced gamma globulin. When fluorescein-labeled anti-gamma globulin is used, all the lymphocytes bind the stain and therefore produce gamma globulin. When specific antigens are substituted for phytohemagglutinin, lymphocytes from sensitized individuals show mitotic activity, but those from nonsensitized individuals do not. Conjugate is bound by only a minority of the cells. Morphologic studies of the lymphocytes demonstrate that in the presence of phytohemagglutinin all the small lymphocytes become large within 24 to 48 hours and that some of these cells resemble plasma cells. In the presence of specific antigens only a minority, 5 to 35 per cent, undergo such morphologic transformation. Phytohemagglutinin is possibly a nonspecific stimulus for gamma globulin production and mitosis. Specific antigens stimulate only a portion of cells.

6678

Hulka, J.F.; Brinton, V.; Schaaf, J.; Baney, C. 1963. Appearance of antibodies to trophoblast during the postpartum period in normal human pregnancies. Nature 198:501-502.

Our original hypothesis that antibody does not appear prior to delivery in normal pregnancies appears to be substantiated. However, our original theoretical explanation, that trophoblasts absorb circulating antibody prior to delivery, does not appear to be entirely satisfactory. If this were true, antibody would appear promptly in the immediate postpartum period. The fact that antibody does not appear until several days after delivery in normal pregnancies suggests that there are other mechanisms at work in the pregnant woman to suppress the appearance of this antibody. One possible mechanism is the suppressive effect of high levels of circulating adrenocorticosteroids in pregnancy, which drop dramatically postpartum Direct FA was used

6679

Martins, A.B.; Moore, W.D.; Dickinson, J.B.; Raffel, S. 1964. Cellular activities in hypersensitive reactions: III. Specifically reactive cells in delayed hypersensitivity: Tuberculin hypersensitivity. J. Immunol. 93:953-959.

Small lymphocytes from lymph nodes of guinea pigs with tuberculin hypersensitivity react specifically with tuberculoproteins, as revealed by fluoresceinated guinea pig antituberculoprotein serum. Fluorescence occurs as a rimming or halo effect in 5 to 20 per cent of such cells. Occasional glowing cells are seen in normal cell preparations at a lower level of intensity of fluorescence. Although the methods employed suggest that antibody globulin adsorbed to cells, or produced by them, is not implicated in the reactive property, this possibility cannot be excluded.

6680

Maurer, P.H.; Pinchuck, P.; Gerulat, B.F. 1965. Antigenicity of polypeptides, poly alpha amino acids: XIV Studies on immunological tolerance with structurally related synthetic polymers. Proc. Soc. Exp. Biol. Med. 118:1113-1118.

Synthetic polymers were used to immunize rabbits and demonstrate degrees of acquired immunological tolerance Direct FA located polymer antigen in spleen imprints Indirect FA demonstrated antibody-containing cells in spleens

6681

Medunitsyn, N.V. 1965 The effect produced by an adjuvant on the process of antibody formation, or immunofluorescent examination Zh. Mikrobiol Epidemiol. i Immunobiol 42.7.113-118. In Russian

Human gamma globulin and a mixture of an antigen with complete or incomplete Freund adjuvant were injected into the posterior paws of guinea pigs. By indirect fluorescent antibody it was shown that when the antigen was administered without the adjuvant, the antibody-forming cells appeared only in the nearby lymph nodes. In reimmunization such cells were revealed in the remote lymph nodes and the spleen The periods of appearance of antibody-forming cells and of the circulating antibodies were reduced following the combined antigen-adjuvant administration Their quantity increased as well. The most intensive antibody-forming process followed the injection of a complete adjuvant; however, participation of the regional lymph nodes in this process could be less than in other immunization conditions

6682

Miller, J.J., III. 1964. An autoradiographic study of plasma cell and lymphocyte survival in rat popliteal lymph nodes. J. Immunol. 92:673-681.

Using tritiated thymidine as a cell label, the rate of disappearance of plasma cells from stimulated and unstimulated popliteal lymph nodes was followed for 6 months in rats. In stimulated lymph nodes there was a rapid early decrease in plasma cell numbers, but a small percentage of labeled plasma cells persisted through the 6-month time point. In the unstimulated nodes, fewer plasma cells were initially labeled, but some of these also persisted throughout the span of the experiment. These cells could not have been formed from any other cell type during the course of the experiment, with the exception of direct transformation of small lymphocytes, because precursor and intermediate types of cells were not sufficiently highly labeled after the first 3 weeks. Some long-lived plasma cells contain, and probably produce, antibody for at least 3 months, the longest time point tested. Throughout the course of the experiment, only two labeled plasma cells were found outside the popliteal nodes, both in the submucosa of the ileum. FA was used as an adjunct to radioactive labeling. After findings by tritiated thymidine were no longer technically optimal, FA could be used to identify antibody-containing cells.

6683

Mitchell, M.S.; Calabresi, P. 1964. Studies on the role of the lymphocyte in the immune response. Yale J. Biol. Med. 36:421-429.

The secondary immune response in lymph nodes not directly primed by a previous localized injection of antigen was investigated. The response in rabbits was studied by various tests including FA. Response involves a morphological change occurring in the medulla of the node, with germinal center activity particularly evident after the 4th day. Conversion of the histological picture from one consisting predominantly of forms resembling 'blasts' to one characterized by mature plasma cells was paralleled by the production of hemagglutination antibody in the serum. An attempt was made to rule out leakage of antigen using methyl green-pyronine and FA. These studies support a hypothesis that the small lymphocyte may act as an immunological messenger, transmitting to remote nodes information that originated in a node sensitized by direct contact with an antigen.

6684

Moore, R.D.; Mumaw, V.R.; Schoenberg, M.D. 1965. Changes in antibody producing cells in the spleen during the primary response. Exp. Mol. Pathol. 4:370-390.

In rabbits given complete Freund's adjuvant or complete adjuvant and diphtheria toxoid there were two types of cells in the speen that contained antibody and gamma globulin. Non-phagocytic mononuclear cells containing 19S gamma-1M antibody and gamma globulin were associated with the appearance and level of circulating 19S gamma-1M globulin antibody. Gamma globulin and 7S gamma-2 antibody in the cytoplasm of the plasma cells were associated with the appearance and level of circulating 7S gamma-2 globulin antibody. Changes in the basophilia and ultrastructure of the cytoplasm of these cells were consistent with increased protein synthesis. The mononuclear cell was present in the red pulp, and the development of the plasma cell occurred primarily in the non-follicular white pulp.

6685

Mori, S.; Moggi, P.; Pratesi, V. 1963. Some immunological problems of infants: III. Different antibody response observed by means of fluorescent technique in sensitized lymphocytes of newborn infants and children. Riv. Clin Pediat. 72:209-213. In Italian.

The authors studied the response to fluorescent diphtheria toxoid of lymphocytes taken from newborn infants and 2- to 4-year-old children immunized against diphtheria and then stimulated 72 hours before blood was taken. Only 30 per cent of lymphocytes of newborn infants but almost all the lymphocytes of older children became fluorescent. Probable reasons for this lesser content of specific antibodies in the lymphocytes of newborn infants are discussed.

6686

Ofumi, T.; Arimori, S. 1964. Role of the thymus and its lymphoreticular cells on antibody formation. Acta Hematol. Jap. 27:176-188.

The thymus is immunologically the most important tissue concerned in producing not only cellular antibodies but also serum antibodies, including autoimmune antibodies. Immunologically competent cells in the thymus are lymphoreticular cells that are the original cells of antibody-containing cells seen in other lymphatic tissues, and they are transformed in various forms in other tissues depending on sensitizing antigens. Specifically, cellular antibody competent cells against bovine serum albumin are lymphoreticular cells, but those against tubercle bacilli are lymphoreticular cells and lymphocytes. Serum antibody competent cells are plasma cells and lymphoreticular cells; autoimmune antibodies are also produced mainly by plasma cells. FA was one method used in the study.

6687

Pernis, B.; Cohen, M.W.; Thorbecke, G.J. 1963. Specificity of reaction to antigenic stimulation in lymph nodes of immature rabbits: I. Morphologic changes and gamma globulin production following stimulation with diphtheria toxoid and silica. J. Immunol. 91:541-552.

Experiments were conducted to test whether antigenic substances rather than nonspecific stimuli are necessary for induction of gamma globulin formation and associated morphologic changes in lymphoid tissue. Diphtheria toxoid was used as the antigen, crystalline silica as the nonantigenic adjuvant. Neonatal rabbits were used as the experimental animals because of their lack of pre-existing stimulation. Stimulated lymph nodes were studied with regard to secondary nodule and plasma cell formation and, in addition, to gamma globulin production in vitro by means of autoradiography of immunoelectrophoretic patterns. Immunofluorescence studies were performed to determine presence of antibody and gamma globulin in lymph node cells. Results indicate that antigenic stimulation is needed for the induction of the specific histologic changes associated with gamma globulin formation. Gamma globulin synthesis was more readily demonstrable than antibody formation in immature rabbits. Several possible reasons for this phenomenon are discussed.

6688

Rubenstein, H.S.; Harter, J.G. 1965. Cells containing antibodies to endotoxin during maximal serum 19S antibody response. Federation Proc. 24:1815:456.

By means of immunofluorescence we have studied rabbit cells containing antibodies to endotoxin at the time when the rabbit's serum 19S antibodies to endotoxin were maximal and 7S antibodies were barely detectable. Rabbits were given six intravenous injections of boiled S. typhosa 0901 (St), McFarland standard No. 5 doses increasing from 0.25 to 1.5 ml over a period of 11 days, followed by bleeding and killing on days 12, 14, and 19. Spleens were fixed in cold ethanol and embedded in paraffin. Five-micron sections were stained for anti-endotoxin by means of three layers: St endotoxin; chicken anti-St, boiled; and rabbit anti-chicken gamma globulin conjugated with fluorescein isothiocyanate. Stained sections were examined under the fluorescence microscope. Most cells containing antibody lined the sinuses of the red pulp or the marginal zone but some appeared free in the sinuses and others were in the white pulp. These cells, with little cytoplasm and large round or elliptical nuclei, resembled hemocytoblasts and immature plasma cells; occasional ones appeared to be disintegrating. Some bizarre cells with abundant cytoplasm and eccentric rectangular or triangular nuclei were encountered, as well as a few cells with abundant cytoplasm and eccentric round nuclei resembling typical mature plasma cells. Complete article.

6689

Sainte-Marie, G.; Coons, A.H. 1964. Studies on antibody production: X. Mode of formation of plasmocytes in cell transfer experiments. J. Exp. Med. 119:743-760.

Cells from lymph nodes of rabbits injected repeatedly with bovine serum albumin were transferred subcutaneously to previously irradiated rabbits, and the recipients were immediately injected with bovine serum albumin. A good antibody response resulted. In a series of such animals killed on successive days, skin samples at sites of cell deposition were removed and examined by immunofluorescence and by light microscopy. In these tissues abundant plasmocytes were found to have multiplied and differentiated in a regular progression from immature, to medium, to mature plasmocytes. During the 6 days of the experiment the small plasmocytes accumulated until they reached 85 per cent of the total plasmocytic population. The mitotic index of the large and medium plasmocytes averaged 11 per cent, implying a generation time of 6.3 hours on the basis of a 1-hour mitotic time. This rate of growth is sufficiently rapid to account for all the plasmocytes on the 6th day as deriving from less than 1 per cent of the population initially transferred. This rate, and the orderly progression in the evolution of the plasmocytic population, make it highly improbable that plasmocytes arise from transformation of lymphocytes, but rather indicate that they spring from specific precursors already present among the transferred cells.

6690

Scheiffarth, F.; Warnatz, H.; Rohde, C. 1963. A fluorescence serologic study of the presence of cell-bound antibodies in transplantation immunity. Med. Exp. 9:115-120. In German.

The authors attempted to demonstrate the presence of cell-bound antibodies in transplantation immunity by means of fluorescence studies.

6691

Schoenberg, M.D.; Stavitsky, A.B.; Moore, R.D.; Freeman, M.J. 1965. Cellular sites of synthesis of rabbit immunoglobulins during primary response to diphtheria toxoid - Freund's adjuvant. J. Exp. Med. 121:577-590.

The present studies are based on previous observations that the intravenous injection of diphtheria toxoid and complete Freund's adjuvant into rabbits resulted in an increased proliferation of cells associated with antibody synthesis; an accelerated, enhanced, and prolonged synthesis of antibody; and a lengthened interval between the appearance of gamma-M and gamma-G hemagglutinating antibodies in the circulation. The molecular species of antibodes that were synthesized by fragments of the spleens were determined

by the incorporation of labeled amino acid into antibody and by binding of radioactive antigen by antibody. These studies were paralleled by determination of the presence and type of antibody within the cell by immunofluorescence. Evidence was obtained that non-phagocytic mononuclear cells in the walls of the sinusoids of the red pulp of the spleen are a major source of 19S gamma-M antibody and that plasma cells in the non-follicular white pulp are a major source of gamma-G antibody. It was hypothesized that the 19S and 7S antibody responses evolved independently with the development of at least two different cell types, a mononuclear cell with capacity for 19S immunoglobulin synthesis and a plasma cell with capacity for 7S immunoglobulin synthesis.

6692

Sercarz, E.E.; Coons, A.H. 1963. The absence of antibody-producing cells during unresponsiveness to BSA in the mouse. J. Immunol. 90:478-491.

Experimentally induced immunologic unresponsiveness to bovine serum albumin was studied in newborn and adult mice. FA was used to examine tissue sections for antigen and for antibody, using a layering method. No antibody-containing cells were found in newborn mice injected daily from birth. Mice injected from the 5th week of life were rendered unresponsive by large, but not small, antigen doses. Circulating antigen, but not antibody or complexes, was demonstrated. Paralysis was specific. Transfer of unresponsive spleen and lymph node cells to a normal mouse did not result in antibody formation upon stimulation by antigen. Responsiveness recovery resulted several weeks following the disappearance of circulating antigen. Immunologically paralyzed animals do not produce antibody. Their cells are specifically inhibited. An immunologically paralyzed cell can become a stimulated cell when antigen concentration falls below an effective inhibitory level.

6693

Stevens, J.A.; Beutner, E.H. 1963. Preliminary report: Immunohistologic studies of rabbit antibodies to rabbit and sheep anterior pituitary preparations. J. Amer. Osteopath. Ass. 62:839.

Rabbits that received multiple injections of crude pituitary globulins (2 M ammonium sulfate precipitate from rabbit anterior pituitary extract) showed antibody titers by indirect immunofluorescent (IIF) staining. Those injected with pituitary extract or suspension did not show immune response by this method. Sera tested with pituitaries of the antibody-producing rabbits also yielded positive results, and some sera fixed complement with pituitary antigen. Similarly, IIF methods showed rabbit and sheep immune response to sheep pituitary globulins in pituitary and in connective and vascular tissues, but not in other organs.

IIF staining of rabbit pituitaries with rabbit antisera to sheep pituitaries showed that both acidophilic and some non-acidophilic cells were stained. BA-44-7201.

6694

Trautwein, G. 1964. The importance of plasma cells as antibody components. Pathol. Vet. 1:423-453. In German.

Humoral antibodies are formed by plasma cells. Plasma cells are immobile connective tissue cells that are formed in the medullary cords of lymph nodes, the red pulp of the spleen, the bone marrow, and in the adventitia of small blood vessels. The theory of the plasmacellular antibody formation is based on clinical and experimental observations that include detection of antibodies in plasma cells with FA. Macrophages and lymphocytes also play a role in antibody formation. Although it is the function of the macrophages to transform corpuscular antigen into soluble immunogenic antigen, the lymphocytes play the role of a co-factor. In infants the thymus lymphocytes transmit substances that are necessary for the development and function of the antibody producing system. The complicated problems of globulin synthesis in the antibody producing cell are explained in the light of the genetic theory of antibody formation advanced by Ehrich.

6695

Urso, P.; Gengozian, N. 1964. Immunofluorescent detection of proliferating human antibody-forming cells. Nature 203:1391-1392.

Proliferative capacity of antibody-containing cells was compared with that of normal cells in the same population. Cells were exposed in vitro and in vivo to tritiated thymidine and subsequently were examined for antibody by FA. Competent human antibody-containing cells proliferate at a greater frequency than the incompetent cells during the early phases of antibody synthesis. The rate of proliferation is reduced to normal levels during the later phases of the antibody response. Mature antibody-containing cells are derived from precursor cells through somatic division. The diffusion-chamber technique in conjunction with FA analysis is feasible for studying cellular dynamics of human antibody-forming tissue after stimulation with an antigen.

6696

Urso, P.; Makinodan, T. 1963. The roles of cellular division and maturation in the formation of precipitating antibody. J. Immunol. 90:897-907.

The role of somatic division in secondary anti-BSA responses by isolated rabbit cells was investigated with tritiated thymidine and the FA techniques and with colchicine, a mitotic inhibitor. The cells were cultured in 0.1-micron porosity diffusion chambers implanted in X-irradiated rabbits and mice. The results showed that not only were antibody-containing cells dividing but that they were dividing at a rate significantly higher than that of the incompetent cells during the log phase of activity. The mean generation time was 12 hours during the log phase and 24 hours in the stationary phase. A direct correspondence was found between the rise in percentage of antibody-containing cells and rise in antibody titer of the culture chamber fluid. We also observed that all the competent cells of the stationary phase were derived from precursor cells through somatic division. Cytomorphologic examinations of antibody-containing cells at intervals after antigenic stimulation suggest a gradual transition in the number and distribution of sites of antibody synthesis.

6697

Wellensiek, H.-J.; Coons, A.H. 1964. Studies on antibody production: IX. The cellular localization of antigen molecules (ferritin) in the secondary response. J. Exp. Med. 119:685-696.

The physical presence of the antigen used to stimulate a secondary antibody response was demonstrated in the cells of popliteal lymph nodes. Rabbits, previously injected with apoferritin (containing no iron) that was prepared from recrystallized horse ferritin, were injected with ferritin 5 weeks later. The antigen was traced by the Prussian blue reaction, by specific antibody, and by electron microscopy. Antiferritin antibody was localized by immunofluorescence, although it was not possible to test cells simultaneously for antigen and antibody Horse ferritin induces a rather weak primary antibody response but a brisk secondary response characterized by the appearance in the medullary cords of numerous plasma cells containing the antiferritin Many intact ferritin molecules were found in the nucleus and cytoplasm of numerous reticular and other phagocytic cells in the sinuses. In decreasing amount, ferritin molecules were also clearly demonstrated in hemacytoblasts and in immature and mature plasmocytes.

6698

Zlotnick, A. 1963. Antibody production by immunologically competent cells transferred to a heterologous host. Lab. Invest. 12:306-315.

Lymph node cells from rabbits sensitized with bovine serum albumin or bovine serum gamma globulin, when restimulated in vitro with homologous antigens and transferred to the cheek pouch of X-irradiated hamsters, produce specific antibody in the recipients. The antibody produced in the hamsters is identical with rabbit gamma globulin. Specific changes in the morphology of the transferred cells during the process of antibody production in the heterologous host are described. Mitotic indices determined daily after injection of colchicine showed a low mitotic activity on the first 2 days after transfer and increased activity on the 3rd and 4th days after transfer. Because of the low mitotic activity on the first 2 days, it is suggested that some of the large cells arise from a transformation of small and medium-sized lymphocytes. The appearance of the large cells is probably due partly to the stimulation with bovine serum albumin or bovine serum gamma globulin, and partly to the stimulation by the host antigens.

B. ANTIGEN-ANTIBODY COMPLEXES

6699

Chiappino, G.; Corbetta, L. 1963. The synthesis of gamma globulins in human palatine tonsils: Immunohistochemical investigations on the nature of the crypt contents. Ann. Laringol. 62:213-221. In Italian.

Immunologic characteristics of gamma globulin precipitates evidenced by immunofluorescence in tonsillar crypts were determined. The precipitates showed fixation of complement and solubilization in an acid environment. They are therefore interpreted as antigen-antibody complexes. The meaning of these antigen-antibody complexes in the activity of the lymphatic-tonsillar tissue is discussed.

6700

Cochrane, C.G. 1963 Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels: I. Structural studies. J. Exp Med 118:489-502.

A short-term model in which circulating antigen-antibody complexes and host complement localized in vessel walls of guinea pigs was analyzed. Localization was accomplished by subjecting the animals to anaphylactic shock The circulating macromolecules, such as antigen-antibody complexes, appeared to localize by being trapped in the vessel wall along the basement membrane that acted as a filter during a state of increased permeability of the vessel This point of localization between the endothelial cell and the basement membrane may represent the earliest focus of inflammation in diseases caused by the deposition of injurious macromolecules such as soluble antigen-antibody complexes from the blood stream. Complexes localized in the vessel walls did not provoke Arthus-type vasculonecrotic reactions even though in both these vessels, and in cutaneous Arthus reactions, antibody, antigen, and host complement were deposited in the vessel walls. In the Arthus vascular reaction, host complement was found microscopically accumulated with the immune reactants in affected vessel walls

6701

Cochrane, C.G 1963 Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels: II Pathogenetic and pharmacodynamic studies J. Exp Med. 118:503-513.

Localization of circulating antigen-antibody complexes in vessels of guinea pigs by means of anaphylactic shock was found to be mediated by histamine that was released at the time of anaphylaxis The source of the histamine may have been the mast cell, as noted in studies employing

a direct attack on the mast cells by octylamine. Platelets played little to no role in guinea pigs in the anaphylactic deposition of circulating complexes. Rat anaphylatoxin caused vascular localization and symptoms of anaphylaxis identical with that brought about by antigen-antibody anaphylaxis. This also was dependent upon release of histamine. Antibody against Forssman antigen in the vessel walls of the guinea pigs also led to deposition of circulating complexes. This was found not to be histamine-dependent. The possible role of local increase in vascular permeability in certain experimental disease states in the localization of circulating complexes is discussed.

6702

Cochrane, C.G. 1963. Factors influencing the localization of circulating antigen-antibody complexes in guinea pigs. Federation Proc. 22:2380:559.

Circulating soluble complexes of bovine serum albumin, BSA, and anti-BSA have been found to localize in small vessels of guinea pigs under the influence of anaphylaxis as shown by fluorescent antibody techniques. In addition, ultrastructural observations showed that circulating macromolecules were trapped along the basement membrane of the vessels. Also, since complexes were strongly concentrated in the vessel wall, it appeared that during a state of increased permeability, the vessels trapped the macromolecules by filtration. Fixation of host complement by the complexes was demonstrated histologically, but studies with decomplemented guinea pigs showed that complement was unnecessary for localization. Studies of the size of circulating molecules capable of lodging in vessel walls revealed that although simple proteins such as gamma globulins were too small to become entrapped, when aggregated by antigen or heat they would deposit in vascular membranes and not pass outside the vessel. This was true even of complexes dissolved in extreme antigen excess and injected into decomplemented guinea pigs, showing that even the smallest antigen-antibody complexes were large enough to become entrapped. This also underlined the fact that complement is unnecessary in the localization. Complete article.

6703

Krakower, C A.; Greenspon, S. 1963. Skin reactions with heterologous anti-tissue sera and the probable sites of the principal antigens. Amer. J. Pathol. 43.55-71

Among the many antigens in skin, those in capillary and venular basement membranes and probably those in the nonfibrillar matrix of its mesenchymal tissues are of significance in accounting for the more intense biologic reactions with heterologous anti-tissue sera. It is apparently only at these two structural sites that the combination of antigen and the appropriate antibody leads to intense and distinctive inflammatory changes. Antibodies that bind with antigens in capillary and venular basement membranes

and antibodies, which on intravenous injection produce a glomerulonephritis, are present in antivascular tissue sera. Such combination leads to a monophasic reaction. The combination of antibodies in antiavascular mesenchymal tissue sera with antigens probably in the mesenchymal matrix of skin leads to a biphasic reaction. Where both antibodies are present in the same antiserum, the effects of those directed against vascular basement membranes obscure the effects of those directed against mesenchymal matrix. The reactions are described. FA demonstrated the sites of antigen-antibody reactions.

6704

Lee, L. 1963. Antigen-antibody reaction in the pathogenesis of bilateral renal cortical necrosis J. Exp. Med. 117:365-376.

In the presence of reticuloendothelial blockade, the intravenous injection of a protein antigen into specifically immunized rabbits or the infusion of soluble immune complexes into normal animals resulted in the production of bilateral renal cortical necrosis. The similarity in the pathogenesis of this lesion and that seen in the classical generalized Shwartzman reaction produced by bacterial endotoxins is indicated by the failure of both lesions to develop in animals pretreated with large doses of heparin, by the finding of heparin-precipitable fibrinogen in the circulation, and by the presence of massive fibrin deposits within the glomerular capillaries. Antigen-antibody reactions in vivo are capable of activating the blood coagulation system, and the mode of action of bacterial endotoxins may have an immunological basis

6705

Linscott, W.D.; Cochrane, C G. 1964. Guinea pig beta-1C globulin: Its relationship to the third component of complement and its alteration following interaction with immune complexes. J. Immunol. 93:972-984.

The beta-1C globulin of guinea pigs was studied. It corresponded to C-prime-3c component of complement, antigenically and functionally. FA was used to demonstrate the presence of this component in Arthus reactions and in antigen-antibody deposits in vessel walls. The actions of various chemicals and natural substances on this complement component are reported

6706

Luporini, G.; Del Giacco, G.S.; Novi, C. 1964. Further research on experimental renal disease produced by antigen-antibody complexes. Boll. Ist. Sieroterap. Milan 43:253-265. In Italian.

Attention was directed by means of immunofluorescence techniques to localization of inoculated complexes and the possible antibody reactions of the host organism in their regard or in regard to the damaged parenchyma. Histological glomerular alterations were pointed out, resulting in an amorphous intracapsular protein exudation; no evident specific lesions at the heart and lung level were seen. The complexes inoculated were demonstrated at the glomerular level In the heart, complexes were located in the perifibrillar seat, but never in the lung parenchyma. Deposition of autologous globulins with an expected antibody activity was seen only in the glomeruli but never in the heart or lung parenchyma.

6707

Paronetto, F. 1964 Immunological observations in experimental liver injury: Factors influencing the localization of antigen-antibody complexes in the liver. Federation Proc 23:1399:234.

To elucidate the role of immunological processes in hepatic injury, the immune response and the significance of mild hepatocellular alterations on the deposition of immune complexes was investigated in mice biweekly injected with CC-14 or allyl alcohol, and weekly with horse serum or bovine serum albumin for 28, 40, and 100 days. They exhibited enhancement of specific antibody production, enlargement of spleen and lymph nodes with numerous antibody-forming cells, high mortality due to anaphylaxis, frequent extensive hepatocellular necrosis with marked inflammatory reaction, and occasional localization in the damaged area of antigen and antibody, presumably in complexes Controls, only immunized or only intoxicated, had no or minimal hepatocellular alteration. Therefore, animals with liver injury develop a quantitative alteration of the immunological reactivity, and a mild hepatocellular alteration favors the deposition or formation of immune complexes in the liver Complete article

6708

Paronetto, F , Popper, H. 1965. Aggravation of hepatic lesions in mice by in vivo localization of immune complexes (Auer hepatitis). Amer. J Pathol 47.549-563

After repeated administration of antigen and carbon tetrachloride for 28, 42, and 63 days, 34 per cent of the mice developed severe hepatic damage characterized by extensive hepatocellular necrosis with neutrophil and

mononuclear cell accumulation and by phlebitis in the tributaries of hepatic veins. In some mice, the lesions, interpreted as an Auer reaction in the liver, contained antigen, antibody, and mouse gamma globulin. They also fixed complement. Levels of circulating antibody did not correlate with the severity of hepatic lesions. Mice injected with either antigen or with carbon tetrachloride alone did not develop diffuse hepatocellular necrosis. Deposition of immune complexes in areas of hepatocellular alteration and increased permeability may enhance the hepatocellular damage. Antigen-antibody complexes injected intraperitoneally into mice with pre-existing hepatocellular damage produced by carbon tetrachloride were visualized only in the Kupffer cells in some mice. In others they were localized in areas of hepatocellular necrosis. Local failure of Kupffer cell phagocytosis associated with increased hepatocellular permeability favors the hepatic deposition of cytotoxic immune complexes that magnify pre-existing minimal hepatic alterations.

6709

Popper, H.; Paronetto, F.; Schaffner, F. 1963. Immunologic hepatic injury. Bull. N.Y. Acad. Med. 39:610-612.

As a portion of this study, FA was used to follow the fate of antigen-antibody complexes injected into bile ducts of rats. Severe injury was seen when the complex was used but not with either portion separately. A hypothesis for immunologic hepatic injury is suggested.

6710

Schafer, H.; Henriquez, M. 1965. Immunopathologic studies of Harder's gland (accessory lacrimal gland) of the rat. Arch. Pathol. Anat. Physiol. 338:3:277-284. In German.

An extract of the intraorbital Harder's gland of the rat was used as antigen to produce antibodies in the rabbit. These antibodies were measured serologically by complement fixation, agar precipitation, and immunoelectrophoresis, then isolated by cellulose ion-exchange chromatography as the gamma globulin fraction. The antibodies were then injected intravenously into healthy rats. After 21 days these rats showed no changes in their Harder's glands. Using FA it could be shown that the immune globulins had reached the glands. On the other hand, an injection 24 hours later of an extract from the Harder's gland that contained the immune globulins or an injection merely of foreign protein called forth pathologic changes in the intraorbital tear glands, in the form of regressive parenchymal damage with nonsuppurative inflammation.

6711

Takahashi, M.; Yoshida, T.O.; Ishii, E.; Kawashima, H.; Mishioka, K. 1965. Fluorescent complement technique: Labelling of guinea pig C'1 with fluorescein isothiocyanate. Jap. J. Exp. Med. 35:51P-52P.

Guinea pig euglobulin and the highly purified first component of guinea pig complement (C'1) prepared by DEAE cellulose column chromatography were labeled with FITC dissolved in carbonate buffer at pH 9.5. The purpose of studies was to know the presence of antigen-antibody complexes in vitro and in vivo using FITC-C'1. As the test antigen-antibody complexes, sheep erythrocyte-rabbit anti-sheep erythrocyte antibody, tanned sheep erythrocyte coated with bovine serum albumin (BSA)-rabbit anti-BSA antibody (BSA - anti-BSA), MM-2 cell (isologous transplantable ascites carcinoma cell in C3H/He mouse)-rabbit anti-MM-2 cell antibody (MM-2-Hetero anti-MM-2), and MM-2 cell-C3H/He mouse anti-MM-2 cell antibody (MM-2-Iso anti-MM-2) were selected for the determination of its specificity and sensitivity. The BSA - anti-BSA and MM-2-Hetero anti-MM-2 were remarkably stained by the FITC-C'1, but two others did not show that the C'1 fixation depended upon a number of C'1 fixing sites on the surface of cell.

6712

Ward, P.A.; Cochrane, C.G. 1965. Bound complement and immunologic injury of blood vessels. J. Exp. Med. 121:215-234.

Rats and guinea pigs were depleted of complement (C) by treatment with heat, aggregated human gamma globulin (agg HGG), symosan, anti-beta-1C globulin, and carrageenin. Although antigen and antibody were bound to vascular structures, Arthus reactions were inhibited. This inhibition was characterized by the lack of C binding to walls of vessels, polymorphonuclear (PMN) cellular infiltrates, and significant vascular damage. When the same animals were observed for several hours, levels of serum C began to rise, C was bound in tissues, PMN infiltrates appeared, and immunologic vasculitis developed. The specificity of C depletion in terms of effects in the first four reacting components of guinea pig C was studied. Various antibodies with different C-fixing capacities in vitro were tested for their ability to induce immunologic vasculitis in normal animals. In rats, only those antibodies that fixed C in vitro possessed biological activity, but in guinea pigs all antibodies tested, regardless of C fixation in vitro, induced Arthus reactions. For a given antibody in rats the vasculitis-inducing property was reflected in its ability to bind C in vascular structures. Rats depleted of circulating PMN by specific antibody were tested for Arthus activity. Although concentrations of immune reactants and C were readily detected in vascular structures, no PMN infiltration occurred and significant vascular damage was averted.

6713

Zinneman, H.H.; Seal, U.S.; Hall, W.H. 1964. Some molecular characteristics of blocking antibodies in human brucellosis. J. Immunol. 93:993-1000.

The localization and vascular effects of rheumatoid factor and of antigen-antibody complexes were studied in the exposed, living rat mesentery following injection of such products, labeled with FITC and unlabeled. Antigen-antibody complexes and rheumatoid factor localize at the intimal surfaces of the exposed mesentery, but localization is blocked by normal but not rheumatoid euglobulin. Rheumatoid factor and antigen-antibody complexes may play a role in development of vascular abnormalities seen in some cases of rheumatoid arthritis.

C. FOREIGN ANTIGEN FATE

6714

Bauer, H.; Horowitz, R.E.; Levenson, S.M.; Popper, H. 1963. The response of the lymphatic tissue to the microbial flora: Studies on germfree mice. Amer. J. Pathol. 42:471-483.

Lymph nodes and spleens from 45 germfree and 52 conventional Swiss-Webster mice of two strains were examined by histologic, histochemical, and immunocytochemical techniques. The number, distribution, histologic, and histochemical characteristics of macrophages in lymph nodes and spleens in germfree and conventional animals did not differ. In lymph nodes the macrophages were found to be related only to the area of drainage, being more prominent in nodes related to the oropharynx and intestine than in those related to the extremities. Reaction centers and immunologically competent cells, however, whether identified as Marschalko plasma cells by conventional microscopy or as gamma globulin-containing cells by immunocytochemistry, were rare in lymph nodes and spleens from germfree animals and abundant in comparable tissues from conventional mice. The presence of a microbial flora affected the immunologic but not the phagocytic function of lymph nodes and spleens.

6715

Bauer, H.; Horowitz, R.E.; Paronetto, F.; Einheber, A.; Abrams, G.D.; Popper, H. 1964. Influence of the microbial flora upon response of serum gamma globulin and lymphatic tissue to irradiation: Studies in germ-free mice. Lab. Invest. 13:381-388.

Germfree and conventional mice received 550 r whole-body irradiation. Histologic, autoradiographic, and immunocytochemical study of lymph nodes and spleens revealed a rapid destruction of lymphocytes, a gradual accumulation of hemosiderin and lipofuscin in macrophages, and an immune response that was manifested by the appearance of newly formed immunoblasts and gamma globulin-containing plasma cells, occurring both in germfree and conventional lymphatic tissue. Since the postirradiation immune response of lymphatic tissue develops both in germfree and conventional animals, it cannot be explained by living microorganisms. Factors such as antigenic or adjuvant effects of tissue breakdown products or a nonspecific stimulation of gamma globulin forming cells by irradiation have to be considered. A significant rise in serum gamma globulin occurred in the germfree and a decline in the conventional mice. Thus, the bacterial flora of the conventional mice influenced the loss of gamma globulin that has been previously attributed to the effect of radiation alone.

6716

Blackwell, J.B. 1965. The effects on the liver of sensitised rats of intravenous injection of antigen. J. Pathol. Bacteriol. 90:259-268.

Liver lesions produced by intravenous injection of human serum albumin into previously sensitized rats have been studied. Animals were killed at intervals from 5 minutes to 48 hours after injection. In the sinusoids clumping of leukocytes was prominent after 30 minutes and appeared to mark sites of accumulation of antigen-antibody complexes. Changes in the liver parenchymal cells were observed as early as 5 minutes after injection. In the initial stages clear vacuoles and eosinophilic globules were found within the cytoplasm of the hepatic cells. The eosinophilic globules stained with acid phosphatase and esterase, and were regarded as lysosomes. By 4 hours areas of midzonal necrosis were discernible. These developed in the majority of livers.

6717

Ciuca, M.; Ciplea, A.G.; Bona. C.: Pozsgi, N. 1965. Degradation of malarial antigen by immunogenesis: Functions of humoral and cellular factors in acquired immunity. Pathol. Microbiol. 28:668-682. In French.

The factors of specific and nonspecific cellular and humoral defense are shown to be permanently linked to the mechanisms of degradation of malarial antigen. In a first phase, the phagocytic activity of the macrophages of the RES is predominant, but in a second phase, in individuals becoming immune the destruction of the parasites is assured by the synergistic intervention of antibodies synthesized by cells of the lymphocyte-plasmocyte type and by macrophages, which remain intensely active in their phagocytic and metabolic activity.

6718

Cuppage, F.E. 1965. Renal changes in the rat following intravenous injection of complete Freund's adjuvant. Lab. Invest. 14:514-528.

Prolonged ultrastructural and functional changes have been produced in the rat kidney following a single intravenous injection of complete Freund's adjuvant. The nature, pathogenesis, and possible significance of these alterations are discussed.

6719

Eisen, A.H.; Cohen, J.J.; Rose, B. 1963. Reaction to tetanus toxoid: Report of a case with immunologic studies. New Engl. J. Med. 269:1408-1411.

A local reaction to tetanus toxoid with delayed onset is reported. Immunologic studies revealed the presence of an immediate wheal and flare and typical delayed hypersensitivity to intradermally administered tetanus toxoid. Attempts to transfer immediate skin reactivity and delayed hypersensitivity passively were unsuccessful with the use of serum and white-cell extracts respectively. A skin biopsy from the site of delayed hypersensitivity showed mononuclear infiltration. FA studies did not indicate the presence of residual antigen or specific antibody-producing cells in this site. Possible pathogenetic mechanisms are discussed. The reaction may be the result of vascular damage produced by the interaction of antigen and antibody at the site of injection.

6720

Horowitz, R.E.; Bauer, H.; Paronetto, F.; Abrams, G.D.; Watkins, K.C.; Popper, H. 1964. The response of the lymphatic tissue to bacterial antigen: Studies in germfree mice. Amer. J. Pathol. 44:747-761.

Lymph nodes from 76 germfree and 76 conventional mice were examined by histologic, histochemical, autoradiographic, and immunocytochemical techniques, at intervals of 2 hours to 14 days after foot pad injection with killed E. coli organisms. All nodes draining the injection site showed transient initial acute lymphadenitis and persistent weight gain. Particulate antigen appeared in sinus macrophages 2 hours after injection in both germfree and conventional nodes but disintegrated more rapidly in the latter. Immunoblasts proliferated first in the intermediate zone of the cortex and later at the corticomedullary junction. This was followed by the appearance of plasma cells, and gamma globulin-containing cells at the corticomedullary junction and in the medullary cords in both germfree and conventional lymph nodes. Circulating antibody developed in all mice after 4 days. The lymphatic tissue of the germfree animal is capable of responding to antigenic stimulation. Previous experience with a microbial flora confers only minor advantages upon conventional animals.

6721

Kabanova, E.A.; Fryazinova, I.B. 1964. Distribution of various types of antigens in the lymphatic system following their subcutaneous injection. Vestn. Akad. Med. Nauk SSSR 19:17-23. In Russian.

Luminescent serological, immunobiological, and serological methods were employed to study the distribution and the duration of native and deposited antigens in lymphatic nodes. The evidence shows that both the native, deposited lipopolysaccharide (LPA) obtained from Gartner's bacteria and the heated vaccines from the same bacteria were equally distributed all over the lymphatic system of rats, although the periods of the antigen stay in the nodes proved different. Native LPA stayed for 2 to 7 months, depending upon the dose; the deposited LPA remained in place for 8 months. The time periods for the diphtheria toxoids appeared to be less protracted, being 48 hours for the native one and 20 days for the deposited. The fact that separate administration of native LPA and of butyro-aqueous emulsion stimulates antibody formation allows us to assume that the effect of the butyro-aqueous emulsion finds its expression chiefly in the nonspecific stimulation of the lymphoid system, and that the building up of depots, as such, with antigens being retained for a protracted period of time, plays but a secondary part in the phenomenon.

6722

Levine, S. 1963. Cerebral white matter: Selective spread of pneumococcal polysaccharides. Science 139:605-606.

Pneumococcal polysaccharides were implanted in rat brain and their distribution was studied by FA. The polysaccharides spread selectively in white matter, frequently extending from anterior to posterior poles. Selective localization of experimental and natural leukoencephalopathies may be related to an innate property of white matter that permits or facilitates spread of noxious agents.

6723

Levine, S.; Zimmerman, H.; Wenk, E.; Gonatas, N. 1963. Experimental leukoencephalopathies due to implantation of foreign substances. Amer. J. Pathol. 42:97-117.

Implants of various materials were [illegible] under the frontal cortex, in the anterior end of the callosal radiation, of the rat brain. Certain inorganic and organic chemicals produced local necrosis and widespread edema of white matter associated with increased permeability of the blood-brain barrier. The occurrence of three different histologic

patterns of injury with the same localization in white matter is explained on the basis of a specific mechanism in white matter that facilitates spread of various substances. Recently, we have applied fluorescent antibody methods to the study of brains implanted with pneumococcal polysaccharides. Specific fluorescence, representing polysaccharide, was selectively distributed in white matter and was co-extensive with the vacuolar lesion described here. These observations provide direct support for the hypothesis that the lesion was due to selective spread of exogenous polysaccharide in white matter.

6724

Miller, J.J., III; Nossal, G.J.V. 1964. Antigens in immunity: VI. The phagocytic reticulum of lymph node follicles. J. Exp. Med. 120:1075-1086.

The localization of antigen in primary follicles and germinal centers of rat popliteal lymph nodes using radioactively labeled antigen was described previously and has been confirmed by direct staining with fluorescent antibodies. A fine web of phagocytic reticulum in primary follicles was found to be responsible for antigen localization in this area. The nature of this web was confirmed by studies of the localization of colloidal carbon. This unique feature of primary follicles is discussed in relation to its importance in the induction of immune responses, our belief being that the great surface area of antigen-retaining cytoplasm in primary follicles is responsible for the appearance of germinal centers in these particular parts of the node.

6725

Moggi, P.; Pratesi, V.; Mori, S. 1963. Some immunological problems of infants: II. The demonstration of specific antibodies in sensitized lymphocytes by means of fluorescent toxoid. Riv. Clin. Pediat. 72:203-208. In Italian.

It was shown by means of the fluorescent antibody technique that lymphocytes taken from subjects first immunized against diphtheria and then stimulated 72 hours before testing contained specific antibodies. The antibody specificity was confirmed by a control test with lymphocytes that had been pretreated with nonconjugated diphtheria toxoid or treated with fluorescent antihuman globulin serum.

6726

Montemagno, U.; Stefano, M.D. 1964. Localization of the A and B blood group substances in human placenta by means of the immunofluorescent technique. Arch. Ostet. Ginecol. 69:319-324. In Italian.

Through the immunofluorescent technique, the authors have studied the localization of A and B group antigens in human placentas. These substances, which are present in the amnion cells, are absent in the trophoblast, which is that portion of the placenta in direct contact with the maternal circulation. Therefore, it would appear that the maternal gravidic compatibility in case of fetal disaffinity of the antigens of the ABO system is to be imputed rather to the absence of such substances in the trophoblast than to a state of immunological inertia of the mother, or of antigenic incompetence of the fetal tissues.

6727

Sainte-Marie, G. 1963. Antigen penetration into the thymus. J. Immunol. 91:840-845.

Bovine serum albumin was injected intramediastinally in rats. It was found that part of the injected antigen reached the mediastinal lymph nodes as well as the adjacent thymus, where it penetrated the medulla and some areas of cortex. In both thymic regions, the antigen was seen on and/or in the cytoplasm of lymphocytes. It is concluded that there is no barrier to prevent the penetration of antigens into the thymic parenchyma, and that antigens that normally drain into the mediastinal nodes also penetrate the thymic parenchyma. This suggests that the failure of the thymus to yield antibody-forming cells does not result from its nonpenetration by antigens.

6728

Zacks, S.I.; Sheff, M.F. 1965 Studies on tetanus toxin: III. Intercellular localization of fluorescent-labeled tetanus toxin and antitoxin in mice. Acta Neuropathol. 4:267-277.

In a series of in vivo and in vitro experiments employing crude and purified tetanus toxin labeled with fluorescent dyes, the following observations were made. Tetanus toxin is bound to muscle and brain, but not to liver, cardiac muscle, spleen, kidney, or lung. Since these tissues will not bind serum albumin or tetanus antitoxin or rhodamine to the same degree, even when these are present at 20 times the concentration of the toxin, this binding indicates tissue selectivity towards tetanus toxin. Mitochondria isolated from brain were stained by RB 200-labeled tetanus toxin, but liver mitochondria were not stained. Treatment of the test animals with either antitoxin or toxin before

sacrifice reduced the apparent amount of toxin bound to muscle in vitro but had little effect on the ability of brain to bind the toxin. After toxin was bound to both muscle and brain in vitro, valences were still available for the binding of antitoxin. Since the amount of antitoxin bound to muscle or brain was greatly increased by prior incubation of the sections with toxin it follows that this is an example of specific immunological binding.

6729

Zubzhitskii, I.N. 1964. Distribution and duration of sojourn of foreign proteins in tissues of nonimmune animals as studied by fluorescence of the antibodies. Dokl. Akad. Nauk SSSR 155:234-237. In Russian.

Direct FA was used to trace foreign antigens inoculated intravenously into mice. The disposition of the various substances is recorded, as is the time of appearance in the mouse tissues. Only antigens of mol wt less than 180,000 are able to penetrate into the cytoplasm and nucleus of hepatic cells and the pulmonary and renal epithelial cells. Heavier molecules penetrate cells of the reticuloendothelium.

D. HYPERSENSITIVITY

6730

Allansmith, M.; Buell, D. 1965. Immunoglobulins in the skin of allergic and nonallergic individuals. J. Immunol. 95:951-958.

Three immune gamma globulins were found in skin specimens from seven allergic and nine nonallergic subjects. The globulins were located in the papillary dermis, and gradually decreased in concentration into the reticular dermis. They were not usually present in the epidermis. No difference in amount of distribution was detected among the groups of subjects.

6731

Cochrane, C.G.; Levenson, H. 1963. Comparison of nonprecipitating and precipitating antibody in provoking Arthus necrotizing vasculitis. Amer. J. Pathol. 43:3a.

Although both precipitating and nonprecipitating antibody are commonly produced following immunization of man and animals, only precipitating antibody has been shown to support the production of necrotizing vascular reactions of the Arthus type. Experiments were designed to determine why nonprecipitating antibody does not sensitize animals for Arthus reactions. Two types of nonprecipitating antibody were studied: rat anti-BSA obtained from active immunization, and rabbit anti-BSA obtained following removal of precipitating antibody by serial absorption. Using fluorescent antibody techniques, the precipitating antibody was found capable of causing deposition of antigen in both the vessel walls and in the heterophils that were crowded in the vessel walls. Gamma globulin and host complement were also found to be concentrated in the vessel walls. Nonprecipitating antibody failed to provoke such deposition both in vessel walls and in the few surrounding leukocytes. Nonprecipitating antibody would not sustain necrotizing vascular reactions because it failed to accumulate immune reactants, complement and heterophils in vessel walls.

6732

Einbinder, J.M.; Walzer, R.A.; Nelson, C.T. 1964. The role of the mast cell in anaphylaxis in the mouse. J. Immunol. 93:165-175.

Those organs showing the greater number of mast cells are not necessarily the ones showing the greater pathologic changes induced by anaphylaxis. Changes in mast cell number, morphology, or products did not appear to be related to the anaphylactic phenomena. Serotonin and histamine,

derived from sources other than the mast cell, are not likely to be the only mediating substances in mouse anaphylaxis. The formation of thrombi increased during anaphylaxis. Mice pretreated with anticoagulants show no significant differences in fatality from untreated mice, although treated animals had fewer thrombi. These thrombi are not antigen-antibody precipitates. FITC-conjugated serum was inoculated into normal and sensitized mice to determine reaction sites.

6733

Fennell, R.H., Jr. 1965. Chronic liver disease induced in rats by repeated anaphylactic shock. Amer. J. Pathol. 47:173-182.

Hyperimmune Holtzman rats were repeatedly subjected to anaphylactic shock. These animals developed scarring of the liver with lobular distortion. Antigen was found to persist in the walls of small vessels in portal areas for as long as 4 weeks after the last antigen injection. In addition to postnecrotic scarring the persistent antigen might act as an injurious agent and provide an additional cause of chronic liver damage.

6734

Horowitz, R.E.; Burrows, L.; Paronetto, F.; Dreiling, D.; Kark, A.E. 1965. Immunologic observations on homografts: II. The canine kidney. Transplantation 3:318-325.

Renal transplants in adult dogs were removed at 12 hours to 7 days after transplantation and studied histologically and immunocytochemically. The rejection of renal homografts is associated with an infiltrate of lymphocytes, hemocytoblasts, and immature and mature plasma cells containing gamma globulin. Beginning at 3 to 4 days the interlobular arteries down to the afferent arterioles show a focal, progressive degeneration with media vacuolization and necrosis, and at 5 to 6 days endothelial proliferation and thrombosis set in. Coincidentally, in the altered vessels small droplets contain only gamma globulin, which can be eluted with an acid buffer and binds in vitro complement; this suggests that the gamma globulin is part of an antigen-antibody complex. Hypothetically, antibody is formed in response to antigen from the graft in the rejection of kidney homografts. Then antigen-antibody complexes, localized or formed in the walls of small blood vessels, cause necrosis and endothelial proliferation that result in thrombosis and tissue necrosis, which terminates the rejection.

6735

Jasin, H.E.; Glynn, L.E. 1965. The antigenic properties of some synthetic poly-aminoacids: II. The antigenicity of polypeptides related to collagen; peptides containing hydroxyproline and acetyl-hydroxyproline. Immunology 8:260-269.

The antigenic properties of some synthetic polymers containing hydroxyproline and acetyl-hydroxyproline have been tested in guinea pigs and rabbits by active cutaneous anaphylaxis, delayed skin hypersensitivity reactions, PCA, tanned cell agglutination, and fluorescent antibody microscopy. The antigenic relationship among these polymers, collagen, and acetylated collagen have been investigated. The results suggest that acetyl-hydroxyproline is a common antigenic determinant in both acetylated copolymers and acetylated collagen. Poly-hydroxyproline and poly-acetyl-hydroxyproline were found not to be antigenic in rabbits or guinea pigs. Rabbit antiserum against acetylated collagen has been used to stain acetylated tissue sections by immunofluorescence. Absorption studies indicate that acetyl-hydroxyproline groups are important antigenic determinants, as shown by the considerable decrease in specific fluorescence when the rabbit anti-acetylated collagen is absorbed with a synthetic polymer containing acetyl-hydroxyproline.

6736

Kemp, H.G.; Pierce, G.E.; Seip, W.F.; Burch, C.C., Jr.; Morrel, S.C.; Edgerton, M.T., Jr.; Barnes, F.W., Jr. 1964. Isotopic and fluorescent studies of proteins of skin grafts and host. J. Cell. Comp. Physiol. 63:121-134.

Fluorescent rabbit antiserum remained attached to 2- and 5-day-old homografts of skin in guinea pigs and was found throughout the collagen bundles of the dermis. Autografts and isografts did not show this. Epidermis effects are not yet entirely clear. It seems possible that certain globulins normally present in the host serum infiltrate the homograft and perhaps are bound to some of its constituents at least as early as 2 days after skin transplantation.

6737

Levenson, H.; Cochrane, C.G. 1964. Nonprecipitating antibody and the Arthus vasculitis. J. Immunol. 92:118-127.

The inability of two nonprecipitating antibodies to produce Arthus vascular inflammatory reactions was studied. In both cases the nonprecipitating antibody did not deposit antigen in the vessel walls in the form of a complex of antigen, antibody, host, and complement, but relatively equal amounts of precipitating antibody did. Rabbit nonprecipitating

antibody interacted with its antigen in the tissues equally as well as precipitating antibody, determined by passive cutaneous anaphylactic reactions, and was abundantly capable of inactivating hemolytic guinea pig complement, with components at least through C 3c taking part. It was proposed that heavy deposition of antigen-antibody complexes, perhaps with host complement, was essential for the accumulation of polymorphonuclear leukocytes and resulting vascular inflammatory damage. FA was used to demonstrate BSA in vessel walls.

6738

Medunitsyn, N.V. 1964. Immunofluorescent investigation of antibody formation in delayed hypersensitivity. Vest. Akad. Med. Nauk SSSR 19:10:39-43. In Russian.

In delayed-type hypersensitivity to soluble antigen, no cells containing antibody were found in the lymph nodes or spleen. Such cells did appear in lymph nodes following intradermal injection of animals with small antigen doses. Following injection of large antigen doses into the plantar pad of an animal paw, a reimmunization effect was observed, accompanied by appearance of antibody circulating and in cells of lymph nodes and the spleen. A connection exists between immediate and delayed types of hypersensitivity.

6739

Medunitsyn, N.V.; Lukmanova, F.F. 1965. Use of the immunofluorescent method for the study of the production of antibodies to pollen antigens Byul. Eksp. Biol. Med. 60:10:85-88. In Russian.

Indirect FA was used to study the process of antibody formation in the popliteal, inguinal, and axillary lymphatic glands and the spleens of guinea pigs at different periods after immunization and re-immunization with an extract of pollen from the herb Phleum pratense. Methods are described for detection of cross immunological reactions between different types of pollen of herbs and trees. The pollen of timothy, cocksfoot, rye grass, fescue, bun, foxtail, and meadow grass possesses general antigenic properties. The pollen of the herb Phleum pratense shows weak cross-reactions with rye pollen and fails to give such reactions with pollen of ambrosia, goose-foot, and trees.

6740

Millman, M.; Wolter, G.H.; Millman, S.; Rosen, R. 1964. A new in vitro test for the detection of antibody in sera of patients allergic to Lolium multiflorum (Italian rye grass). Ann. Allergy 22:136-145.

An in vitro technique using antihuman globulin as a tracer in antigen-antibody reactions is described (indirect FA). This technique was evaluated and compared with results of skin tests obtained from all patients tested with the same antigen. Using Lolium multiflorum as pollen-extract antigen, the results from the in vitro tests and the skin tests compare very favorably.

6741

Movat, H.Z.; Uriuhara, T. 1964. The vascular changes responsible for progression of the Arthus reaction. Federation Proc. 23:2678:548.

Direct and reversed passive Arthus lesions were studied by light, fluorescence, and electron microscopy in 1- to 24-hour-old lesions in rabbits. In the direct Arthus lesion antigen-antibody precipitates accumulated within the first 2 hours in the wall and lumen of venules. The lumina were often occluded by the precipitates. The occlusion of lumina was further enhanced by accumulation of leukocytes, platelets, and fibrin. Leukocytes accumulated also in the vessel wall and around the vessels. The platelets often showed swelling and degranulation. In the reversed passive Arthus reaction antigen-antibody precipitates and leukocytes accumulated only in the vessel wall and not in the lumen. The endothelium showed gaps and disruption and also necrosis in advanced lesions. In leukopenic animals treated with nitrogen mustard there was no gross swelling and hemorrhage, yet there were ultrastructural alterations in the endothelium at sites of antigen-antibody accumulation. Antigen-antibody complex formation and accumulation seems to initiate the Arthus reaction, but leukocyte accumulation is essential for development of the gross lesions. The role of leukocytes, that of complement and of platelets, is being investigated in vivo and in vitro. Complete article.

6742

Paronetto, F.; Horowitz, R.E.; Sicular, A.; Burrows, L.; Kark, A.E.; Popper, H. 1965. Immunologic observations on homografts: I. The canine liver. Transplantation 3:303-317.

Canine liver homografts were placed in the pelvis with the host liver in place to study rejection without complicating hepatic failure. The host lymph nodes and spleen exhibited an immune response with gamma globulin - containing cells also noted in the graft. Gamma globulin

bound in vivo was found in hepatocytes, bile ducts, vessels, and macrophages of the graft but not in the host liver. Complement and host serum globulin were bound to the same structures in vitro. The antigen to which serum globulin was bound seems to be a carbohydrate. Dogs with liver damage following vascular occlusion of a lobe in situ had circulating antinuclear and antiductular binding substances but no specific localization of gamma globulin or complement fixation in the damaged liver. These studies suggest one mechanism in the rejection of liver homografts, which starts with antigen release from the graft, proceeds by antibody production by the host and by the formation or localization of antigen-antibody complexes in hepatocytes, which are destroyed by a cytotoxic reaction.

6743

Rappaport, B.Z. 1964. Antigen-antibody reactions in allergic human tissues: III. Immunofluorescent study of allergic nasal mucosa. J. Immunol. 93:792-797.

In antigen-challenged nasal mucosa of seasonal hay fever patients, antigen, presumably bound to antibody, was identified in the cytoplasm of mucosa and gland epithelial cells, in histiocytes, pericytes, capillary endothelial cells, and fibrocytes. In histamine-challenged tissue coated with antigen in vitro, fluorescence was demonstrable in surface epithelium and in gland cells, histiocytes, and pericytes, but not in fibrocytes or endothelial cells. Fluorescent staining also suggests the possible presence of antigen and antibody in eosinophils of the allergic nasal mucosa.

6744

Rappaport, B.Z.; Walker, J.M.; Booker, B.F. 1964. Purification of highly conjugated precipitins and globulins containing reagins with silk hydrolysate. J. Immunol. 93:782-791.

Highly conjugated human globulins containing reaginic antibodies against egg albumin, ragweed pollen, and cottonseed and conjugated rabbit precipitins against egg albumin and ragweed pollen were purified by adsorption with silk hydrolysate. The purified antibodies were still highly conjugated and gave satisfactory specific staining of antigen in allergic human skin. The reduction in nonspecific staining was probably due to adsorption of noncovalently bound dye by the silk hydrolysate. The presence of antibody-bound antigen in epithelial cells of challenged, allergic skin was confirmed. The epithelial cells of histamine-challenged, allergic skin were capable of binding specific antigen when this was supplied in vitro. The human reaginic globulins and the rabbit precipitins gave similarly effective staining and inhibition results, and the localization of antigen demonstrable with either antibody was the same.

6745

Rauch, H.C.; Raffel, S. 1964. Cellular activities in hypersensitive reactions: IV. Specifically reactive cells in delayed hypersensitivity: Allergic encephalomyelitis. J. Immunol. 93:960-964.

A basic protein of low molecular weight isolated from bovine nerve tissue induces allergic encephalomyelitis in guinea pigs when injected in small amounts in Freund adjuvant. This protein has been shown to reside in the myelin sheaths of the animal of origin as well as of guinea pigs and man. The responses that follow the administration of nerve tissue to guinea pigs include a specific reactivity of lymphocytes of nodes and spleen for this purified antigen and delayed skin reactivity to it, in the absence of circulating antibodies detectable by ordinary serologic methods. The relationship of lymphocytic and skin reactivity and of disease induction to a purified protein antigen of the myelin sheath strongly suggests a contingency of the lesions upon the reactivity of the cells. However, the intervention of cell-bound or cell-produced antibodies cannot yet be excluded as a basis for the pathologic process.

6746

Spear, G.S.; Kihara, I. 1963. Forssman antigen in the guinea pig desensitized to Forssman antibody: An immunofluorescent study. Bull. Johns Hopkins Hosp. 112:270-278.

Sheep erythrocytes and guinea pig organs are two sources of Forssman antigens. Antiserum against sheep erythrocytes, when injected intravenously into normal guinea pigs, produces he rophile shock, a reaction similar in a number of respects to ordinary anaphylaxis but characterized additionally by marked pulmonary edema. It is assumed that the injected Forssman antibody reacts with inborn Forssman antigen. Guinea pigs can be specifically desensitized to Forssman antiserum. The present experiments were designed to study one possible mechanism, a change in tissue antigen. Forssman antigen was studied by FA in sections of kidneys, heart, and lungs and in cultures of trypsinized kidney cells, in normal guinea pigs, and in guinea pigs desensitized for Forssman antibody. Antigen in the desensitized animals was not demonstrably different from that in the normal controls, suggesting that depletion or alteration of tissue antigen is probably not the mechanism by which desensitization is achieved.

6747

Stutman, O.; Zingale, S.B. 1964. Immunological reactivity of thymic autografts in the rat. Arth. Rheum. 7:755-756.

A large number of rats were studied on whom thymus gland autografts were implanted in the subcutaneous tissue on the 5th day of life. These animals, during adult life, showed a normal histologic structure of their lymphatic organs, produced normal levels of circulating antibodies after immunization with diphtheria toxoid, and rejected first-set skin homografts; their lymphatic cells induced runt disease in newborn recipients. All these parameters were markedly depressed in the thymectomized controls. The autografted thymus revealed a cellular response comparable to the one observed in the lymph nodes of normal animals after immunization. The most remarkable feature of this cellular response, demonstrated by immunofluorescence techniques, was a marked plasma cell proliferation producing specific antibody. In some animals, structures like germinal centers were observed in the autografted immunized thymus. The autograft undergoes an initial depletion of its lymphocytes and is then repopulated with normalization of its architecture. It may be postulated that the graft is repopulated by lymphocytes of nonthymic origin that are responsible for the cellular changes observed.

6748

Stutman, O.; Zingale, S.B. 1964. Immunological reactivity of thymic autografts in the rat. Proc. Soc. Exp. Biol. Med. 117:389-393.

The immunological reactivity of the thymus was studied in young adult rats in which the gland was removed from its normal location at the fifth day of life and implanted in the subcutaneous tissue. As compared with control groups, these animals showed a normal histological structure of their lymphatic organs and also produced normal levels of circulating antibodies after immunization with diphtheria toxoid. The autografted thymus, by contrast with the normally located gland, revealed a cellular response comparable to the one observed in the lymphatic organs of normal animals after parenteral immunization. The most remarkable feature of the cellular response of the autografted thymus was a marked plasma cell proliferation producing specific antibody, as was demonstrated by differential cell counting and immunofluorescence studies.

6749

Wernatz, H.; Scheiffarth, F.; Abou-Rebyeh, L. 1965. Serologic studies of cell-bound antibodies in immunotransplantation. Med. Pharmacol. Exp. 13:177-183. In German.

Mononucleocytes from inbred strains of mice sensitized by intraperitoneal injection of homologous extracts of spleen and kidney and homologous skin grafts were shown by means of serological techniques, the antiglobulin consumption test, and serum fluorescence examinations to be capable of binding homologus tissue antigens specifically.

6750

Watkins, E., Jr.; Adams, H.D., Jr. 1964. Tissue transplantation research. Lahey Clin. Bull. 13:128-135.

The replacement of a diseased organ by transplantation of a healthy one from another individual would revolutionize current medical practice. Initial steps have been taken in solution of this transplantation problem by the successful exchange of simpler tissues. Further progress in this field of research is dependent upon deeper understanding of the immune mechanisms that result in rejection of a transplant when a genetic difference exists between the transplant and its host. FA is especially useful in following the immune response to the new tissue.

6751

Wilson, W.E.C.; Talmage, D.W. 1965. Erythrocyte chimerism and acquired immunologic tolerance. J. Immunol 94:150-156.

Three strains of hybrid mice were used. Mice of two strains were injected with spleen and bone marrow cells from the third strain during the neonatal period. Then they were grafted with skin from the third strain after maturation. FA demonstrated isoantibody specific for the third strain on erythrocytes from mice of the other two strains, 53 of 54 mice that had retained the grafts for 100 days. Erythrocyte chimerism accompanies immunologic tolerance acquired in newborn mice injected with viable hematopoietic cells.

E. MISCELLANEOUS STUDIES

6752

Colberg, J.E.; Dray, S. 1964. Localization by immunofluorescence of gamma globulin allotypes in lymph node cells of homozygous and heterozygous rabbits. Immunology 7:273-279.

The cellular production of two rabbit gamma globulin allotypic specificities, A4 and A5, determined by allelic genes was investigated by the fluorescent antibody method. The 7S gamma globulin fractions of precipitating antisera were conjugated to fluorescein isothiocyanate and to lissamine rhodamine B sulphonyl chloride. Frozen sections of lymph nodes from 18 rabbits, A4-A4 and A5-A5 homozygotes and A4-A5 heterozygotes, were studied after exposure to the fluorescent antibody conjugates. The conjugates, each specific for antigenic determinants of 7S gamma globulin, reacted specifically with the cytoplasm of plasma cells and intrinsic cells of the germinal centers. The rabbit anti-A4 conjugate reacted only with lymph node cells of A4-A4 and A4-A5 rabbits; the rabbit anti-A5 conjugates reacted only with cells of A5-A5 and A4-A5 rabbits; the horse antirabbit gamma globulin conjugates reacted with cells of all three genotypes. By a variety of techniques, identical cellular localization of the two allotypes, A4 and A5, was found in the A4-A5 heterozygotes. Less than 1 per cent of the cells in any heterozygous lymph node section contained one allotype without the other.

6753

Eisen, H.N 1964. Determination of antibody affinity for haptens and antigens by means of fluorescence quenching. Methods Med. Res. 10:115-121.

This is a theoretical discussion of FA used in the study of hapten reactions. First additions of hapten were found to cause an excessive amount of quenching. The reasons for this are not fully understood.

6754

Genova, R.; Vaccaro, R. 1964. Contribution to the knowledge of autohemagglutination from blister fluid by the immunofluorescent technique. Riv Clin. Pediat 73:199-203. In Italian.

The immunofluorescent technique was used to study the nature of autohemagglutination in blister fluid. From the results it was concluded that the antibodies of the fluid were not of the gamma globulin type. The agglutination of the erythrocytes in saline with blister fluid was in favor of interpreting such antibodies as complete.

6755

Harris, T.N.; Dray, S.; Ellsworth, B.; Harris, S. 1963. Rabbit gamma globulin allotypes as genetic markers for the source of antibody produced in recipients of Shigella-incubated lymph node cells. Immunology 6:169-178.

Rabbits homozygous for each of an allelic pair of allotypes of gamma globulin A4 and A5 were used as donors and recipients of transferred antigen-incubated lymph node cells, the cells of donors of one allotype being in each case transferred to recipients of the other. When agglutinins to Shigella appeared in the sera of the recipient animals, the allotype of the agglutinin was determined by adsorbing it to Shigella on a glass slide, then treating the preparations with fluorescein-conjugated rabbit anti-A5 gamma globulin and anti-A4 gamma globulin antisera, respectively. In each case the reactions of the recipients' sera were positive for gamma globulin of the donor allotype but not of their own. Positive reactions were given only by sera above a certain range of agglutinin titer. After a sufficient decline of the agglutinin level in the sera of the recipients, these animals were actively immunized with Shigella. The agglutinins that now appeared in these rabbits gave positive reactions with the fluorescent antibody against their own allotype. These data indicate that in moderately X-irradiated rabbits given antigen-incubated rabbit lymph node cells, the antibody that subsequently appears in the recipient's serum has been synthesized by the donor's cells.

6756

Matsuhashi, N. 1964. Serological study of human serum. Jap. J. Allergy 13:240-244. In Japanese.

Serological studies show that antibodies in globulin or 19S gamma globulin, depending on its source, has its antigenic specificity in a particular antigen antibody reaction. The human antibodies appeared in various fractions obtained by DEAE column were treated with mercaptoethanol and tested with various antigens.

6757

Maurer, P.H. 1965. Antigenicity of polypeptides, poly-alpha-amino acids: XIII. Immunological studies with synthetic polymers containing only D- or D- and L-alpha-amino acids. J. Exp. Med. 121:339-349.

Polymers consisting solely of dextro-alpha-amino acids are not immunogenic in rabbits, guinea pigs, man, and mouse. The same polymers of levulo-alpha-amino acids are very effective antigens. This has been attributed to the importance of metabolizability of a polymer in contributing to its immunogenicity. In the glu-lys-ala series of polymers,

the immunogenicity of a polymer of two L-amino acids and a D-amino acid appears to be governed by the immunogenicity of the two L-amino acids. However, some of the specificity may be directed toward configurations containing the D-amino acid. It has been noted that injections of rabbits with polymers of D-amino acids have resulted in a reduced response against the isomeric L polymer.

6758

Meyers, H. 1964. The effect of complement on the permeability of cell membranes in immune reactions using the immunofluorescent technique. Federation Proc. 23:2422:505.

Rabbit erythrocytes coated with human gamma globulin were incubated with fluorescein-labeled antihuman gamma globulin. Fresh human serum was added as the source of complement. The ghosts of the lysed red cells showed intense fluorescence. Using unlabeled material, the red cell ghosts were exposed to fluorescein-labeled antibodies against human complement or complement components 1, beta-1C, and -1A. Fluorescence of the red cell ghosts indicated the presence of complement and its components on or in the red cell membrane. Labeled antibodies to the complement components were added to coated red cells and antihuman gamma globulin. When complement was added to this complex, no hemolysis occurred, but amorphous fluorescent masses were seen in the free space, indicating a reaction between complement and its antibody. No fluorescence was seen on the ghosts. These experiments indicate that an antibody against a coat on the cell membrane can affect the integrity of that cell only with the interplay of complement. Complete article.

6759

Michelazzi, L.; Baldini, I.; Novelli, A.; Nanni, G. 1965. Immune response induced by RNA-immunocarrier extracted from heterologous immune sera. Nature 205:194.

It is possible to elicit an immune response by RNA immunocarrier extracted from sera of heterologous species. Indirect FA demonstrated that the material adsorbed onto guinea pig erythrocytes.

6760

Michelazzi, L.; Nanni, G.; Baldini, I.; Novelli, A. 1964. Presence of 'RNA immuno-carrier' in immune rabbits' serum. Experientia 20:447-449.

Increases in RNA content of serum and gamma globulin of immunized animals were observed. Transfer of RNA from immunized to non-immunized animals allows prompt specific antibody production in the recipient. FA was one method used to demonstrate antibody production.

6761

Paronetto, F.; Popper, H. 1964. Enhanced antibody formation in experimental acute and chronic liver injury produced by carbon tetrachloride or allyl alcohol. Proc. Soc. Exp. Biol. Med. 116:1060-1064.

Two injections of carbon tetrachloride produce enhancement of agglutinins in mice that receive various amounts of heterologous red cells after the first injection. Prolonged administration of carbon tetrachloride or allyl alcohol enhances antibody levels against horse serum and increases susceptibility to anaphylactic shock. Carbon tetrachloride is effective whether given before or during immunization, or if continued after immunization. In mice treated with carbon tetrachloride, the weight of the spleen increases and the lymphoid system exhibits proliferation of cells containing gamma globulin. In immunized mice treated with carbon tetrachloride or allyl alcohol, these cells produce specific antibodies that indicate a quantitative alteration of the immunological reactivity. Liver injury seems to have an adjuvant effect.

6762

Pernis, B.; Chiappino, G.; Kelus, A.S.; Gell, P.H.G. 1965. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissues. J. Exp. Med. 122:853-876.

The cellular localization of allotypes in rabbit lymphoid tissues has been studied by immunofluorescence. In heterozygous animals the double staining for two allotypes controlled by allelic genes has shown the existence of two populations of plasma cells, one containing one allotype and the other the alternative one. The localization in different cells of immunoglobulins marked by allelic allotypic specificities has been confirmed by microspectrography of single cells. An exception to this rule was given by the presence in the germinal centers of lymphoid follicles of apparently uniform mixtures of products of the two allelic genes. Double staining for two allotypes controlled by genes at different loci showed, instead, the presence of many cells containing both allotypes;

the number of these cells was highest in doubly homozygotes, in the other it was consistent with random association of non-allelic specificities. In addition, double staining for one allotype and gamma-G globulins in the lymphoid tissues of rabbits homozygous, at the a or at the b locus, has shown the presence of cells containing immunoglobulins that lack one allotype.

V. NATIVE ORGAN AND TISSUE ANTIGENS

A. ORIGIN AND DISTRIBUTION

6763

Aoki, S. 1964. Studies on sero-immunological differences between the synovial and cartilagenous tissues by means of the fluorescent antibody technique. Bull. Osaka Med. Sch. 10:1-21.

FA was used to clarify the sero-immunological difference between the synovial and the cartilagenous tissues in arthrosis. Emulsions of tissues of rabbits, human beings, and dogs were mixed with Freund's adjuvant to immunize rabbits. The value of the antibody was measured by Boyden's sensitized erythrocyte agglutinating reaction. There was an elevation of the homo- and hetero-immunizing values. Identification of the tissues was tried by FA and microfluorophotometry to determine the specific immunological factors in both tissues. Differentiation of the synovial tissue from the ordinary connective tissue was possible. There were two joint tissues, the synovia and the articular cartilage, manifesting their difference serologically.

6764

Barnes, G.W.; Soanes, W.A.; Mamrod, L.; Gonder, M.J.; Shulman, S. 1963. Immunologic properties of human prostatic fluid. J. Lab. Clin. Med. 61:578-591.

The antigens of human prostatic fluid have been compared with those of other tissues by immunochemical methods. Prostatic fluid contains at least four or five distinctive antigens, as well as other antigens shared with blood serum. By direct immunodiffusion and by inhibition of immunodiffusion, it was demonstrated that some specific prostatic fluid antibodies were not affected in their homologous reaction by the presence of plasma or plasma constituent. Pooled prostatic fluid itself, however, inhibited the homologous reaction completely. By immunoelectrophoresis the reaction of prostatic fluid with antiprostatic fluid serum was found to be dissimilar to that obtained with prostatic fluid against antiserum to human serum. The electrophoretic fraction participating in the formation of the major homologous precipitin band was located in and about the slow beta globulin zone. The specificity of the reaction of rabbit antihuman prostatic fluid serum was confirmed by indirect FA staining of frozen sections of the prostate gland. Staining reaction involved chiefly acinar cells and luminal materials.

6765

Barth, R.F.; Russell, P.S. 1964. The antigenic specificity of spermatozoa: I. An immunofluorescent study of the histocompatibility antigens of mouse sperm J. Immunol. 93:13-19.

Experiments were undertaken to determine whether histocompatibility antigens were detectable on spermatozoa and whether it might be possible to differentiate spermatozoa bearing X and Y chromosomes by the fluorescent antibody technique. C57BL-6 female mice were immunized with skin grafts and lymphoid cells from C57BL-6 males in an attempt to produce antibody specific for Y-determined antigens. Despite accelerated second-set rejection of male skin grafts, it was not possible to detect antibody to Y-determined antigen in female C57BL-6 mice immunized with male tissue. It was possible to demonstrate the presence of H-2 antigens on the cell membranes of lymphoid cells but not spermatozoa by the fluorescent antibody technique. Using sera from both male and female C57BL-6 mice that had been immunized with sperm or testis, it was possible to demonstrate, by both direct and indirect fluorescent staining techniques, antibody specific for the acrosome of mouse, rat, and guinea pig sperm. It is suggested that acrosomal antigen may be a cell-specific antigen of spermatozoa.

6766

Beloshapkina, T.D.; Abelev, G.I. 1965 Immunohistochemical characteristics of insoluble mouse liver cell antigens. Folia Biol. 11:6:472-477.

The insoluble fraction of normal mouse liver cell ghosts was isolated and its antigenic properties were tested by indirect FA. In unfixed liver sections and sections fixed with alcohol and acetone, stained with immune anti-cell ghost sera, characteristic fluorescence of liver cell membranes was found in unfixed and acetone-fixed sections, but not in alcohol-fixed sections. In alcohol-fixed sections the same sera gave fluorescence of connective tissue components (the walls of Kupffer cells and the vascular endothelium). After destruction of the connective tissue by collagenase, cell membrane fluorescence did not disappear in acetone-fixed preparations. On treating a suspension of isolated liver cells with anti-cell ghost antibodies, surface fluorescence of the cells appeared in unfixed and acetone-fixed smears, but not in alcohol-fixed specimens. It was concluded from the findings that immune serum against cell ghosts contains antibodies against connective tissue and also against the surface antigens of the cells themselves. From their nature, these antigens are probably associated with phospholipoproteins.

6767

Beutner, E.H.; Djanian, A.; Witebsky, E. 1964. Immunohistologic studies of rabbit antibodies to rabbit anterior pituitary preparations. J. Histochem. Cytochem. 12:15-16.

Rabbit immunized with rabbit anterior pituitary preparations suspended in complete Freund adjuvant developed antibodies to rabbit anterior pituitary antigen(s). Indirect immunofluorescent staining (IF) revealed antibody titers of 1:30 to 1:300 in 8 of 16 immunized rabbits. Staining occurred in the cytoplasm of acidophilic cells of the anterior pituitary. The localization appeared precise in most experiments. Most positive sera also fixed complement with rabbit anterior pituitary antigens. The active component was precipitable with 2 M ammonium sulfate. Complement fixation and IF staining failed to occur with any of the other rabbit endocrines or other rabbit tissue tested. Tanned cell agglutination with cells coated with pituitary extracts yielded positive results with some of the sera containing pituitary antibodies. IF staining occurred with sections of the antibody-producing rabbits thus revealing the autoantibody nature of the serologic reaction. However, no pathologic changes could be observed in the pituitaries of the antibody-producing rabbits. Species specificity studies revealed specific IF staining with pituitary sections of guinea pig, hog, beef, and dog, but not with human or monkey pituitaries. Similarly, hog and beef pituitary antigens fixed complement but monkey and human pituitary antigens did not. Complete article.

6768

Beutner, E.H.; Djanian, A.; Witebsky, E. 1964. Serological studies on rabbit antibodies to the rabbit anterior pituitary. Immunology 7:172-181.

Rabbits immunized with suspensions or extracts of rabbit anterior pituitary in Freund's adjuvant may develop specific antibodies to components of the rabbit pituitary. Immunofluorescent staining with such antisera occurred in isolated cells of the anterior pituitary. These correspond to cells stained with acid fuchsin, i.e., acidophils or alpha cells. Some of the pituitary antisera fix complement with pituitary extracts. A tanned-cell hemagglutination test using pituitary extracts as coating antigen yielded positive reactions with some of the pituitary antisera. Immunofluorescent staining revealed that antisera reacted with the pituitary of the antibody-producing rabbit. No direct or indirect evidence of pathological changes in the autoantibody-producing animals could be found.

6769

Billen, J.R.; Griffin, J.W.; Waldron, C.A. 1964. Investigations for pyronin bodies and fluorescent antibodies in 5:5 diphenylhydantoin gingival hyperplasia. Oral Surg. 18:773-782.

This study indicates that pyronin bodies are found in conjunction with plasma cells and are more readily discernible in the gingival tissue of patients with Dilantin hyperplasia but are not pathognomonic of Dilantin hyperplasia. Pyronin bodies are seen as readily with hematoxylin and eosin as with other staining methods. A specific antigen-antibody reaction is discredited on the basis of direct staining with fluorescein-conjugated gamma globulins. The connective tissue substance from patients with Dilantin gingival hyperplasia exhibited antigenic properties that were probably located in the reticulin or cementing substance. In the connective tissue of patients with periodontitis the degree of fluorescence was less and the morphology of the antigen site was not so definitive in nature as in Dilantin tissue.

6770

Boss, J.H. 1963. Antigens in membranous and fibrillar structures of human tissues: An immunofluorescence microscopical study of their widespread nature. Arch. Pathol. 76:434-445.

Fluorescein-labeled antihuman kidney and antiplacental sera, known to localize in vitro in the epithelial and mesenchymal basement membranes of the kidney and placenta, have been used to demonstrate the occurrence of identical or very similar antigens in various human tissues. The data indicate that such antigens are widely distributed throughout the body. They reside within basement membranes, argyrophil fibers, sarcolemma, and neurilemma. It appears that every organ has its characteristic membranous-fibrillar antigen distribution pattern.

6771

Boss, J.H. 1963. Observations on the species-nonspecificity of the human renal and placental basement membrane antigens. Experientia 19:517-518.

The direct FA test was used to demonstrate relationships between antigens of human basement membranes. Antigens used in cross-absorption tests were human, rabbit, guinea pig, rat, and mouse kidney. Conjugated antisera to human renal or placental membrane antigens stained several species of kidneys. Placenta and kidney antigens cross-absorbed each other's antibody from the conjugate.

6772

Boss, J.H. 1963. The antigen distribution pattern of the human placenta: An immunofluorescent microscopic study using the kidney as an experimental model. Lab. Invest. 12:332-342.

The distribution pattern in the human placenta of antigens capable of eliciting the production of antibodies in the rabbit is reported. These antigens reside in the fetal vascular and in the trophoblastic basement membranes. They are identical or very similar to the basement membrane antigens of the kidney. Thus, the placental and renal antigens, as well as the anti-placental and anti-kidney antisera, are mutually interchangeable in immunologic and immunohistologic reactions. An attempt to demonstrate circulating anti-placental antibodies in the serum of normal pregnant women and of pre-eclamptic patients is described. Two immunohistologic approaches have failed to show the presence of antibodies capable of localization in the placenta. Any immunologic response to the conceptus may be of the delayed hypersensitivity type.

6773

Boss, J.H.; Craig, J. 1963. The distribution patterns in the rat placenta of antigens common to the rat glomerulus as revealed by immunofluorescent techniques. Amer. J. Pathol. 42:443-454.

The renal glomerulus and the placenta in the rat have a common antigen, as demonstrated by the fluorescent antibody techniques. This has been demonstrated by direct staining of the tissues with labeled rabbit antirat glomerular serum, or by indirect methods following the intravenous injection of antirat glomerular serum, or by indirect methods following the intravenous injection of antirat glomerular serum. The localization of the latter was achieved by staining the tissue either with labeled antirat gamma globulin prepared in the goat or with labeled rabbit antirat gamma globulin more than 5 days after the original injection. The common antigenic sites in the placenta were the basement membranes of the labyrinth and trophoblast, Reichert membrane, and the yolk sac.

6774

Cain, H. 1963. Fluorescence-optical findings in various renal vascular processes. Verhandl. Deut. Pathol. 47:361-365. In German.

It has been demonstrated in comparative acridine orange fluorochrome and immunofluorescence investigations that the development of hyalinizing vascular renal processes may be both produced and, with respect to their further course, be determined by various factors. Besides disturbances occurring in the vascular wall (changes in the serum protein balance) the activities of mucopolysaccharidases (proteolysis of fiber protein) both absorption and repair processes, as well as immunological reactions, play an important part in renal vascular hyalinization. Accordingly, the histochemically recognizable spectrum of arterial hyalin can be very wide.

6775

Campbell, J.C. 1965. An immuno-fluorescent study of lens regeneration in larval Xenopus laevis. J. Embryol. Exp. Morphol. 13:171-179.

The appearance and localization of lens antigens in lens regenerating from the cornea of larval Xenopus laevis were investigated by FA. The cornea of the normal eye was not stained, but lens antigens were detected in the cytoplasm of cells of the inner layer of the corneal epithelium overlying the pupillary space with 24 hours of lens removal. In the cells of the lens vesicle, before fiber formation, cytoplasmic labeling was weak, except for small areas of cells at the posterior margin of the vesicle from which the primary lens fibers are formed. The most intense fluorescence appears over the fibers and is stronger in older fibers, whether primary or secondary. The lens epithelium is unlabeled. The limitations of the technique are considered, and results are discussed in the light of other immunological data on normally developing and regenerating lens.

6776

Del Giacco, G.S.; Novi, C.; Scotti, G. 1963. Immunofluorescence research on anti-kidney antibody fixation. Boll. Ist. Sieroterap. Milan 42:541-554. In Italian.

Immunofluorescence techniques were used to study the fixation of antikidney antibodies parenterally injected in rats. Sera are quickly fixed on renal glomeruli; after 5 minutes it is possible to see glomerular fluorescence. The preferential fixation of anti-kidney serum occurs at the level of the basal membrane of the glomerular capillaries, but it is also possible to see it within vascular structures, both renal and extra-renal, and in lung, liver, and spleen arterial walls, but with lower intensity. The

serum lasts for a long time fixed at the glomerular level. It is possible to detect the presence of homologous rat globulins at the glomerular level. The lesions, histologically detectable (as was anticipated from the preliminary data), were a rather scarce and aspecific anhistic exudation in the Brown capsule, sometimes with hypernucleosis.

6777

Emmelot, P.; Bos, C.J.; Benedetti, E.L.; Rumke, P. 1964. Studies on plasma membranes: I. Chemical composition and enzyme content of plasma membranes isolated from rat liver. Biochim. Biophys. Acta 90:126-145.

Plasma membranes were isolated from rat liver. Electron microscopy showed that the preparations were not contaminated by other identifiable cellular components. Antibodies prepared against the isolated membranes were preferentially fixed by the plasma membranes of liver tissue. Indirect FA was used. The chemistry of the plasma membranes is discussed in detail.

6778

Engelgart, N.V. 1963. Immunological histochemical characteristics of one of the organ-specific antigens of mouse liver. Biul. Eksp. Biol. Med. 56:97-101. In Russian.

A method is described for obtaining monospecific antibodies to one of the organ-specific antigens of mouse liver. With the aid of these antibodies, the antigen content of the liver and of normal mouse organs was determined by double diffusion in gel. The fluorescent antibody method was used to locate this antigen in the cytoplasmic granules of hepatic parenchyma cells.

6779

Feltkamp, T.E.W.; Fruyff, K. 1965. Autospermagglutinins: Immunofluorescent studies. Ann. N.Y. Acad. Sci. 124(Pt.2):702-708.

FA studies with sera of infertile males were done on testicular sections and sperm smears. A positive correlation was found between agglutination and fluorescent staining. Various parts of the sperm, sometimes in different combinations, were involved in the fluorescent staining. The correlation with agglutination type was not clear.

6780

Field, E.J.; Ridley, A.; Caspary, E.A. 1963. Specificity of human brain and nerve antibody as shown by immunofluorescence microscopy. Brit. J. Exp. Pathol. 44:631-634.

Rabbit antihuman brain serum attaches to guinea pig myelin, both central and peripheral. Rabbit antihuman nerve serum combines only with peripheral nerve and nerve roots, and this combining power is less than that of the serum against brain. The possible significance of these findings is briefly discussed.

6781

Green, H.N.; Ghose, T. 1964. Localization of liver specific antibody in normal and 3'-DAB-treated rats. Nature 201:308-309.

This study was designed to determine the nature of the localization of antibodies against livers of normal rats and rats treated with 3'-methyl-4-dimethylaminoazobenzene (3'-DAB). Antisera were produced in rabbits. Indirect FA was used to stain tissues in vivo and in frozen sections of liver and other organs. Comparative results between normal and treated animals indicated that the 3'-DAB binds to proteins of the hepatic cells and acts as a hapten. It changes the specificity of the liver specific antigen.

6782

Hong-Bigh-Vuong; Nahon, E.; Lacour, F.; Lacassagne, A. 1964. Cancerology: Immunofluorescence study of the ribosomic antigens in the abdominal cells of mice. Compt. Rend. 259:1361-1364. In French.

Antiribosome antibodies from mouse abdominal cells, conjugated with fluorescein isothiocyanate, demonstrated the ribosomic antigens in cytoplasm and a smaller quantity in the nucleus of fixed abdominal cells.

6783

Kornguth, S.E.; Anderson, J.W. 1965. Localization of a basic protein (immunohistology) in the myelin of various species with the aid of fluorescence and electron microscopy. J. Cell Biol. 26:157-166

Alanine was the N-terminal amino acid of a basic protein of low molecular weight that was isolated from either human or guinea pig brain. Antibodies prepared against the guinea pig protein were labeled with either fluorescein or ferritin. Studies showed that an immunohistochemically similar protein is in the myelin sheaths of central and peripheral nervous tissues of chicken and frog and a variety of mammalian species. Loss of integrity of the myelin during processing enhanced the antigen-antibody reaction.

6784

Lobo, B.A.; Abreu, W.M.; Santa-Rosa, G.L. 1963. Immunohistological study of the testicle and epididymis of the guinea pig. Int. J. Fertil. 8:539-546.

Cryostatic sections of the testis and epididymis were submitted to FITC labeled sera (normal, anti-testis, anti-spermatozoa, anti-seminal vesicle). The sections of testis stained by anti-testis sera and anti-spermatozoa sera reveal the acrosomic material of spermatozoa and granules in spermatids. Both were faintly stained when treated by anti-seminal vesicle and normal sera. Epididymis sections stained by anti-testis sera and anti-spermatozoa sera showed the gametes' heads to be intensively stained. The anti-seminal vesicle sera showed acrosoma; the normal sera revealed the tails. The collagenous fibers were visible only in the sections stained by labeled anti-testis sera.

6785

Maisel, H.; Harmison, C. 1963. An immuno-embryological study of the chick iris. Anat. Rec. 145:256-257.

Immunochemical analysis of the chick iris reveals three kinds of antigens. One group consists of proteins specific for the iris. The second group is identical immunologically and electrophoretically to chick lens alpha, beta, and gamma crystallins. The third consists of a protein with serum specificity. From sedimentation coefficients it was concluded that iris alpha and gamma crystallins closely resemble the corresponding lens proteins in molecular size. However, iris beta crystallin is a considerably smaller molecule than the lens protein. Analysis of the pattern of appearance of iris antigens during development showed that the alpha crystallin antigen was the first to arise, being detected in the presumptive retina and iris of 72-hour chick embryos. Beta and gamma crystallin antigens were apparent in iris extracts of 8-day embryos. One specific iris antigen was detected in ten-day extracts, at a time when the iris musculature is established, but the other was noted in 14-day extracts. Complete article

6786

Mancini, A.M.; Costanzi, G.; Tison, V.; Levin, S. 1964. Antigenicity of the human arachnoid. Lancet 2:1295-1296.

Antigens of the reticular type are present in the human arachnoid connective tissue. We found an antigenic difference between reticulin and collagen. We found no evidence of nerve tissue antigens in the arachnoid.

6787

Mancini, R.E.; Barquet, J.; Paz, M.A.; Vilar, O. 1965. Histoimmunological study of collagen during tendon fibrillogenesis. Proc. Soc. Exp. Biol. Med. 119:656:660.

The localization of soluble and insoluble collagen fractions during the development of normal chick embryo tendon and granulation tissue in injured adult chick tendon was studied by the immunohistochemical technique. Antisoluble collagen antibody reacted only with the cytoplasm of young fibroblasts and with extracellular young collagen fibers. Reticulin fibers that precede the collagen and interfibrillar mucopolysaccharides did not react nor did other tissue structures. Anti-insoluble collagen antibody did not react with fibroblasts and reticulin fibers but did react with collagen bundles.

6788

Mancini, R.E.; Davidson, O.W.; Vilar, O.; Nemirovsky, M.; Bueno, D.C. 1964. Achrosomal antigenicity in rat testes. Fert. Steril. 15:695-700.

FA fluorescence using either one- or two-step techniques was comparable. Antibodies responsible for the FA reaction are in the globulin. Globulins from completely sensitized rats reacted specifically with the acrosomes of the germinal cells of normal rats, as established by controls. Results point to the acrosome as the only cellular depot of testicular antigen. The in vivo experiment showed that immune globulins do not diffuse through the basal membrane into the tubules in quantity enough to be detected by FA.

6789

Mancini, R.E.; Paz, M.; Barquet, J.C.; Vilar, O.; Davidson, O.W. 1964. Histoimmunological studies on chicken collagen. J. Histochem. Cytochem. 12:37-38.

Young and adult chicken leg tendon collagen was fractionated according to Jackson. Complement-fixing antibodies against insoluble and neutral salt soluble fractions were obtained in rabbits and guinea pigs, respectively. Gamma globulins were separated and used in both direct and indirect FA. Immunohistologic procedures were carried out in fresh frozen sections of adult and young chicken tissues, as well as in 10- to 20-day-old embryos. Low titer antibodies were observed following sensitization with both antigens. When connective tissues from adult chickens were analyzed, both antigens appeared to be located in the collagen fibers only. A brighter fluorescence was elicited by the insoluble fraction. FA gave similar results, but the fluorescence corresponding to the neutral salt soluble fraction increased. When embryonic tissues were studied, only the neutral salt soluble fraction was observed in an intracellular location. The insoluble one appeared in the intracellular substance, and showed a very weak positive fluorescence. Similar negative results were obtained when the immune gamma globulins were absorbed in vitro in the presence of an antigen excess, and later used for FA. Complete article.

6790

Mancini, R.E.; Paz, M.; Vilar, O.; Davidson, O.W.; Barquet, J. 1965. Histoimmunological detection of collagen fractions. Proc. Soc. Exp. Biol. Med. 118:346-350.

The soluble and insoluble fractions of collagen from chicken leg tendon were used as antigens to induce corresponding antibodies in adult rabbits and guinea pigs. Antibodies were checked immunoserologically and the globulins or its gamma fraction labeled with fluorescein isothiocyanate and purified with Sephadex. Adequately controlled FA technique was used to detect the presence of the antigen in different types of connective tissue of adult chickens. It was observed that there was cross-reaction between the neutral-soluble and the citrate-soluble fractions. Although a direct reaction occurred with the insoluble collagen fraction, fluorescent antibodies against these different fractions reacted similarly with the collagen fibers and bundles but not with reticulin and elastic fibers, mucopolysaccharides, basement membranes, nor with matrix of cartilage and bone. Intensity of fluorescence was higher with the fibers of dense connective tissue as compared with loose or mucoid type of connective tissue.

6791

Marena, F. 1963. Research on the immunofluorescence of the crystalline lens. Ann. Ottalmol. 89:1087-1089. In Italian.

By the FA technique, the author demonstrated that globulins from the serum of normal individuals may be fixed on heterologous crystalline lenses.

6792

Metzger, R.S. 1963. Immunological studies on the nature of pancreas-specific isoantigens. Federation Proc. 22:2017:498.

Pancreas-specific isoantigens in rabbits have been previously described. Complement-fixing pancreas-specific isoantigens have also been found in monkeys immunized with pooled monkey pancreas extract and Freund adjuvants. Immunofluorescence studies of rabbit pancreas isoantigens show them to be located in the cytoplasm of acinar cells. Fluorescence with some isoantigen-antibody systems is often limited to the apex of the acinar cells and lacking in basal cytoplasm; in other systems there is diffuse cytoplasmic fluorescence of the entire acinar cell cytoplasm. Isoantigens were not detectable in secretions of the pancreas. There were no changes noted in the pancreas of normal rabbits receiving isoimmune serum and or cells. A group of rabbits was partially pancreatectomized and immunized with an extract of their own pancreas incorporated in Freund adjuvants. No iso- or auto-antibodies were elicited in this group and there were no histological changes in the remaining pancreas. Complete article.

6793

Metzger, R.S. 1964. Immunologic studies of pancreas-specific isoantigens. J. Immunol. 93:176-182.

Rhesus monkeys immunized with pooled rhesus monkey pancreas extract and Freund complete adjuvant developed pancreas-specific isoantibodies, and the electrophoretic mobility of the isoantigens is similar to that described in rabbits. Passive transfer of antiserum or immune lymph node and spleen cells to recipients whose pancreas had the necessary isoantigens produced no histologic changes or functional alterations of the pancreas in the recipients. Autoimmunization of rabbits with their own pancreases incorported in Freund complete adjuvant failed to elicit auto- or iso-antibodies, and the pancreases of these immunized animals were normal grossly and histologically. Immunofluorescent studies showed the rabbit pancreas isoantigens to be located in the cytoplasm of the apex of the acinar cells. The pancreas isoantigens of both rabbits and rhesus monkeys were detected in pancreatic secretions.

6794

Möller, G. 1963. Phenotypic expression of isoantigens of the H-2 system in embryonic and newborn mice. J. Immunol. 90:271-279.

Previous studies indicated that red blood cells and spleen cells of newborn A, C3H, and CBA mice were resistant to humoral H-2 antibodies during the first 3 days after birth. It was demonstrated in the present work that this difference in timing with regard to the phenotypic expression of the various H-2 components was preserved when they were brought together on the same F1 hybrid erythrocytes or spleen cells. It was demonstrated by the indirect fluorescent antibody technique that H-2 antigens were present on all spleen cells of newborn mice, but the frequency of positively stained cells diminished rapidly with decreasing age in embryos and no antigenic cells could be detected in 15-day-old embryos.

6795

Moeller, G. 1964. Fluorescent antibody technique for demonstration of isoantigens in mice, p. 58-69. In H.N. Eisen (ed.) Methods in Medical Research, Vol. 10, Section I. Year Book Medical Publishers, Inc., Chicago, Ill.

Detailed procedures for reagent preparation and application are given. Other serologic procedures for study of isoantigens may be employed, but the limitations of these procedures can be overcome to a large extent by use of FA. This method is applicable to most cell types and to a wider range of isoantigens than those represented by the H-2 system. In addition, it can detect antigens at the cellular level.

6796

Nairn, R.C. 1963. International Congress of Clinical Pathology: Immunofluorescence. Brit. Med. J. 2:1258.

This is a brief report of a discussion by Dr. Nairn on the subject of FA detection of organ-specific antigens.

6797

Nozaki, M.; Foster, L.; Sery, T.W. 1963. Reaction of eye tissues to heterologous antiglomerular antibodies. Arch. Ophthalmol. 70:86-95.

In vivo localization of rabbit antirat glomerular antiserum, when injected into normal rats intravenously, was detected in small blood vessels of the choroid, optic nerve, and conjunctiva by staining the tissue sections with fluorescein-conjugated antirabbit gamma globulin. However, an attempt to inject the fluorescein-conjugated rabbit antirat glomerular globulin into normal rats failed to demonstrate specific immunological reactivity to eye tissues. The complement fixation test revealed the presence of antibodies in antirat glomerular antiserum to crude antigen preparations from the rat kidney cortex, uvea, lung, and liver.

6798

Nozaki, M.; Foster, L.; Sery, T.W. 1963. Uveal and other ocular tissue reactions to heterologous antilens capsule antibodies. Invest. Ophthalmol. 2:641-647.

The immune reactivities of heterologous antibovine whole lens, lens substance, and lens capsule antibodies to the host uveal and other ocular tissues were compared by the fluorescein-conjugated antibody technique. Fluorescein-conjugated antibovine lens capsule antibody reacted specifically with the lens capsule and the walls of the vascular tree in iris, ciliary body, retina, choroid, and optic nerve. Fluorescein-conjugated anti-whole-lens antibody showed specific staining in both the lens capsule and lens substance. The walls of the vascular tree were also stained specifically, but less than the staining obtained by the anti-lens-capsule antibody. Fluorescein-conjugated antibovine lens substance antibody reacted with the lens substance but not with the capsule or other eye structures. Eye sections treated with fluorescein-conjugated normal rabbit gamma globulin did not exhibit any specific stainings. The significance of the antigenicity of the lens capsule, its relationship to basement membrane structures, and its possible source as a stimulus to lens-induced uveitis are discussed.

6799

Okada, T.S. 1964. Cellular distribution of some microsomal antigens in embryonic chicken kidneys. Exp. Cell Res. 33:584-587.

Distribution of substituents of deoxycholate extracts of chicken kidney microsomes were studied by FA. Fraction K45 proteins were precipitated from the extracts by 25 and 45 per cent saturation ammonium sulfate. Fraction E refers to those from 60 and 80 per cent saturation ammonium sulfate precipitation. Antisera to K45 and E were conjugated and used.

Methods for fixation of specimens and elimination of nonspecific fluorescence are detailed. Staining patterns using one or both conjugates on tissue sections are described. FA demonstrated differing cellular distribution of K45 and E antigens.

6800

Okada, T.S. 1965. Development of kidney-specific antigens: An immunohistological study. J. Embryol. Exp. Morphol. 13:285-297.

Kidney-specific antigens can be isolated from the microsomal fraction of adult chicken kidneys in a semipurified form. Highly kidney-specific antiserum that was obtained by injecting the isolated antigens into rabbits was conjugated and used as a histochemical reagent to study the development of immunological kidney specificity in mesonephros of developing chicken embryos. In the fully differentiated mesonephros at stages 27 to 30, specific fluorescence appeared only in the apical part of the cells of proximal secretory epithelium. These patterns were the same in earlier stages. First, intense fluorescence occurred, coinciding with the first histological organization of epithelium, at stages 20 to 21. At these initial stages, however, a fluorescent dot was sometimes detected inside the nucleus, besides the inner border of the epithelial cells. By combining the antibody staining technique with autoradiography, it was revealed that the percentage of the cells incorporating H3-thymidine in the fluorescent positive cells was about twice as high as that in the negative cells at stages 22 and 25. This result indicates the higher proliferation rate of the antigen-containing cells.

6801

Perkins, E.S. 1963. The antigenic relationships of ocular and other tissues Trans. Ophthal. Soc. UK 83:271-278.

Agar gel diffusion tests were used to demonstrate immunologic relationships among a number of tissues. FA was used to confirm and extend these results. A table of cross-reactions is given in the text. Antigenic relationships were demonstrated not only between tissues of ectoderm origin but also between tissues of ectoderm and endoderm origin.

6802

Perkins, E.S.; Wood, R.M. 1963. Antigenic components of guinea pig tissues. Exp. Eye Res. 2:255-264.

Rabbits were immunized with extracts of ocular and systemic tissues of guinea pigs. The immune sera were tested against a wide range of guinea pig tissues, using the Ouchterlony technique, immunoelectrophoresis, and the fluorescent antibody method. All the sera showed antibodies to other tissues in additon to those used for immunization. Many of the cross-reactions demonstrated by agar diffusion were due to serum components in the tissue extracts. However, some cross-reactions, notably with antilens serum, were not due to serum components. The fluorescent antibody technique again showed numerous cross-reactions between ocular and other tissues even after absorption of the immune serum with normal guinea pig serum.

6803

Pierce, G.B.; Sri Ram, J. 1965. The localization of epithelial basement membrane antigens in the kidney of the mouse. Federation Proc. 24:1692:434.

We have demonstrated that three types of murine epithelium, parietal yolk sac and mammary and granulosal cell epithelium, synthesize a common basement membrane antigen in vitro in the absence of connective tissues. This epithelial basement membrane antigen (EBM) was immunologically and chemically distinct from basement membrane antigens of the vasculature or from reticulin. Utilizing the fluorescent antibody technique, EBM was shown to be only a small part of glomerular and tubular basement membranes. Antisera to renal basement membranes (anti-renal BM) contain anti-EBM as a minor component that can be removed by absorption with EBM, leaving antibodies that react with reticulin and vascular basement membranes. The anti-reticular and anti-vascular components can be removed only by exhaustive absorption with their antigen. This absorbed antiserum contains anti-EBM that stains the thickened basement membrane of parietal yolk sac epithelium. However, the intensity is too low to refract in the thin renal BM. EBM as well as endothelial basement membranes and reticulin have been localized in the kidney with ferritin-labeled antibodies. Complete article.

6804

Pressman, D. 1963. Certain aspects of tissue-specific antigens, p. 363-376. In Canadian Cancer Conference, Vol. 5. Academic Press, New York.

Antibodies have been prepared that have the property of localizing in vivo in the particular tissues against which they were formed. The localizing antibody appears to be directed against the vascular bed of the tissues and the differences in localizing properties reflect differences in the vascular beds in different organs and tissues. The localization was shown by radiolabeling and FA. Antibodies prepared against a particular tissue will contain antibodies that will localize in other tissues as well. However, antibodies with different localizing properties can be partially separated. Antibodies are also formed that do not localize when injected in vivo. These antibodies may not be able, in vivo, to come in contact with their respective antigens. It has been possible to separate components from individual tissues, for example kidney, which are capable of neutralizing the kidney-localizing activity. Antibodies have also been prepared against various transplanted and chemically induced animal tumors. In the case of the transplanted tumors, antibodies can be prepared that localize to a high degree in the tumors. These antibodies are directed against fibrin that appears to be laid down rapidly during tumo growth. Antibodies prepared against chemically induced hepatoma were shown to localize in liver as well as in hepatoma. Antibodies can localize preferentially in the hepatoma. Use has been made of a paired-label technique in which antibody preparations are labeled with one isotope of iodine and the control preparations are labeled with another. Mixing these two preparations and injecting them simultaneously permits determination of differences in localizing properties of the antibody and control preparation in any particular portion of the tumor or tissue. FA studies were also carried out to determine from which tissue certain tumors were derived. Adult rhabdomyosarcomas lost the ability to produce myosin, whereas those classified as embryonal rhabdosarcomas still contained myosin.

6805

Rauch, H.C.; Raffel, S. 1964. Immunofluorescent localization of encephalitogenic protein in myelin. J. Immunol. 92:452-455.

A protein preparation extracted from bovine spinal cord induces allergic encephalomyelitis in guinea pigs. Normal human, guinea pig, and bovine spinal cord tissue sections were exposed to rabbit anti-encephalitogenic protein serum. Similar control sections were treated with normal rabbit serum, absorbed immune rabbit serum, or buffered saline. Following the application of fluorescein isothiocyanate - labeled goat antirabbit gamma globulin, only those sections exposed to the specifically immunized

rabbit serum showed fluorescent myelin structures. It is concluded that the encephalitogenic protein is localized in myelin, the target tissue of experimental allergic encephalomyelitis, and further that it lacks species specificity.

6806

Rothbard, S.; Watson, R.F. 1965. Immunologic relations among various animal collagens. J. Exp. Med. 122:441-454.

Complement fixation and in vivo immunofluorescence with cross-absorption studies showed that acid-soluble collagens prepared from rat, mouse, guinea pig, chicken, carp, and man exhibit species specificity. Rat and mouse collagens were indistinguishable and cross-reacted with guinea pig collagen. Cross-reactions also occurred between the collagens of rat and man and chicken and man. Tissue specificity, or an antigen common to all of the collagens, was not demonstrated. There was complete agreement in the results of the two immunologic methods. The findings in this study support the conclusion that collagen in some form is present in the renal glomerular basement membranes.

6807

Stevens, J.A.; Beutner, E.H. 1964. Immunohistologic studies of rabbit antibodies to rabbit and sheep anterior pituitary preparations. J. Histochem. Cytochem. 12:38-39.

Twenty-eight rabbits received multiple injections of globulins prepared by precipitation with 2 M ammonium sulfate of rabbit anterior extracts in complete Freund adjuvant. In contrast to rabbits immunized with rabbit pituitary suspensions or extracts, all rabbits that received anterior pituitary globulins developed significant antibody titers as revealed by indirect FA. Some sera also fixed complete with pituitary antigens. Sera tested with pituitaries of the antibody-producing rabbits themselves also yielded positive staining results. Four rabbits received multiple injections of sheep pituitary globulins in complete Freund adjuvant. FA staining of both sheep and rabbit pituitary sections with the antisera revealed specific reactivity of significant titers. No specific FA activity could be demonstrated on other rabbit organs. However, FA staining of other sheep organs revealed reactions with connective tissue element and blood vessels. Histologic studies of FA staining of rabbit pituitaries with rabbit antisera to sheep pituitaries revealed staining in acetophilic cells as well as some non-acetophilic cells. Studies of the pituitary antibody-producing rabbits again revealed the autoantibody nature of these rabbit antibodies to sheep pituitary antigen. Complete article.

6808

Sulitzeanu, D.; Bernecky, J.; Yagi, Y.; Pressman, D. 1963. Soluble antigens from rat liver connective tissue. Proc. Soc. Exp. Biol. Med. 114: 468-472.

The work described here has revealed the presence of a large number of antigenic components in a soluble fraction obtained from an originally insoluble residue of rat liver. The major source of these antigens appears to be the liver connective tissue. The material used for their preparation was an extensively washed insoluble residue of rat liver, morphologically appearing as bundles of fibers, with no cells present. The antigens were absent from the rat plasma. One of them, No. 3, was not found in the supernatant of the liver homogenate; the others were found there in much smaller concentrations. Both the original antiserum prepared against liver sediment and a purified antibody fraction derived from it reacted only with the connective tissue elements of the liver and kidney. The presence of some of these antigens in both liver and kidney indicates that they are probably widespread constituents of the connective tissue and are not specific for the liver. The use of sonic vibration for solubilizing connective tissue components while maintaining their antigenic reactivity may open the way to an analysis of the composition of connective tissue unattainable heretofore.

6809

Szent-Gyorgyi, A.G.; Holtzer, H. 1963. Reactivity of myosin to antibodies in cross-striated chick myofibrils: I. Muscle at rest length. Biochim. Biophys. Acta 74:709-721.

The amount of myosin that reacted in striated chick myofibrils at rest length with antibodies prepared against myosin, light meromyosin, heavy meromyosin, and actin was quantitatively determined. This was done using relative insolubility of the antibody-antigen complex in a solvent that extracts myosin readily from the myofibril. Extraction of myosin was followed by determining the antibody concentration from fluorescence measurements and other methods. Antibodies prepared against myosin and light and heavy meromyosin reacted extensively with myosin in the myofibril. Antibody prepared against actin fixed only small amounts of myosin. There are more antigenic sites against anti-myosin than against anti-light and less against anti-heavy meromyosin. These antibody preparations contained antibodies that reacted at different sites. The experiments rule out the possibility that steric hindrance or blocked antigenic sites significantly influence the results. Distribution of myosin in the A-band is discontinuous. At rest length, a narrow central region does not react with anti-myosin or anti-light meromyosin. Migration of myosin within the A-band may be possible.

6810

Takata, C.; Albright, J.F.; Yamada, T. 1964. Lens antigens in a lens-regenerating system studied by the immunofluorescent technique. Develop. Biol. 9:385-397.

Appearance and localization of lens antigens in the cell population engaged in Wolffian lens regeneration in adult Triturus viridescens was studied with the immunofluorescent technique. Paraffin sections of the regenerating cell population at various stages of regeneration were treated with the gamma globulin fraction of rabbit antiserum against newt lens, conjugated with FITC. The normal pigmented iris and regenerates of Sato stages II and III were completely negative. At stage IV, some of the regenerates indicated cytoplasmic fluorescence in the prospective primary lens fiber cells. In subsequent stages the cytoplasm of lens fiber cells always showed fluorescence, but the majority of the lens epithelium cells were negative. At stage X, when the lens epithelium becomes one cell thick, the cytoplasm of all lens epithelium cells was positive, as was that of all lens fiber cells.

6811

Takata, C.; Albright, J.F.; Yamada, T. 1964. Study of lens antigens in the developing newt lens with immunofluorescence. Exp. Cell Res. 34:207-210.

Organ-specific lens antigens appear in the cytoplasm of primary lens fiber cells when the lens placode is transformed into the lens vesicle; they later increase in amount with differentiation of lens fibers. In the lens epithelium, lens antigens become detectable later, when its regular epithelial arrangement has been attained. The stage of first appearance of lens antigens demonstrated in the present experiment approximately coincides with that determined with the precipitin technique in the frog embryo. Occurrence of lens antigens detectable with our method is correlated with differentiative events of lens-forming ectoderm cells, but not with lens-inducing activities of the optic cup or with lens potency of the ectoderm cells.

6812

Takata, C.; Albright, J.F.; Yamada, T. 1965. Lens fiber differentiation and gamma crystallins: Immunofluorescent study of Wolffian regeneration. Science 147:1299-1301.

From the adult lens of Triturus viridescens, a fraction of proteins was isolated that corresponded to gamma crystallins of higher vertebrates. Tests by immunoelectrophoresis indicated that the antiserum against this fraction reacted with gamma crystallins but not with alpha or beta crystallins. From this antiserum an FA reagent was prepared for detection of gamma crystallins from newts. In the normal lens of the adult newt, these

crystallins were detected in fiber cells and fiber material but not in the epithelial cells. During transformation of the iris into the lens after lens removal, the staining reaction was negative in the regenerating tissue up to the time the prospective primary fiber cells elongated. Subsequently, without exception those cells in fiber differentiation indicated a gamma crystallin reaction. When the secondary fiber cells were produced at the equatorial zone of the regenerating lens, they also showed a gamma crystallin reaction. Thus, gamma crystallins characterized fiber differentiation.

6813

Wilken, H. 1964. Immunohistological demonstration of the gamma globulin in the tissue of the human placenta. Folia Histochem. Cytochem. 2:157-167.

Placentas of healthy and gestotic pregnant women were studied by FA. Staining was applied first in the form of the direct demonstration of antigens with labeled heterologous antiplacental sera. The indirect method was chiefly used. The placenta sections were treated first with non-pregnancy, pregnancy, and gestosis serum and then stained with fluorescein-coupled antiglobulinic serum. No differences as to intensity and localization of the color reaction are found between normal and gestotic placentas. Treatments with non-pregnancy, pregnancy, and toxicosis sera gave practically identical staining results. The alpha globulins demonstrated with the labeled antiglobulin serum showed a characteristic localization in the placental tissues. These occur mainly in the following structures of the placenta: decidua graviditatis, villous epithelium, villous stroma, amnion epithelium, tunica media, and adventitia of the fetal blood vessels.

6814

Willson, J.T.; Katsh, S. 1964. Localization of spermatozoal antigens by fluorescent antibodies. Anat. Rec. 148:411.

FA localization of antigenic sites of guinea pig epididymal spermatozoa has been accomplished. Both direct and indirect methods were used on living epididymal spermatozoa as well as on dried sperm smears and testicular prints. The results were the same in both methods and were specific. Spermatozoa from the distal portion of the epididymis displayed brilliant green fluorescent acrosomes and less intensely stained midpieces and principal pieces when treated as dried smears. Treated living spermatozoa fluoresced as did the dried preparations but in addition developed small clumps of fluorescent material that adhered to the cell membrane of the head, midpiece, and principal piece. The main difference in the staining of the acrosomes of epididymal and testicular spermatozoa was that in the latter only the inner acrosomes displayed the intense fluorescence. Kinoplasmic droplets also fluoresced. Cross-reactivity was found with human but not with rat spermatozoa.

6815

Winick, M.; Greenberg, R.E. 1965. Chemical control of sensory ganglia during a critical period of development. Nature 205:180-181.

Sensory ganglia from chick embryos undergo a sequence of differentiative phases. Between the 5th and 6th day, a change occurs wherein they develop a characteristic response to nerve-growth-promoting protein manifested on the 7th day whether left in vivo or cultured in vitro. At 13 days, another change occurs in which ganglia lose their capacity to respond to NDG only if left in vivo. NGF does not seem to effect the initial change, but the loss of reactivity may be under control of the nerve growth factor. The developmental sequence described in these investigations provides a model system for the correlation of morphological and biochemical events during various phases of differentiation of sensory ganglia. FA demonstrated the permeability of ganglia by nerve-growth-promoting protein.

6816

Woolf, N.; Pilkington, T.R.E. 1965. The immunohistochemical demonstration of lipoproteins in vessel walls. J. Pathol. Bacteriol. 90:459-463.

The application of FA to a study of the distribution of lipoproteins within the arterial intima shows that beta-lipoproteins are present in a variety of patterns of localization in atherosclerosis. In uncomplicated lesions, the lipoprotein may be derived from circulating plasma as a result of a filtration process.

B. PATHOLOGY

6817

Barland, P.; Sandson, J.; Janis, R. 1964. Immunofluorescent studies of normal and osteoarthritis articular cartilage. Arth. Rheum. 7:290.

The distribution of the proteinpolysaccharides in human articular cartilage has been studied by indirect FA. Antisera were prepared in rabbits to the light (PP-L) and heavy (PP-H) proteinpolysaccharides extracted from normal human articular cartilage. Antisera to PP-H and PP-L gave similar patterns of fluorescent staining. In normal articular cartilage the chondrocyte cytoplasm and lacunar contents stained intensely, while the matrix stained faintly. The staining in the matrix was granular and characteristically distributed around the chondrocytes in the various zones of articular cartilage. Prior treatment of the cartilage sections with testicular hyaluronidase at 37 C for 18 hours produced a marked change in the staining of the normal matrix. The staining became more intense, widely distributed, and more homogeneous. These changes correlated with loss of metachromatic staining of the same areas. The pattern obtained with osteoarthritic cartilage differed from normal. In the superficial zone, which exhibited loss of metachromasis, the FA was intense, homogeneous and widely distributed. Polysaccharides in cartilage matrix are depolymerized in osteoarthritis.

6818

Boss, J.H. 1965. Basement membrane antigens under pathological conditions: Alterations in the immunohistologic appearance of membrane antigens in the placenta of the D2-hypervitaminotic rat. Arch. Pathol. 79:340-344.

Hypervitaminosis D2 in pregnant rats induces severe lesions in the placental allantoic villi characterized by the interstitial deposition of an abnormal foreign substance associated with degeneration of the trophoblastic cells and disappearance of stainable basement membranes. Examination of such placentas by FA reveals progressive aberrations in the distribution pattern of the basement membrane antigens. Irregularities and discontinuities in the specific fluorescence of the basement membranes constitute the earliest recognizable changes. In the most advanced lesions allantoic villi devoid of demonstrable basement membranes are encountered

6819

Franklin, R.R.; Dukes, C.D. 1964. Antispermatozoal antibody and unexplained infertility. Amer. J. Obstet. Gynecol. 89:6-9.

In an attempt to establish the frequency of occurrence of antispermatozoal antibody in women whose fertility status had been determined, 89 patients were studied for the presence of circulating antispermatozoal antibody. Such an antibody was found in 78.9 per cent of the patients with no demonstrable organic cause for infertility, in 10.37 per cent of those with organic reason for infertility, in 11.8 per cent of the patients of known fertility, and in 4.27 per cent of the patients of unknown fertility. The results of the study strongly suggest that vaginally deposited spermatozoa can provide an antigenic stimulus resulting in the elaboration of circulating antispermatozoal antibodies in susceptible women. Statistically, the results indicate a strong correlation between the existence of circulating antispermatozoal antibody and unexplained infertility.

6820

Geld, H., van der. 1964. Anti-heart antibodies in the postpericardiotomy and the postmyocardial-infarction syndromes. Lancet 2:617-621.

Anti-heart antibodies were detected in sera of 13 of 15 cases with the postpericardiotomy syndrome and in 8 of 14 cases with the postmyocardial-infarction syndrome using indirect FA and the antiglobulin-consumption test. Anti-heart antibodies were also found after cardiac surgery in sera of patients without concomitant signs of the postpericardiotomy syndrome.

6821

Hammarstrom, S.; Lagercrantz, R.; Perlmann, P.; Gustafsson, B.E. 1965. Immunological studies in ulcerative colitis: I. Colon antigen and human blood Group A- and H-like antigens in germfree rats. J. Exp. Med. 122:1075-1086.

Sera from patients with ulcerative colitis contain antibodies that hemagglutinate sheep red cells sensitized with phenol-water extracts from colon, cecum, or feces of germfree rats. Minor concentrations of such antibodies are also present in a certain fraction of normal human sera. Hemagglutination and hemagglutination inhibition experiments with human erythrocytes and with the rat extracts showed that the latter contained an antigen similar to human blood Group A antigen. In contrast, a blood antigen like Group B could not be detected in these extracts. However, experiments with eel serum indicated that these extracts also contained an antigen similar to the H antigen of the human ABO system. Hemagglutination inhibition experiments indicated that A, H, and colon antigen were widely distributed throughout the gastrointestinal tract of the germfree rats. The colon

antigen was enriched in the extracts from colon, cecum, and feces. Fluorescent antibody staining provided evidence that both the colon antigen and the A antigen were present in similar sites of the colon and cecum mucosa, particularly in goblet cells of the crypts, and in the mucus.

6822

Kirsner, J.B. 1965. The immunologic response of the colon. J. Amer. Med. Ass. 191:809-814.

Present data do not demonstrate an immune basis for ulcerative colitis, although clinical and laboratory features continue to implicate hypersensitivity influences, perhaps as secondary factors. The colon is capable of generating a variety of immunologic reactions, but attempts to produce unequivocally an immune colitis by the injection of antisera to animal colon remain inconclusive. Although results vary, at least several types of antigens are present in the colon, and circulating reactants (antibodies) apparently directed to constituents of normal and colitis colon mucosa, are demonstrable in patients with ulcerative colitis, and very occasionally in patients with other diseases. The antibodies may represent a secondary immunologic response to preexisting, nonimmunologic injury of the colon. Bacteria or bacterial components, perhaps acting as haptenes, may contribute to the possible antigenicity of the colon tissue. Hypersensitivity mechanisms of the cell-bound type, perhaps involving components of the cellular infiltrate, may participate in the pathogenesis of ulcerative colitis. Finally, immunosuppressive therapy may provide another, albeit indirect, approach to the study of possible immune factors in ulcerative colitis.

6823

Kolker, I.I. 1963. A study of the rate of anti-kidney antibodies binding by a homologous organ with the microfluorescent method. Zh. Obshch. Biol. 24:3:226-229. In Russian.

When using FA it was shown that anti-kidney antibodies that were passively injected into the blood were capable of very rapid localization in a homologous organ, and especially in ball capillary clusters. Simultaneously the rapid removal of localized antibodies from the blood was noted. During the first 20 to 30 minutes the majority of such antibodies were removed. Subsequently they continued to circulate in blood in a very small amount for some time.

6824

Marena, F. 1963. Demonstration of anti-lens antibody in the sera of cataract patients. Ann. Ottalmol. 89:907-910. In Italian.

The FA test was used to demonstrate the existence of anti-lens antibodies in the serum of cataract patients. FA was also used to demonstrate the capacity of the crystalline bodies of cataract patients to fix specific antiglobulin.

6825

Mills, P.V.; Shedden, W.I.H. 1965. Serological studies in sympathetic ophthalmitis. Brit. J. Ophthalmol. 49:29-33.

Sera from five patients with sympathetic ophthalmitis were examined by each of three immunological techniques in an attempt to demonstrate antibodies for uveal tissue. The techniques used were fluorescent immunology, passive cutaneous anaphylaxis, and complement fixation. No evidence of circulating antibodies for uveal tissue was obtained.

6826

Rauch, H.C.; Raffel, S. 1963. Immunofluorescent localization of encephalitogenic protein in myelin. Federation Proc. 22:332:216.

Normal human, guinea pig, and bovine spinal cord tissue sections were exposed to rabbit anti-encephalitogenic protein serum. Similar control sections were treated with normal rabbit serum, absorbed immune rabbit serum, or buffered saline. Following the application of goat antirabbit gamma globulin labeled with fluorescein isothiocyanate, only those sections exposed to the specifically immunized rabbit serum showed fluorescent myelin structures. It is concluded that the encephalitogenic protein is an organ-specific antigen common to various species. It is localized in the myelin, the target tissue of experimental allergic encephalomyelitis. Complete article.

6827

Roitt, I.M.; Ling, N.R.; Doniach, D.; Couchman, K.G. 1964. The cytoplasmic auto-antigen of the human thyroid: I. Immunological and biochemical characteristics. Immunology 7:375-393.

The reaction between the thyroid microsomal antigen and Hashimoto sera was studied by quantitative complement fixation. Iso-fixation curves indicated a heterogeneity among individual sera and cross-absorption experiments suggested that this could be attributed to antibodies with differing abilities to fix complement with the same antigenic determinants. The results of equilibrium density gradient centrifugation, electron microscopic and immunofluorescent studies were consistent with an association of the antigen with small smooth-surfaced vesicles in the microsomal fractions. The microsomal antigen may provide a model for organ-specific components in other tissues able to provoke or sustain autoimmune diseases.

6828

Tan, E.M.; Kaplan, M.H. 1963. Immunological relation of basement membrane and a serum beta globulin in the mouse: Demonstration of renal basement membrane alteration in mice injected with streptolysin S. Immunology 6:331-344.

Antiserum to a fraction of mouse beta globulin isolated by electrophoresis and column chromatography was found reactive by immunofluorescence with renal basement membrane of glomeruli and tubules, and with basement membrane and connective tissue in other organs. This method for staining renal basement membrane was applied to the study of renal lesions produced in mice by streptolysin S. In lesions characterized by early changes of acute tubular necrosis a marked decrease in ability to stain tubular basement membrane was observed. Reappearance of staining property in basement membrane was linked to re-regeneration of diseased tubules. Repeated injections of streptolysin S produced alterations in staining also of glomerular basement membrane.

VI. OTHER TISSUE SUBSTANCES

6829

Anderson, G.F.; Barnhart, M.I. 1964. Intracellular localization of prothrombin. Proc. Soc. Exp. Biol. Med. 116:1-6.

Microsomal localization of prothrombin identifies the intracellular sites for synthesis. Prothrombin-deficient animals lacked this localization, but normal and vitamin K1 - stimulated animals displayed it. Microsomes are evidently involved in synthesis of specific proteins.

6830

Anderson, G.F.; Barnhart, M. 1964. Prothrombin synthesis in the dog. Amer. J. Physiol. 206:929-938.

Liver parenchymal cells were found to be the sites for production of prothrombin. Dogs were employed and their rate of prothrombin synthesis was modified as desired. FA was a valuable aid in evaluating liver cell function. Under normal conditions there is periodic synthesis of prothrombin with only 10 per cent of the parenchymal cells actively participating at any one time. Prothrombin-deficient or normal dogs can be stimulated to more vigorous activity by giving vitamin K 1. In such circumstances all the liver parenchymal cells were able to synthesize prothrombin. A regulatory mechanism promotes synthesis, storage, or release of prothrombin by liver parenchymal cells. No other cell types of the liver, spleen, or bone marrow appeared capable of synthesizing prothrombin

6831

Barnes, G.W.; Soanes, W.A.; Mamrod, L.; Gonder, M.J.; Shulman, S. 1963. Immunologic properties of human prostatic fluid. J. Lab. Clin. Med. 61:578-591

The antigens of human prostatic fluid have been compared with those of other tissues by immunochemical methods Prostatic fluid contains at least four or five distinctive antigens, as well as other antigens shared with blood serum By direct immunodiffusion and by inhibition of immunodiffusion, it was demonstrated that some specific prostatic fluid antibodies were not affected in their homologous reaction by the presence of plasma or plasma constituent. Pooled prostatic fluid itself, however, inhibited the homologous reaction completely. By immunoelectrophoresis the reaction of prostatic fluid with antiprostatic fluid serum was found to be dissimilar to that obtained with prostatic fluid against antiserum to human serum. The electrophoretic fraction

participating in the formation of the major homologous precipitin band was located in and about the slow beta globulin zone. The specificity of the reaction of rabbit antihuman prostatic fluid serum was confirmed by indirect FA staining of frozen sections of the prostate gland. Staining reaction involved chiefly acinar cells and luminal materials.

6832

Barnes, G.W.; Soanes, W.A.; Mamrod, L.; Gonder, M.J.; Shulman, S. 1963. Immunologic studies of prostatic fluid. Federation Proc, 22:2020:498.

The antigens of human and canine prostatic fluid, PF, have been studied by immunologic methods, using gel diffusion, passive agglutination, and immunofluorescence to study the heterogeneity and the specificity of these antigenic mixtures. In the human system, PF was shown to contain at least four or five characteristic antigens, as well as other antigens that are shared with blood serum. By immunoelectrophoresis the reaction of PF with anti-PF serum was found to be dissimilar to that obtained with PF against antiserum to human serum. The fraction participating in the formation of the major homologous precipitin band was located in and about the slow-beta-globulin zone. The specificity of the reaction of rabbit antihuman PF serum was confirmed by indirect immunofluorescent staining of frozen sections of the prostate gland. The staining reaction involved chiefly acinar cells and luminal materials. Tanned cell hemagglutination has shown positive reactions between these antisera and PF-coated cells. In the canine system, PF was shown to contain two components in preliminary studies. Complete article.

6833

Barnhart, M.I. 1965. Prothrombin synthesis: An example of hepatic function. J. Histochem. Cytochem. 13:740-751.

Prothrombin, a plasma protein valuable in blood coagulation, appears to be produced exclusively by liver parenchymal cells. FA procedures were applied to various organs from bovine, canine, and human sources. Since anti-prothrombin was species specific, each species required its own fluorescent antiprothrombin as a cellular marker. Reticuloendothelial cells were not reactive with fluorescent antiprothrombin. In normal individuals prothrombin synthesis occurred in a cyclic asynchronous fashion with significant quantities of prothrombin contained in only 10 to 30 per cent of the hepatocyte population. Essentially all hepatocytes were able to synthesize prothrombin when stimulated by vitamin K 1. The rate of prothrombin synthesis depended upon both the level of plasma prothrombin and the amount of vitamin K 1 available to the hepatocytes. From quantitation of plasma prothrombin during drug alteration of prothrombin synthesis in dogs the average prothrombin replacement time was calculated as 9.5 hours.

6834

Baum, J.; Simons, B.E., Jr.; Unger, R.H.; Madison, L.L. 1963. Localization of glucagon in the alpha cells in the pancreatic islet by immunofluorescent technics. Diabetes 11:371-374.

Localization of the site of glucagon production has been indirect in all previous studies. Immunofluorescent techniques were utilized to identify the site of origin of glucagon. A specific rabbit anti-beef-pork glucagon antiserum was used to identify glucagon in the pancreatic islet. FITC labeled goat antirabbit antiserum was the fluorescent tag that permitted visualization of the site of the anti-glucagon-glucagon reaction in bovine pancreas. Appropriate control studies demonstrated the specificity of the reaction. In serial sections alpha cells were located by the dark field illumination technique. The fluorescent tag showed glucagon to be located in those fields identified as alpha cells in adjacent 4-u sections. This could be differentiated from the beta cells identified by anti-insulin antiserum. These data provide the first direct evidence for the production of glucagon in the alpha cells of the pancreatic islet.

6835

Berns, A.W.; Blumenthal, D.S.; Blumenthal, H.T. 1963. Effect of heterologous insulin antibody on the developing cells of the islets of Langerhans of the chick embryo. Federation Proc. 22:220:197.

Rabbit anti-insulin globulin was injected into chick embryos every 3 days from incubation to hatching. Controls consisted of non-injected embryos and those receiving normal rabbit globulin. Embryos were sacrificed at hatching and at 3 or 4 days after hatching. Blood was obtained for glucose determinations and the pancreas was fixed for histochemical and fluorescence microscopy study. There was a moderate increase in blood glucose of the passively immunized animals and a decrease in the average size of the islet cells, as well as a reduction in the average number of cells per islet. However, there was no evident change in the density of granules per beta cell. The embryos injected with immune serum showed binding of fluorescein-conjugated insulin by islet beta cells that could be specifically inhibited with nonfluorescent insulin. Controls showed binding of fluorescent anti-insulin serum by the beta cells and to a lesser degree by the passively immunized embryos. Both normal- and immune-serum-injected embryos showed binding of goat anti-RGG by beta cells. There was no binding of fluorescent snake venom, used as a protein control, by the beta cells of embryos injected with immune serum. Complete article.

6836

Berns, A.W.; Owens, C.T.; Blumenthal, H.T. 1964. A histo- and immunopathological study of the vessels and islets of Langerhans of the pancreas in diabetes mellitus. J. Gerontol. 19:179-189.

A comparison of the frequency of various types of vascular lesions in the pancreas of diabetic and nondiabetic groups revealed a threefold increase in endothelial proliferative vascular lesions, and a smaller increase in thrombotic and atheromatous vascular lesions in the diabetics. There was a slight increase in atheromatous lesions when hyalinization of the islets was also present. In a high percentage of cases hyaline islets were noted to specifically bind fluorescein-conjugated insulin as well as fluorescein antihuman globulin. This occurred in both diabetic and nondiabetic groups. Similar binding was noted in the proliferative vascular lesions. Maturity-onset diabetes may be an age-related autoimmune disease with altered insulin as the responsible antigen. In this context the proliferative vascular lesions are considered to be an expression of an immune state, and the hyaline (amyloid) deposits in the islets as an age-related phenomenon representing a deposition of an insulin-insulin antibody complex.

6837

Blau, S.; Janis, R.; Hamerman, D.; Sandson, J. 1965. Cellular origin of hyaluronateprotein in the human synovial membrane. Science 150:353-355.

Bright FA staining, which indicated the presence of hyaluronateprotein, was observed in the lining cells of the synovial membrane following application of rabbit antiserum to hyaluronateprotein and a fluorescein-labeled antiserum to rabbit gamma globulin. Staining was shown to be specific and due to antigenic determinants on or closely associated with the protein moiety of hyaluronateprotein.

6838

Blau, S.; Janis, R.; Hamerman, D.; Sandson, J. 1965. Localization of hyaluronateprotein in synovial lining cells by immunofluorescent methods. Arth. Rheum. 8:432.

Hyaluronate in synovial fluid arises from lining cells in the synovial membrane. Lining cells are present on the surface of the synovial membrane. Cytochemical methods visualize higher oxidative and hydrolytic enzyme activities in the lining cells than in subsynovial fibroblasts. Both light and electron microscopic studies indicate a complex fine structure of the lining cells compatible with synthetic and secretory activities. The purpose of this study was to localize hyaluronate in cells of the synovial membrane. Synovial membrane was obtained from normal joints. Antisera to HP, absorbed

with normal human serum, were layered on the slides. Fluorescein-labeled antirabbit gamma globulin was added. Bright fluorescence was localized to lining cells and their intercellular spaces, and occasionally to blood vessel walls. Appropriate controls were negative. Absorption of antisera with whole normal HP almost completely abolished fluorescence. When antisera to normal HP were absorbed with either the polysaccharide or the protein part of normal HP, only the protein part abolished fluorescence. This shows that antigenicity resides in the protein. Hyaluronate-protein is present in the lining cells of the synovial membrane and is presumably synthesized there.

6839

Blumenthal, D.S.; Berns, A.W.; Blumenthal, H.T. 1964. Anti-insulin serum effects on islets of Langerhans of chick embryo. Arch. Pathol. 77:107-112.

Chick embryos were divided into three groups, one serving as noninjected controls, the second as normal serum injected controls, and the third as an anti-insulin serum injected group. The anti-insulin serum was obtained from a male rabbit immunized against alum-precipitated insulin, and normal serum was obtained from a male rabbit of comparable weight. The anti-insulin injected group showed a higher average blood sugar level and a larger average size of the beta cells of the islets of Langerhans than the control groups. However, the average islet size was smaller in the experimental than in either control group, and there was no difference between the three groups in the density of beta cell granulation. By fluorescence microscopy it was possible to demonstrate that the anti-insulin globulin enters the beta cells where it is bound by the beta granules.

6840

Briard, A.A.; Quinn, L.Y. 1964. Demonstration of antipenicillinase Bacteriol. Proc M112:65.

It is possible to demonstrate the presence of circulating and noncirculating antipenicillinase by complement fixation and fluorescent antibody techniques. Further study indicates that it may be possible to produce antipenicillinase in primary cultures of nonimmunized rabbit spleen, and to demonstrate the presence of the antibody with fluorescent antibody techniques. Rabbits were immunized with the enzyme penicillinase by a series of six injections of concentrated penicillinase (20 million units) followed by a booster of the same concentration. Circulating antipenicillinase was demonstrated in the serum by complement fixation The use of various adjuvants did not stimulate the production of antipenicillinase. Noncirculating antipenicillinase was demonstrated by fluorescent antibody techniques. Various lymph nodes of the head and neck region, the spleen,

and the liver of immunized rabbits were sectioned and FA-treated. Circulating antipenicillinase titers ranged from 1:16 to 1:128. Noncirculating antipenicillinase was demonstrable with fluorescent antibody techniques in all the above-mentioned tissue. Antipenicillinase was also present in the primary rabbit spleen culture.

6841

Burstein, R.; Berns, A.W.; Hirata, Y.; Blumenthal, H.T. 1963. A comparative histo- and immuno-pathological study of the placenta in diabetes mellitus and in erythroblastosis fetalis. Amer. J. Obstet. Gynecol. 86:66-76.

In study comparing the normal placenta with that in diabetes and erythroblastosis, proliferative vascular lesion was demonstrated that binds fluorescent insulin specifically in the diabetic placenta and fluorescent anti-Rh serum in the erythroblastotic placenta. Neither the vascular endothelium nor the syncytial knots bind the fluorescent reagents; the antigen-antibody complex evidently diffuses through these cells and is trapped in basement membrane structures. Serological studies indicate that in both disease states maternal antibodies cross the placental barrier and enter the fetal circulation. Binding of fluorescent reagent by placental tissue in cases with negative titers indicates the presence of fixed tissue antibody. Pathological and clinical evidence suggests that the latter is more directly related to the occurrence of placental lesions than is circulation antibody. In the case of diabetic subjects who have never received insulin, the binding of fluorescent insulin may be indicative of an autoimmune phenomenon.

6842

Craig, J. 1964. The distribution of surface active material in the lungs of infants with and without respiratory distress. Biol. Neonatorum 7:185-202.

Isolated surface active material from human lungs obtained at autopsy was used for preparation of an antibody in rabbits. This was used to localize, by FA, surface active material in the lungs of stillborn and liveborn immature infants dying in the first few days of life. The surfactant was absent from most very immature infants. In slightly older premature infants' lungs it was present in the cells of the proximal alveolar walls; in infants with body weights above 1,300 grams it commonly appeared in the alveolar spaces as well. In infants with hyaline membranes, the membranes appeared to contain the material, although the remainder of the lung was completely negative in severe cases.

6843

Craig, J.M. 1965. Histological distribution of ferritin. Arch. Pathol. 79: 435-440.

Ferritin has been localized in the tissues of the newborn in the reticulo-endothelial cells, the liver parenchymal cells, and the renal tubules with fluorescein-labeled antibody prepared in the rabbit, using crystalline human ferritin as antigen.

6844

Dallenbach-Hellweg, G.; Battista, J.V.; Dallenbach, F.D. 1965. Immunohistological and histochemical localization of relaxin in the metrial gland of the pregnant rat. Amer. J. Anat. 117:433-450.

The presence of relaxin in uteri, placentae, and ovaries of pregnant rats was studied with fluorescein labeled anti-relaxin serum and confirmed by histological and histochemical techniques. In sections of uteri from day 12 of pregnancy stained with these antisera, the cytoplasm of the granular cells of the mesometrial decidua gave off fluorescence indicating anti-relaxin binding. On day 15, 17, and 19, the granular cells of the metrial gland and occasional lakes in nearby vascular lumina fluoresced. No other specific fluorescence was evident in the uteri or placentae. The fluorescent regions of these cells corresponded exactly with the cytoplasmic aggregates of granules in the metrial gland and mesometrial decidua. Histochemical studies of these granules, which bound with anti-relaxin, revealed a chemical composition congruent with that known for relaxin. The dissolution of connective tissue fibers, believed to be a main function of relaxin, was observed in the last days of pregnancy within the metrial gland. The relaxin-containing cells in the human and in the rat differ only in their localization in the tissue, which depends on their phylogenetic adaptation to different placentational needs.

6845

Della Corte, F.; Biondi, A. 1964. Localization of anti-FSH fluorescent antibody in the hypophysis of pig (Sus scrofa L. var. domestica Gray). Riv. Biol. 57:359-368. In Italian.

By means of FA followed by staining of the same sections by PAS, Galgano II, and Galgano-Della Corte methods, one can demonstrate that the anti-FSH antibody of pig labeled with fluorescein isothiocyanate is localized in the major portion of the beta cells of the hypophysis of the pig. There is also a nonspecific fluorescence in some alpha cells. It is obtained also when a nonspecific fluorescent globulin is used. It does not disappear when the section is preliminarily treated with unmarked antibody.

6846

Dvorak, H.F.; Cohen, R.B. 1965. Fluorescent antibody and histochemical study of rabbit muscle phosphorylase. Lab. Invest. 14:559-560.

FA has been employed to localize rabbit muscle phosphorylase (alpha-1,4-glucan-orthophosphate glucosyl-transferase) within striated muscle cells. The findings are correlated with classical histochemical studies of phosphorylase activity. Pooled sera, obtained from guinea pigs sensitized against rabbit phosphorylase alpha, twice crystallized, in complete Freund's adjuvant gave a single line of precipitation with the enzyme in Ouchterlony plates at 1:32. Such sera, tagged with FITC and applied to thin frozen sections of rabbit striated muscle, produced a specific, granular, fluorescing precipitate in the cytoplasm between myofibrils. Appropriate controls were satisfactory. After absorption of tagged, specific antisera with rabbit liver powder, staining was specific for striated muscle. Application of antisera to sections of rabbit muscle before incubation in substrate resulted in significant inhibition of histochemically demonstrable phosphorylase activity, whereas control sera had little or no inhibitory effect. These findings confirm the localization of phosphorylase and the inhibition of phosphorylase activity in tissue sections by specific antibody as demonstrated by studies in vitro. This permits the morphologist to evaluate and correlate enzyme activity and enzyme protein in tissue sections.

6847

Dvorak, H.F.; Cohen, R.B. 1965. Localization of skeletal muscle phosphorylase using a fluorescent antibody technique and its correlation with histochemical observations. J. Histochem. Cytochem. 13:454-460.

FA was employed to localize rabbit muscle phosphorylase within striated muscle cells Pooled sera from guinea pigs sensitized against 2X crystallized rabbit phosphorylase alpha in complete Freund's adjuvant gave a single line of precipitation with the enzyme in Ouchterlony plates. Such sera, tagged with FITC and applied to thin frozen sections of rabbit striated muscle, produced a specific, granular, fluorescing precipitate that was localized to the sarcoplasm between myofibrils. Preincubation with untagged specific antisera prevented specific staining. Appropriate control sera, similarly tagged, produced only slight nonspecific fluorescence. With properly absorbed specific antisera, staining was specific for striated muscle as opposed to rabbit liver or smooth muscle. The FA findings were correlated with those obtained by classical histochemical studies of phosphorylase activity.

6848

Ehinger, B. 1965. The cytological localization of rat pancreas ribonuclease by means of fluorescent antibodies. Histochemie 5:145-153.

The occurrence of ribonuclease in murine and bovine pancreas has been studied with FA. Results are identical in the two species. Staining occurred in the ergastoplasmic (basal) zone and in the nucleoli of the acinar cells. In the islets of Langerhans, only very faint or no nucleolar staining occurred. Extraction experiments indicate that ribonuclease might be present in at least two forms in the pancreas, one readily dissolvable and one that is more strongly bound to the cytoplasm.

6849

Ehinger, B.; Lagerstedt, S. 1963. Some histochemically important properties of ribonuclease isolated from fixed tissues. Histochemie 3:307-314.

As part of this study FA was employed. Specific fluorescence was seen in the pancreas, in the ergastoplasmic zones, and in the nucleoli. The clear pictures given by the fluorescent antibody technique, as adopted in the present paper, may indicate the actual site of the RNAase molecules. More critical work is necessary for elucidating the occurrence of various artifacts and the optimum conditions for staining.

6850

Emmart, E.W.; Spicer, S.S. 1964. The application of fluorescent antibody technique to the localization of endogenous prolactin in normal pituitary tissue and in a tumor of the pituitary. J. Histochem. Cytochem. 12:16.

The pleomorphism of the cellular components of the pituitary, together with the fact that muliple hormones are known to be present in the pituitary, limits the interpretation of those cellular changes demonstrated by various histochemical staining techniques. Using FA endogenous prolactin has been localized precisely in the acidophils of the anterior pituitary. By the same procedure prolactin has been demonstrated in the tumor tissue of a transplantable rat pituitary tumor. Complete article.

6851

Emmart, E.W.; Schimke, R.T.; Spicer, S.S.; Turner, W.A. 1963. The localization of glyceraldehyde 3-phosphate dehydrogenase in kidney tissue by means of fluorescent antibody. Exp. Cell Res. 30:460-475.

Rabbit muscle glyceraldehyde 3-phosphate dehydrogenase, highly purified by recrystallization, has been used as an antigen in guinea pigs. Antisera have been obtained that readily precipitate the enzyme and inhibit its activity. An immunochemical evaluation of the precipitability of this preparation with antisera revealed that after simple diffusion in agar gel, pH 7.2, and during electrophoresis in barbital buffer, pH 8.6, the enzyme dissociates into two components, each having a different motility. Separation and immunochemical evaluation of these components have shown that both are enzymatically active and are inhibited by the same antisera. It seems, therefore, that the appearance of multiple precipitins in gel and in saline dilutions of the antigen is not due to heterologous contaminants. The localization of the enzyme in the rat kidney by the fluorescent antibody technique has been described.

6852

Emmart, E.W.; Spicer, S.S.; Bates, R.W. 1963. Localization of prolactin within the pituitary by specific fluorescent antiprolactin globulin. J. Histochem. Cytochem. 11:365-373.

It has long been recognized that the pleomorphic changes that occur in the pituitary gland during estrogenic cycles in the experimental animal are probably associated with hormonal activity. Although various histochemical staining techniques demonstrate the particular cells concerned in these changes, the presence of a particular hormone cannot be directly demonstrated by these methods. Using ovine prolactin as an antigen with Freund's adjuvant, it has been possible to produce precipitating antisera capable of inhibiting the action of prolactin on the mucosa of the pigeon crop sac. By means of fluorescent antiprolactin globulin, prolactin has been demonstrated in the cytoplasm of the acidophilic (alpha) cells of the pars distalis of the cat and rat pituitary.

6853

Finck, H. 1963. Localization of amylase by fluorescent antibody. Anat. Rec. 145:229.

Thrice crystallized hog pancreatic amylase was used to produce antisera in chickens. The gamma globulin fraction of immunized chicken serum inhibited amylatic activity of solutions of the enzyme. When tested against the enzyme in various gel diffusion procedures, immune gamma globulin produced a single precipitation zone. Fluorescein-labeled immune gamma globulin was used to detect amylase in sections of frozen-dried or chemically fixed hog pancreas, liver, and parotid glands. All acinar and hepatic parenchymal cells were positive. The patterns of cytoplasmic fluorescence varied from uniformly diffuse to discrete (zymogen granules) in pancreas and parotid glands; hepatic parenchymal cell cytoplasm was uniformly and diffusely fluorescent. Appropriate controls showed that the localizations were specific. Complete article.

6854

Fisher, J.M.; Rees, C.; Taylor, K.B. 1965. Antibodies in gastric juice. Science 150:1467-1469.

The presence in gastric juice of specific antibody has been demonstrated. It is mainly an IgG antibody reacting with the cytoplasm of gastric cells. It has been detected in patients with atropic gastritis, with or without pernicious anemia, whose sera contain antibodies to parietal-cell cytoplasm. Evidence is presented that associated circulating antibody to cytoplasm of thyroid acinar cell does not appear in the gastric juice.

6855

Fisher, J.W.; Taylor, G.; Porteous, D.D. 1964. Erythropoietin localization in the glomeruli of sheep kidney using a fluorescent antibody technique. Blood 24.846.

The renal site of erythropoietin production was studied by FA. Anti-erythropoietin serum was prepared by injecting young rabbits intradermally with sheep erythropoietin in complete Freund's adjuvant. Antigen-adjuvant emulsion containing 56 units of erythropoietin was injected intradermally (0.1 ml into each footpad) on the first day. Injections were repeated one week later into four sites on the back of the neck. The animals were bled 2 weeks later. The anti-erythropoietin serum showed eight precipitin lines when tested by immunoelectrophoresis. Only one line was seen after absorption with diluted normal sheep serum. Marked neutralization of the biological effects of erythropoietin was produced by the anti-erythropoietin serum. Frozen sections of normal sheep kidney were treated with the absorbed antiserum and conjugate

in an indirect FA test. There was intense fluorescence of the capillary walls of the glomerular tufts. Glomeruli in control sheep kidney sections or other capillaries in the kidney not associated with glomerular tufts failed to show this specific fluorescence. The intense fluorescence seen in the capillary walls of the glomerular tufts suggests that erythropoietin is either produced or stored in these cells.

6856

Fisher, J.W.; Taylor, G.; Porteous, D.D. 1965. Localization of erythropoietin in glomeruli of sheep kidney by fluorescent antibody technique. Nature 205:611-612.

The site of erythropoietin elaboration is the glomerular tuft. The intense fluorescence of the capillary wall suggests that erythropoietin is either produced or stored in these cells. The fact that the erythropoietin antibody was predominantly antiglomerular, with complete absence of renal tubular staining, is good evidence that the renal tubules are not a site of production of erythropoietin.

6857

Fogel, M.; Koffler, D. 1964. Immunofluorescent localization of LH and FSH in fresh-frozen and formalin-fixed sections of the human adenohypophysis. Federation Proc. 23:1855:411.

Antisera to human chorionic gonadotropin, HCG, and human menopausal gonadotropin, HMG, were prepared in rabbits and assayed for purity by immunoelectrophoresis following absorption of contaminating antibodies to follicle-stimulating hormone, FSH, from anti-HCG serum and of contaminating antibodies to luteinizing hormone, LH, from anti-HMG serum. The antigenic properties of LH and FSH in fresh-frozen and formalin-fixed tissue were studied by the fluorescent antibody technique. LH was localized by purified anti-HCG serum in both frozen and formalin-fixed paraffin-embedded tissues; however, the intensity of fluorescence was somewhat decreased in the formalin-fixed material. FSH localization by purified anti-HMG serum was similarly noted in fresh-frozen tissue, but could not be demonstrated in formalin-fixed preparations. This contrast in staining qualities between anti-HCG serum and anti-HMG serum in formalin-fixed tissues further demonstrates a difference in immunological specificity between these antisera. Complete article.

6858

Frank, G.M.; Samosudova, N.V.; Kryukova, M.Ye.; Kalamkarova, M.B.; Ogievetskaya, M.M. 1963. Localization of muscle protein in isolated myofibrils by fluorescent antibody techniques. Biofizika 8:5:569. In Russian.

Photographs of FA-labeled myofibrils indicate the redistribution of proteins of the actomyosin complex during extension and contraction. The change in the localization of antibodies corresponds to the sliding model of Huxley only to a certain degree. This conformity is expressed in the expansion and contraction of the central fluorescent bands of the A-disc, coincident with the H-zone, and in the expansion and contraction of the fluorescent bands of the I-disc. On the other hand, the behavior of the proteins under study during the stage of strong contraction does not conform to the picture of the actin filaments pushed into the anisotropic disc. The discrete distribution of antibodies in myosin and its components indicates the existence of heterogeneity of the myosin filaments along their length within the anisotropic disc.

6859

Glynn, A.A.; Parkman, R. 1964. Studies with an antibody to rat lysozyme. Immunology 7:724-729.

An antibody to rat lysozyme was prepared and its activity and specificity were studied by inhibition and double diffusion techniques. By means of this antibody, together with fluorescent antirabbit globulin, the distribution of lysozyme in rat and mouse polymorphs and macrophages has been examined and some changes that follow phagocytosis were demonstrated.

6860

Goldstein, M.N.; Burdman, J.A. 1965 Studies of the nerve growth factor in submandibular glands of female mice treated with testosterone. Anat. Rec. 151:199-207.

The effect of testosterone propionate administration on the quantity and localization of a nerve-growth protein was studied in the submandibular glands of adult female Swiss mice. Testosterone treatment for 14 to 24 days resulted in an increase in the quantity of a nerve-growth-promoting protein, approaching those levels found in normal untreated adult male Swiss mice. The nerve-growth-promoting protein was localized by immunofluorescent techniques in the cytoplasm surrounding the zymogenic granules of the serous tubular portions of the submandibular glands in both control and treated animals. The role played by the submandibular gland in the synthesis and storage of this factor is discussed.

6861

Hachmeister, U.; Kracht, J. 1965. Antigenic properties of beta-1-24-corticotropin. Arch. Pathol. Anat. Physiol. Klin. Med. 339:3:254-261. In German.

Using rabbit antisera produced against the synthetic beta-1-24-corticotropin, the antigen-containing cells in the anterior lobe of the pig hypophysis and of other species (man, dog, cat, rabbit) were FA stained. The results were comparable to those that were obtained with labeled antisera against hypophyseal ACTH, but were more precise because antibodies against impurities were excluded. Restaining with the periodic acid - Alcian blue - PAS - orange G technique disclosed that the fluorescing cells of the anterior lobe are identical with the R cells. The fluorescence was restricted to the granula-containing cytoplasm. In the region of the Golgi complex it was fainter, whereas the cell nucleus failed to stain. For immunohistological studies on cell bound corticotropic substances it seems recommendable to use such specific antibodies.

6862

Hansson, H.P. 1965. Demonstration of carbonic anhydrase by means of fluorescent antibodies in human erythrocytes. Life Sci. 4:965-968.

An immunofluorescence method is described for simultaneous demonstration of two iso-enzymes of carbonic anhydrase, HCA B and HCA C, in human erythrocytes. By means of a mixture of anti-HCA B globulin conjugates with lissamine rhodamine B, and anti-HCA C globulin conjugated with fluorescein isothiocyanate, both enzymes could be localized within each single erythrocyte.

6863

Hargis, G.K.; Yakulis, V.J.; Williams, G.A.; White, A.A. 1964. Cytological detection of parathyroid hormone by immunofluorescence. Proc. Soc. Exp. Biol. Med. 117:836-839.

The fluorescent antibody technique has been used to detect and to localize parathyroid hormone in the chief cells of bovine, human, and rat parathyroid tissue. Immunochemical cross-reaction has been found among these species. The oxyphil cells of human parathyroid tissue did not reveal specific fluorescence. This observation is regarded as further evidence that the oxyphil cells do not normally synthesize or store parathyroid hormone.

6864

Hartroft, P.M.; Sutherland, L.E.; Hartroft, W.S. 1964. Juxtaglomerular cells as the source of renin: Further studies with the fluorescent antibody technique and the effect of passive transfer of antirenin. Can. Med. Ass. J. 90:163-166.

Use of the fluorescent antibody technique indicates that the source of renin is the juxtaglomerular cell and not macula densa or other structures of the renal cortex. This concept is strengthened by the demonstration of juxtaglomerular cells containing granules in the fetal pig metanephros and fish mesonephros by fluorescent staining. Passive transfer of antirenin to sodium-deficient dogs blocked sodium retention, illustrating the importance of the renin-angiotensin system in the handling of sodium by the kidney. In these animals, staining with fluorescein-labeled antiglobulin suggests that antirenin entered the juxtaglomerular cell itself, although irreversible damage did not result.

6865

Hirschman, A.; Dziewiatkowski, D.D. 1964. Localization of chondromucoprotein in calf and rat epiphyseal cartilage using the fluorescent antibody technique. Anat. Rec. 148:291-292.

Direct FA was used to stain sections of epiphyseal cartilage. The cells in all of the zones of epiphyseal cartilage were positive. Reserve zone matrix was weakly positive, proliferative zone matrix was positive, and hypertrophic zone matrix was positive, but became weaker and then negative on approaching the spongiosa. Bone appeared to be negative except for some intrinsic fluorescence. Both calf and rat cartilage reacted similarly with anti-calf PP-L antibodies, as would be expected from the cross-reaction demonstrated by the Ouchterlony technique. There is a change in or loss of mucoprotein just prior to calcification.

6866

Hurlimann, J.; Thorbecke, G.J.; Hochwald, G.M. 1965. The liver as the site of C-reactive protein (CRP) formation. Federation Proc. 24:280:177.

Fluorescent antibody studies have suggested that radioactive CRP (CxRP) is formed by degenerating muscle whereas incorporation of C14 amino acid into CRP in vitro occurred in liver from appropriately stimulated animals only. Therefore CxRP and CRP production was studied again with rabbit and monkey tissues in vitro at varying times after different

in vivo stimuli. Autoradiography of immunoelectrophoretic patterns prepared with concentrated culture fluids and carrier was used to demonstrate incorporation of C14 lysine and isoleucine into CRP. Tissues from normal animals did not incorporate C14 amino acid into CRP. Liver tissue taken 24 hours after injection of pneumococci, endotoxin, paratyphoid vaccine, or turpentine actively produced CxRP or CRP. Occasional fetal monkey or human livers and regenerating monkey liver also produced CRP. Among the many tissues from treated animals that did not form CxRP was degenerating muscle from the injection site of paratyphoid vaccine. Such muscle tissue neither labeled CxRP nor influenced the ability of the liver to form CRP when cultured together. A correlation existed between the appearance of increased CxRP in the serum, increased production of other serum proteins, and formation of CxRP by liver. Complete article.

6867

Kalamkarova, M.B.; Samosudova, N.V.; Kryukova, M.Ye.; Ogievetskaya, M.M. 1963. Localization of muscle contractile proteins after denervation by labeled antibodies. Biofizika 8:6:696-698. In Russian.

After denervation a change is observed in the localization of muscle contractile protein in the femur of a chicken. The absence of fluorescence of the central bands of the anisotropic disc, corresponding to the localization of myosin and its subunits, is apparently connected with a decrease in the amount of these proteins and their replacement by proteins with other immunological properties. No changes were detected in the localization of antiactin.

6868

Kent, S.P. 1963. A study of mucins in tissue sections by the fluorescent antibody technique: III. The specificity of antibody to salivary gland mucins and the effect of chemical alterations of mucins on the specificity of the antibody. Ann. N.Y. Acad. Sci. 106:389-401.

Cow submaxillary mucin, human submaxillary mucin, the capsule of Cryptococcus, and blood group substances A and H were observed by the fluorescent antibody technique in formalin-fixed, paraffin-embedded tissue sections. The effect of a variety of chemical alterations of these antigens on their reactions with specific antibody was studied. The following are suggested: Free carboxyl and hydroxyl groups are essential to the reaction of the above antigens with specific antibody. Cow submaxillary mucin contains sialic acid, which is essential to the reaction of this mucin with its specific antibody; blood-group-specific substance A either does not contain sialic acid or if sialic acid is present it is not necessary for the serologic reaction; the introduction of phenylhydrazine or p-hydrazenobenzoic acid into the antigens following periodic acid oxidation often restores the reaction of the antigen with its specific antibody, but it is associated with a number of cross-reactions not seen in the controls.

6869

Kent, S.P. 1963. Study of tissue mucins using the fluorescent antibody technique: II. The preparation and specificity of human submaxillary gland mucin antibody. J. Histochem. Cytochem. 11.273-282.

Mucin was extracted from human submaxillary glands and purified. Antibodies to the mucin were produced in rabbits and labeled with FITC. The labeled antibody was used to demonstrate mucin in paraffin embedded tissue sections of human submaxillary glands. The antibody solution reacted with the mucin in human submaxillary glands but not with the submaxillary glands of other species. Cross-reactions were noted with epithelial mucins in human sublingual glands, lingual glands, submucosal glands of the trachea, gallbladder, pancreatic ducts, Bartholin glands, and cervical gl ds. The organ specificity of the antibody solution was improved by absorbing the antibody solution with sections of Bartholin glands, i.e. the cross-reactions noted with the Bartholin glands was eliminated but the antibody solutions continued to react with human submaxillary glands. The relationship of the antibody to human submaxillary mucins to blood group factors is discussed. Possible ways of obtaining antibodies with a high degree of organ specificity are noted.

6870

Keutel, H.J. 1964. Localization of uromucoid in human kidney and in sections of human kidney stone with the fluorescent antibody technique. J. Histochem. Cytochem. 13:155-160.

Fluorescein-labeled antibodies were used to demonstrate uromucoid. This urine-specific mucoprotein is demonstrably present only in the epithelial cells of the proximal segments of the normal human renal tubules and in the matrix of human kidney stones of all the common crystalline compositions.

6871

Keutel, H.J. 1965. Localization of uromucoid in human kidney and in sections of human kidney stone with the fluorescent antibody technique. J. Histochem. Cytochem. 13:155-160.

FA was used for the demonstration of uromucoid. This urine-specific mucoprotein is demonstrably present only in the epithelial cells of the proximal segments of the normal human renal tubules and the matrix of human kidney stones of all the common crystalline compositions.

6872

Keutel, H.J.; King, J.S., Jr.; Boyce, W.H. 1964. Further studies of uromucoid in normal and stone urine. Urol. Int. 17:324-341. In German.

The immunological technique has been applied to investigation of the urinary mucoprotein that has received several names in the literature according to the procedure used for its isolation. The following conclusions may be drawn: There is only one urine-specific mucoprotein (uromucoid) that can be separated by different methods. Uromucoid and its split products are immunologically identical. Uromucoid shows the same immunological reaction in normal urine, stone urine, and stone matrix. Sialic acid may or may not be found, according to the isolation procedure used. Kidney tissue (cortex and medulla) is the apparent source of uromucoid. Uromucoid, marked with fluorescent dyes, could be shown to participate in in vitro urine crystallization caused by bacterial alteration of the urine.

6873

Koffler, D.; Fogel, M. 1964. Immunofluorescent localization of LH and FSH in the human adenohypophysis. Proc. Soc. Exp. Biol. Med. 115:1080-1082.

Purified antisera to HCG and HMG, specific for LH and FSH, have been demonstrated to localize in cells of the human adenohypophysis by the fluorescent antibody technique.

6874

Lambotte, R.; Salmon, J. 1963. Transfer of amniotic fluid into maternal circulation. Bull. Soc. Roy. Belge Gynecol. Obstet. 33:269-278. In French.

Two specific elements were shown in human amniotic fluid by means of immunoelectrophoresis. Secretory origin was proved by FA. The passage of these elements into the maternal circulation during the respective phases of labor has been demonstrated. The hypothesis of an eventual maternal auto-immunization and its consequences is considered.

6875

Loewi, G. 1965. Localization of chondromucoprotein in cartilage. Ann. Rheum. Dis. 24:528:535.

Antibody to porcine cartilage chondromucoprotein has been used to stain sections of costal, articular, and tracheal cartilage by FA. The results have been compared with Alcian blue uptake under controlled conditions. The presence of chondromucoprotein was shown in the lacunar rim. In the surrounding matrix, staining was less marked, but was associated with the interterritorial regions. Alcian blue staining gave, in some instances, the reverse of the fluorescent staining pattern, in that there was strong staining of a fairly wide peri-lacunar zone, but very little uptake in interterritorial regions. Treatment of sections with hyaluronidase greatly increased FA staining both in intensity and extent. At the same time Alcian blue uptake was abolished. The action of hyaluronidase was most marked on unfixed sections. The hyaluronidase effect may be attributed to degradation and removal of polysaccharide providing for increased access of antibody to the protein moiety of chondromucoprotein. Regional staining differences could be due to different composition of the chondromucoprotein polymer in the various territories but could also be accounted for by the property of excluded volume.

6876

Loewi, G.; Muir, H. 1965. The antigenicity of chondromucoprotein. Immunology 9:119-127.

Antibody was obtained in rabbits following immunization with porcine chondromucoprotein and complete adjuvant. Several constituents of the antigen were revealed only by prior hyaluronidase digestion; an antigenic constituent revealed without hyaluronidase treatment was found to be shared by chondromucoproteins from several other species. Cross-reactivity was confirmed by tanned-cell hemagglutination inhibition and by delayed hypersensitivity reactions in guinea pigs. Electrophoresis of chondromucoprotein gave some separation of the immunologically distinct constituents. The results suggest that chondromucoprotein may be made up of several species-specific proteins as well as a polysaccharide peptide of common occurrence in several species. No evidence for antibody directed against chondroitin sulphate was found. The antibody has been used for the localization by immunofluorescence of chondromucoprotein in sections of tissue.

6877

Lupulescu, A.; Simionescu, L.; Merculiev, E. 1963. The role of estrogenic hormones in the development of immunological rabbit goiter. Endokrinologie 44:335-346. In German.

Massive immunization with thyroid antigen to rabbits elicits a parenchymatous microfollicular craw with reduced colloid. The craw development seems to be traceable to the hypersecretion of thyreotropic hormone due to blockage of synthesis of thyroid hormone through autoantibody. The serological tests show no significant difference between the test groups; the radioiodine uptake changes according to the actual test model. The presence of intracellular anti-thyroid antibodies in the immunological craw may be shown with the help of a marking with fluorescein isothiocyanate. The simultaneous dispensation of estradiol benzoate in large amounts leads to an arrest of the craw developing processes and to a change in development in the direction of a colloid craw. The simultaneous bilateral ovarial extirpation leads to a predominance of lesions similar to those observed in Hashimoto's thyroiditis, which points to the possible role of a gonadotropic hypersecretion in the etiology of this disease. These tests show the role of hormones in the development of immunological thyroid diseases.

6878

Mancini, A.M.; Costanzi, G.; Zampa, G.A. 1964. Human insulin antibodies detected by immunofluorescent technique. Lancet 1:726.

FA tests using gamma globulins from diabetic patients were made against sections of normal human, bovine, and pork pancreas, and other organs. Positive reactions were obtained only on human pancreas with sera from 8 of 14 insulin treated patients and one of three non-treated patients.

6879

Mancini, A.M.; Zampa, G.A.; Vecchi, A.; Costanzi, G. 1965. Histo-immunological techniques for detecting anti-insulin antibodies in human sera. Lancet 1:1189-1191.

FA for detecting anti-insulin antibodies in human sera is described. Human pancreas samples obtained within 3 hours of death were used as tissue-insulin antigen. Lyophilized pancreatic sections were treated with FITC sera, indirect FA, complement and anti-complement reaction, reaction with fluorescent complement, and reaction with sera absorbed with insulin. By these procedures 29 sera from diabetics and three from normal subjects were examined. Gamma globulins from sera of diabetics, treated with insulin or untreated, fix to beta cells of fresh human pancreas but not to bovine or pork pancreas. Gamma globulins from normal subjects do not fix to beta cells of human pancreas. Complement increases fluorescent reactions. These gamma globulins are anti-human insulin antibodies.

6880

Mancini, R.E.; Alonso, A.; Barquet, J.; Alvarez, B.; Nemirovsky, M. 1964. Histoimmunological localization of hyaluronidase in the bull testis. J. Reprod. Fertil. 8:325-330.

A highly purified and potent hyaluronidase preparation extracted from bull testis was employed to induce heterologous antibody by sensitizing adult rabbits. Specific anti-enzyme antibodies were demonstrated by the Ouchterlony method, by passive cutaneous anaphylaxis, hemagglutination, and complement fixation tests. In order to localize the cellular site of the accumulation of the enzyme, direct and indirect immunofluorescent techniques were applied to tissue sections and to isolated germ cells of adult bull testis. Specific fluorescence adequately checked by control reactions appeared in the perinuclear area of spermatids, in the acrosomes of spermatozoa, and with less certainty in the cytoplasm of other cells tentatively identified as spermatocytes. Nonspecific fluorescence could be seen in the basal cell line of the seminiferous tubules.

6881

Masson, P.; Heremans, J.F.; Prignot, J. 1965. Immunohistochemical localization of the iron-binding protein lactoferrin in human bronchial glands. Experientia 21:604-605.

FA techniques localized lactoferrin in serous and mucinous acinar cells of the bronchial glands.

6882

McGarry, E.E.; Ambe, L.; Nayak, R.; Birch, E.; Beck, J.C. 1964. Studies with antisera to pituitary hormones. Metabolism 13:Suppl.:1154-1164.

Specific antisera to hormones have been used to elucidate specificity of biological activity in an in vitro system. Fluorescein-conjugated antisera have been used to localize hormones in human pituitary tissue. Certain cells were found to fluoresce with antisera both to ACTH and to TSH. Several immunochemical techniques failed to demonstrate a cross-reaction to explain this finding.

6883

Midgley, A.R., Jr. 1963. Immunofluorescent localization of human pituitary luteinizing hormone. Exp. Cell Res. 32:606-609.

Luteinizing hormone was localized immunohistochemically to certain granular periodic acid-Schiff positive cells in the anterior lobe, in the pars tuberalis, and in the cell nests in the posterior lobe of the human pituitary gland. This study was performed with rabbit antiserum specific for human chorionic gonadotropin, which was shown to cross-react in gel diffusion analysis with purified human pituitary luteinizing hormone and to be capable of neutralizing its biological activity.

6884

Midgley, A.R., Jr. 1964. Immunofluorescent identification and histochemical characterization of cells containing human pituitary luteinizing hormone and follicle-stimulating hormone. J. Cell Biol. 23:59A-60A.

With the use of direct and indirect FA with rhodamine-labeled and fluorescein-labeled hormone-specific neutralizing antisera, the immunologically reactive portions of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were localized to different cells in frozen, freeze-dried, freeze-substituted, and formalin-fixed water-washed paraffin-embedded human anterior pituitary glands. FA specificity was established with reciprocal blocking and absorption controls. To determine the histochemical properties of cells containing either LH or FSH, fluorescent-positive cells were photographed. The cover slips were removed, the slides were re-stained by a variety of histochemical procedures, and the cells previously photographed were located again in the re-stained sections. Cells reacting with antibody to LH were found to be lightly aldehyde-fuchsin and periodic acid-Schiff (PAS) positive, whereas cells reacting with antibody to FSH were usually more intensely stained by the PAS procedure. The intensity of histochemical staining failed to correlate with that of fluorescence, and most of the PAS-positive cells were fluorescent negative. With the performic acid, Alcian blue, PAS, orange G sequence of Adams, cells containing LH appeared blue, whereas cells containing FSH appeared purple or purple-blue. Fluorescent-positive cells have not stained with orange G. Complete article.

6885

Moritz, K.-U.; Ambrosius, H. 1964. Immunohistological detection of thyroglobin in the thyroid gland. Biol. Zentralbl. 83:3:337-347. In German.

Thyroglobin behaves electrophoretically like alpha globulin. Antiswine thyroglobin serum gives a slight reaction with the antigens of other mammals. The form and size of the microscopic precipitate of localization studies differ in swine and rats. Thyroxin does not inactivate the antiserum. Cytochemically, RB 200 is as satisfactory as FITC and is superior to DANS. BA-46-71874.

6886

Mosca, L.; Chiappino, G. 1964. Localization of corticotrophin and luteinizing hormone production in human pituitary. Lancet 2:1016-1017.

The fluorescent antibody technique was applied to glands of patients who had died of non-endocrine diseases. Sections treated with rabbit antiserum anti-human-chorionic-luteinizing hormone (HLH) and then with goat fluorescent antibody antirabbit gamma globulins displayed anti-HLH gamma globulins in most of the delta cells located in the same areas as the corticotrophin cells, notably in the posterior areas of the pars anterior and in the basophilic invasion of the neural lobe. These findings, together with some recent data, support the hypothesis that in man the source of both corticotrophin and luteinizing hormone must be in the family of delta cells.

6887

Murakami, M. 1964. Mechanism of lipid deposition on arterial wall. Jap. Heart J. 5:397-398.

Although the encrustation theory is still acceptable for the pathogenesis of lipid in the arterial wall, one can add additional important mechanisms. Fibrin and beta-lipoprotein are found in association in arterial walls.

6888

Murakami, M.; Sekimoto, H.; Tatsuguchi, M.; Yasuda, Y.; Masuda, S.; Matsumoto, K.; Shinagawa, T.; Genda, A.; Ikeshima, T.; Ozaki, H.; Yamada, T.; Murakami, M. 1964. Studies on the role of fibrinogen on lipid deposition into the aorta. Jap. Heart J. 5:108-114.

The purpose of this study was to determine whether the presence of fibrinogen has an effect on the deposition of beta-lipoprotein into the aorta. The results obtained with the fluorescent antibody technique indicated that accumulations of beta-lipoprotein in the intima were associated with the presence of fibrin. It is suggested that fibrinogen enhances the deposition of lipid complexes from abnormally lipaemic sera.

6889

Mustakallio, K.K.; Levonen, E. 1964. Histochemical demonstration of heparin-precipitating lipids in atherosclerotic aortas. J. Atherosclerosis Res. 4:370-372.

In frozen tissue sections an acetone-fast lipid compound was able to precipitate heparin in the presence of calcium in low concentration. This precipitate was demonstrated with toluidine blue. Adquately controlled, this method may prove suitable for the study of intimal changes during atherogenesis. FA conjugates against human beta-lipoprotein were also used.

6890

Nishimura, E.T.; Kobara, T.Y. 1963. Some observations on the distribution of catalase in mouse tissues by fluorescent antibody technique. Federation. Proc. 22:3038:669.

Liver catalase of high specific activity was prepared from Strong A mice and used as the antigen for preparing the antimouse catalase rabbit serum as described previously. Rhodamine B isocyanate was used to conjugate the antimouse catalase globulin, RCG, and the untreated control globulin, RNG, from rabbit sera. The specificity against mouse catalase was established by Ouchterlony double diffusion tests before and after rhodamine conjugation. After freeze-substitution, tissues of Strong A mice were embedded in Carbowax 1000, sectioned at 2 microns, stained and examined by UV microscopy. Sections were stained with RCG; RNG; after initial blocking, with unconjugated antimouse catalase globulin followed by RCG; unstained. Results showed intense and specific staining of nuclei and cytoplasm of hepatic cells, but not veins, arteries, erythrocytes, or connective tissue. Kupffer cells did not show specific staining. Kidney revealed intense cytoplasmic staining of the tubular epithelial cells of proximal and distal segments. The collecting ducts, glomeruli, erythrocytes, blood vessels, and adipose tissue remained essentially unstained. Complete article.

6891

Nishimura, E.T.; Kobara, T.Y.; Drury, J.J. 1964. Localization of catalase in tissues by the fluorescent antibody technique. Lab. Invest. 13:69-76.

An immunofluorescent method for the localization of catalase in mouse and human hepatic and renal tissues is described. The specificity of the staining reaction was established by the successful blocking of immunologic-reactive sites in the cells by their initial exposure to unconjugated anticatalase globulin. Additional support of specificity was obtained in mouse kidneys, where the fluorescent dye was bound mainly to proximal and distal tubular epithelium of the collecting ducts in the medulla, which exhibited no specific fluorescence.

6892

Pansky, B.; House, E.L.; Cone, L.A. 1965. An insulin-like thymic factor: A preliminary report. Diabetes 14:325-332.

Insulin was traced in the thymus of the mouse, pig, and calf. It was also located in the pancreas of the pig. Both direct and indirect FA tests were employed. Insulin was found in reticular cells and in Hassal bodies.

6893

Parker, J.W.; Elevitch, F.R.; Grodsky, G.M. 1963. Binding of fluorescent insulin to intracellular antibodies in guinea pigs immunized with insulin. Proc. Soc. Exp. Biol. Med. 113:48-53.

Fluorescein isothiocyanate - insulin conjugates, both bovine and porcine, containing two to three chromatographic and electrophoretic fractions, showed a 70 to 80 per cent retention of capacity to bind insulin antibodies produced in guinea pigs, compared with unmodified crystalline insulin. The conjugates and unconjugated insulin with iodine-131 behaved similarly, electrophoretically and chromatographically, in the presence of antibody. Direct staining with fluorescent insulin of frozen sections of lymphoid tissues from guinea pigs immunized with insulin produced specific cytoplasmic fluorescence in plasma cells, which were present in increased numbers. Non-lymphoid tissues from the same animals contained no specifically staining elements beyond occasional scattered plasma cells. No differences in staining were observed when lymphoid tissues from animals immunized with bovine insulin were stained with porcine fluorescent insulin and vice versa.

6894

Parker, M.L.; Jarett, L.; Schalch, D.S.; Kipnis, D.M. 1965. Rat growth hormone: Immunofluorescent and radioimmunologic studies. Endocrinology 76:928-932.

The cross-reactivity that exists between antisera to porcine and rat growth hormones and the successful radioiodination of rat growth hormone have permitted the development of a sensitive radioimmunoassay for the latter. Human growth hormone does not cross-react in this system. By fluorescent techniques the anti-PGH serum has been found to stain the acidophiles of the rat pituitary, a finding that provides further evidence of cross-reactivity with rat growth hormone.

6895

Pierce, G.B., Jr.; Midgley, A.R., Jr. 1963. Histogenesis and function of syncytiotrophoblast. Amer. J. Pathol. 43:10a.

The theory that cytotrophoblast secretes chorionic gonadotropin (HCG) may be invalid in view of our recent FA demonstration of this hormone in the syncytiotrophoblast. Choriocarcinoma has been examined electron microscopically and by FA to determine whether this localization represented either production of hormone by these giant cells or storage of hormone produced elsewhere. Ultrastructurally the syncytiotrophoblastic giant cells were found to be truly syncytial and were honeycombed by enormous numbers of dilated cisternas of endoplasmic reticulum (ER). The ER corresponded in distribution and size to the droplets of HCG observed in the immunofluorescent studies. As no support was found for the contention that the HCG had been phagocytosed, the hormone must have originated in the endoplasmic reticulum of the syncytiotrophoblast. Cytotrophoblast had only scant profiles of a tubular ER. Gradations in the degree of development of ER could be found between cyto- on the one hand and syncytiotrophoblast on the other. Syncytiotrophoblast may be derived by differentiation from cytotrophoblast and had, as the differentiated cell type of trophoblast, a principal function, the secretion of chorionic gonadotropin. Complete article.

6896

Posrovitz, J. 1964. Collective antigens and Rh factor in human spermatozoa. Dapim Refuiim 23:445-446. In Hebrew.

The serological methods described allowed determination of human blood type of the subject in the smallest quantity of human spermatozoa. This is of great significance in juridicial medicine. It was also determined that the Rh factor is present in the sperm.

6897

Querci, V. 1965. Medicolegal interest of the immunofluorescent pregnancy test. Minerva Med. 85:61-64. In Italian.

An immunological test for pregnancy based on the antigenic property of chorionic gonadotropin is described. The method involves agglutination of erythrocytes, on which gonadotropins have been adsorbed, in contact with antigonadotropin labeled with fluorescein isocyanate. Acquisition of fluorescence by the erythrocytes indicates absence of gonadotropin in the urine. Studies were carried out on urine and sera of 15 women who had had abortions during the first trimester. The immunofluorescence method yielded positive results for a longer time period after the abortions than other methods, thereby recommending it in situations of obstetrical and medicolegal interest which cannot be solved unequivocally by means of the usual tests.

6898

Querci, V.; D'Antona, N. 1964. Utilization of immunofluorescence in the diagnosis of pregnancy. Minerva Med. 84:15-16. In Italian.

As classical tests for biological diagnosis of pregnancy are not always reliable, the use of the antigenic property of chorionic gonadotropin was tested. Good fluorescence of the red blood corpuscles was observed after treatment with antigonadotropinic serum mixed or not mixed with urine deprived of the specific antigen. An absolute absence of fluorescence was obtained in the prepared specimens of red blood corpuscles treated with antigonadotropinic serum previously strengthened with the urine of a pregnant woman.

6899

Roy, A.K.; Neuhaus, O.W.; Gardner, E. 1965. Studies on rat urinary proteins. Federation Proc. 24:2091:507.

Rat urinary proteins were identified by immunological and electrophoretic techniques. Immunoelectrophoretic separations developed with rabbit antisera against total rat serum, or total urinary proteins, revealed at least 15 different components, of which six were immunologically identical with their serum counterparts, i.e., albumin, alpha-1b, -c, -f, beta, and gamma globulins. Thirty-seven per cent of the protein in rat urine migrated in paper and agar electrophoresis as a single band in the inter-alpha region but did not react with antitotal serum antiserum in the immunoelectrophoretic system. This major urinary protein was isolated in a pure form using ammonium sulfate fractionation and DEAE-cellulose chromatography. It was found to be homogeneous by electrophoresis, ultracentrifugation, and immunological reaction.

The sedimentation constant and molecular weight were shown to be 2.2S and 26,800 respectively. Hexose, hexosamine, and sialic acid levels of 2 per cent, 1.6 per cent, and 1.4 per cent, respectively, were found; the amino acid composition was also determined. Extracts of perfused kidney reacted strongly with an antiserum specific for this protein. Of other tissues tested only the liver gave a positive reaction. Fluorescent antibody labeling yielded similar results. Complete article.

6900

Rubin, W.; Fauci, A.S.; Sleisenger, M.H.; Jeffries, G.H. 1965. Immunofluorescent studies in adult celiac disease. J. Clin. Invest. 44:475-485.

To investigate the possible role of immunologic processes in the pathogenesis of adult celiac disease, duodenal and/or jejunal biopsies from nine patients with adult celiac disease and from five control patients were studied by immunofluorescent techniques. Immunoglobulins were demonstrated in mononuclear cells in the lamina propria of all tissue sections. Gamma-1A was the major immunoglobulin present. No in vivo or specific in vitro fixation of beta-1C globulin component of complement was observed in these tissues. In specimens from all subjects, the beta-1C globulin component of complement was fixed in vitro at the superficial margin of the epithelial cells and in goblet cells, a distribution corresponding to mucin. This nonspecific complement fixation may be secondary to a nonspecific in vitro fixation of immunoglobulin, particularly gamma-1M globulin, which was fixed in the same areas. Autoantibodies reactive against epithelial cell cytoplasm could not be demonstrated. The cytoplasm of the intestinal epithelium from celiac patients bound gliadin, but not casein, bovine serum albumin, or ovalbumin.

6901

Schwarz-Speck, M.; Maeder, E. 1964. Immunological studies with natural and synthetic ACTH in subjects allergic to ACTH. Int. Arch. Allergy Appl. Immunol. 24:Suppl:29-47. In German.

In an examination of 16 sera of patients by the gel-diffusion method, precipitating antibodies against natural ACTH preparations and swine-hypophysis extract were found in two patients with clinical ACTH allergy and positive skin tests. Synthetic peptides gave no precipitation and no precipitation inhibition. The antibodies shown with FA in at least one of the two cases appear to confirm the results obtained with the precipitation method. The precipitating antibodies were directed against accompanying material from the hypophysis and not against the ACTH molecule.

6902

Sciarra, J.J.; Kaplan, S.L.; Grumbach, M.M. 1963. Localization of antihuman growth hormone serum within the human placenta: Evidence for a human chorionic growth hormone - prolactin. Nature 199:1005-1006.

The human placenta as early as the 12th week of gestation contains a protein localized in the syncytial cytoplasm of the villous trophoblast that partially cross-reacts immunologically with rabbit anti-HGH serum. Studies to be reported elsewhere suggest that this protein is a chorionic growth hormone (prolactin) and may represent an important metabolic hormone of pregnancy. It is noteworthy that immunofluorescent investigations of human chorionic gonadotropin in the human placenta indicate that this hormone is also present in the syncytiotrophoblast.

6903

Sciarra, J.J.; Tallberg, T.; Ely, C.A. 1964. Immunohistochemical localization of human chorionic gonadotropin (HCG) in the human placenta. Anat. Rec. 148:400.

Rabbit antiserum to HCG was produced by prolonged immunization with a commerical preparation in Freund's adjuvant. The antiserum was exhaustively absorbed with lyophilized human serum and an extract of normal female urine. Immunoelectrophoretic analysis using a highly purified HCG preparation indicated a single complex precipitin line. Localization of HCG in the human placenta was achieved by both direct and indirect FA. For the direct procedure rabbit anti-HCG was conjugated with FITC. Conjugated antisera were purified by filtration through Sephadex G-25 and by absorption with rat and rabbit liver powders. Representative areas of the villous portion of several first, second, and third trimester human placentae were examined. Tissue was processed by the freeze-drying method and sectioned at 1.5u although cryostat sections and sections of formalin fixed tissue gave essentially the same localization. Specific fluorescence due to HCG was found in the syncytial layer of the villous trophoblast, at all stages of gestation from 12 weeks to term. No fluorescence was observed in the cytotrophoblast or in the villous connective tissue. Fluorescence was specific and was localized in the cytoplasm of the syncytiotrophoblast.

6904

Shapland, C.G. 1964. Studies on a human thyroid protease. J. Med. Lab. Technol. 21:1-20.

FA was used to identify fractions of thyroid extract being separated.

6905

Shimazaki, M. 1963. Mutual relationship between cells of anterior pituitary and target organs. Folia Endocrinol. Jap. 39:746-748. In Japanese.

This study was designed to elucidate the functional status and morphology of the anterior pituitary body. Physiologic experiments involving castration, hormone administration, and adrenalectomy are described. Results from these experiments indicated that acidophile cells might be producers of prolactin. Antibovine prolactin antibody was produced by immunizing rabbits, and FITC was conjugated to the globulin fraction. Fluorescence was observed at the sites corresponding to acidophile cells.

6906

Simons, B.E., Jr.; Baum, J.; Unger, R.H.; Madison, L.L. 1963. Direct localization of glucagon in the alpha cells of the pancreatic islet by immunofluorescent techniques. Texas Rep. Biol. Med. 21:468.

The availability of a specific antibody to glucagon made possible this study designed to localize the site of production of glucagon within the pancreatic islet cells. The immunofluorescent 'sandwich technique' was utilized. FITC labeled goat anti-rabbit gamma globulin was used to localize the site of reaction of rabbit anti-beef glucagon in beef pancreas. Control studies with rabbit anti-bovine serum albumin failed to show specific fluorescence. In serial sections, alpha cells were first located by the dark field illumination technique. The fluorescent staining showed glucagon to be located in those cells identified as alpha cells in adjacent 4-micron sections. This could be distinguished from the beta cells localized with anti-insulin antiserum. With this technique, the presence of glucagon was confirmed in the alpha cells of other species. These data provide the first direct evidence for the production of glucagon in the alpha cells of the pancreatic islet. Complete article.

6907

Sutherland, L.E.; Hartroft, P.M. 1963. Further studies of juxtaglomerular cells with the fluorescent antibody technique. Federation Proc. 22:2317:548.

Immunological studies of juxtaglomerular, JG, cells, begun by Edelman and Hartroft, were extended. A new and easier method of tissue preparation based on freeze substitution was adopted. This technique gave the advantage of thin sections of 1 micron or less, an impossibility with the cryostat, and more consistent histologic results than the freeze-dry method formerly used in this work. High-titered anti-hog renin made in dogs, conjugated with fluorescein isothiocyanate, selectively stained granules in JG cells of dogs and rabbits. In hog kidney, however, this antirenin, which had been absorbed with liver and bone marrow powders, stained tubular droplets as well. By absorbing with low-renin hog kidney powder instead, only specific staining of JG granules remained. In a search for other antigens, a substance reacting with anti-canine globulin was found in cytoplasm of canine JG cells. This staining was diffuse in contrast to discrete granule staining with antirenin. Complete article.

6908

Sutherland, L.E.; Hartroft, P.M.; Bailey, J.D.; Balis, J.U. 1963. Juxtaglomerular cells in familial electrolyte-losing syndrome in infants. Amer. J. Pathol. 43:20a-21a.

Four infants, 6 months to 1 year of age, failed to thrive and suffered dehydration with alkalosis and low serum levels of K, Na, Cl, and Mg. Mental deterioration ensued as the disease progressed. Three of the cases were siblings, including identical twins, and the fourth infant was the product of a consanguineous union. In necropsy kidney sections, many glomeruli were immature. Others were well developed. The most conspicuous finding in all these cases was extreme hyperplasia and hypergranulation of juxtaglomerular cells. Vacuoles were present in the basal portions of macula densa cells, indicating hyperactivity, but nuclei in this portion of the distal tubule were flattened and the cytoplasm tenuous. The zona glomerulosa of the adrenals was hypertrophied and fasciculata narrow. Kidneys were obtained immediately after death for electron microscopy and FA studies. Renin content was assayed at 50 times normal adult values. Anti-hog renin will not neutralize human renin, nor will hog renin form angiotensin from human hypertensinogen. In this case, however, fluorescein-conjugated anti-hog renin stained granules in juxtaglomerular cells. Hog renin also formed angiotensin from the hypertensinogen in the serum of this patient. Complete article.

6909

Thiede, H.A.; Choate, J.W. 1963. Chorionic gonadotropin localization in the human placenta by immunofluorescent staining: II. Demonstration of HCG in the trophoblast and amnion epithelium of immature and mature placentas. Obstet. Gynecol. 22:433-443.

Antisera to human chorionic gonadotropin (HCG) was prepared by injecting highly purified HCG in complete Freund's adjuvant intradermally into adult male rabbits. The resulting antibody was characterized by both agar gel diffusion and immunoelectrophoresis. Quick frozen sections of immature and term placenta were examined for the localization of HCG by indirect FA. The viability of the tissue used was confirmed by quantitative serum titers for HCG and growth in tissue culture. Extensive control procedures were employed. Histological identification of the placental cells involved was determined by counterstaining the fluorescent sections with histochemical stains. Specific fluorescence was confined to the trophoblast of all placentas as well as the amnion epithelium. The HCG appeared to be concentrated in the syncytiotrophoblast and the amnion.

6910

Thiede, H.A.; Choate, J.W. 1964. A comparison of frozen and chemically fixed tissues for localization of HCG in the human placenta by the immunofluorescent antibody technique. J. Histochem. Cytochem. 12:17.

The effect of chemical fixation on placental tissues subsequently used for the localization of human chorionic gonadotropin (HCG) by FA staining was studied. Blocks of immature placental tissue obtained at the time of therapeutic abortion, as well as tissue from term placentas, were either quick-frozen or placed in 10 per cent formalin. The viability of the tissue was confirmed by quantitative serum titers for HCG and by growth in tissue culture. Antiserum was prepared by intradermally injecting adult male rabbits with highly purified HCG in complete Freund's adjuvant. FA studies utilizing this highly specific antibody with quick-frozen tissue localized the maximum fluorescence to the syncytiotrophoblast and to the amnion epithelium of all placentas examined. Formalin fixed tissues consistently failed to exhibit any specific fluorescence when incubated with anti-HCG and fluorescein conjugate. Greatly reduced fluorescence was noted in sections treated with ether and alcohol. Complete article.

6911

Thiede, H.A.; Choate, J.W.; Bindschadler, D.D. 1963. Chorionic gonadotropin localization in the human placenta by immunofluorescent staining: I. Production and characterization of antihuman chorionic gonadotropin. Obstet. Gynecol. 22:310-315.

The production of a potent and specific antiserum to HCG in rabbits is described. The in vitro activity of the antibody was characterized by ring precipitin titers, agar-gel diffusion, and immunoelectrophoresis. These studies established the presence of a potent precipitating antibody that reacted specifically with HCG. The antigen was concentrated in the Beta-1 fraction and formed a single precipitation line with the rabbit antibody in agar gel diffusion. The antihormonal activity of the antiserum was demonstrated by quantitative bioassay. The problems associated with the application of such an antiserum for detection of HCG, including its use in immunofluorescence studies, are discussed.

6912

Toreson, W.E.; Feldman, R.; Lee, J.C.; Grodsky, G.M. 1964. Pathology of diabetes mellitus produced in rabbits by means of immunization with beef insulin. Amer. J. Clin. Pathol. 42:531.

New Zealand rabbits immunized against beef insulin developed antibodies that bound insulin. Two of five such animals manifested a diabetic state that became apparent within 1 month and persisted indefinitely. Blood sugar levels rose to 300 to 400 mg per 100 ml. Glycosuria and lipemia were apparent, and there was weight loss, polyphagia, and polydipsia. Biopsies of the pancreas at the 5th month revealed complex lesions of the islets. Extraordinary infiltrations of the islets and interlobular stroma with mononuclear cells and increased reticulum were seen. There was extensive degranulation, glycogen infiltration, and dissolution of beta cells (hydropic degeneration). Small islets composed almost entirely of alpha cells were noted. With histochemical methods and FA, insulin could not be demonstrated in the islets of these immunized diabetic rabbits. Insulin was present in the islets of the immunized, non-diabetic animals. These islets displayed normal histology. Glucose tolerance studies did not demonstrate diabetic characteristics. Immune mechanisms may constitute a pathogenic factor in diabetes mellitus. Complete article.

6913

Torgersen, O.; Myren, J.; Oystese, B. 1963. Functional aspects of gastric biopsy. Verhandl. Deut. Ges. Pathol. 47:308-311. In German.

A brief report is given of the use of immunofluorescence in the study of the excretion of proteins from the human gastric mucosa, and of the evaluation of histamine-stimulated parietal cell secretion by means of dehydrogenase methods.

6914

Walker, J.G.; Doniach, D.; Roitt, I.M.; Sherlock, S. 1965. Serological tests in diagnosis of primary biliary cirrhosis. Lancet 1:827-831.

The serum of 32 patients with primary biliary cirrhosis gave granular cytoplasmic staining in unfixed tissue sections, using fluorescein conjugates of antihuman gamma globulin and anticomplement in the double layer immunofluorescent test. The staining reactions showed no organ- or species-specificity, and cells known to be rich in mitochondria were preferentially stained. Possible confusion with the specific thyroid and gastric parietal cell antibodies could be avoided by using rat kidney as the substrate. The immunofluorescent reaction was positive in all 32 patients with primary biliary cirrhosis and negative in all other cases. The conventional complement fixation test with tissue homogenates was less sensitive and immunologically less specific. The latex agglutination test failed to distinguish reliably between primary biliary cirrhosis and main bile duct obstruction. The results of this investigation do not clarify the etiological role of the cytoplasmic antibodies in primary biliary cirrhosis, but suggest that they do not arise merely as a consequence of damage to the bile ductules or as sequelae of biliary obstruction.

6915

Winick, M.; Greenberg, R.E. 1965. Appearance and localization of a nerve-growth-promoting protein during development. Pediatrics 35:221-228.

A nerve-growth-promoting protein (NGF) has been identified in a number of vertebrate species, including the human fetus. This protein can be localized to the axial regions or the sympathetic chain directly, and wherever found is immunologically similar to NGF from mouse salivary gland. During development NGF appears concomitant with morphologic differentiation of the sympathetic chain and always in association with it. These studies further implicate the participation of the nerve-growth-promoting protein in the regulation of growth of sympathetic ganglia.

6916

Wolf, J.E., Jr. 1963. A fluorescein-antibody study of the pituitary gonadotrophic hormones. Texas Rep. Biol. Med. 21:451-452.

The purposes of this research were: (1) to localize the gonadotrophic cells responsible for elaboration of the follicle stimulating hormone (FSH) and the luteinizing hormone (LH), (2) to study the antigenic characteristics of the hormones and, if possible, (3) to determine the distribution pattern of the hormone-producing cells in the hypophysis. Indirect FA was used. Frozen-dried and quick-frozen sections of sheep, hog, and rat pituitary glands were treated with rabbit anti-sheep FSH and anti-sheep LH globulin preparations, and subsequently incubated with fluorescein-labeled goat anti-rabbit globulin. The product of these immunologic reactions was an antigen-antibody complex that fluoresced. With this technique, FSH and LH producing cells have been localized in the hypophyses of both sheep and hog. The successful localization of intracellular gonadotrophic hormones in these two animals offers evidence that two distinct gonadotrophic cells are responsible for FSH and LH secretion. Antigenic components of the gonadotrophic hormones are specific characteristics of the hormones, per se, and not necessarily species specific. Complete article.

6917

Yata, J. 1964. Studies on the growth hormone in child pituitaries by the FA technique: II. Changes observed in Simmonds' disease, dwarfism, nutritional disturbances, and during treatment with large doses of corticosteroid. Acta Paediat. Jap. 68:207-215. In Japanese.

Intrapituitary growth hormone (GH) was stained by FA. Changes occurred in two cases of Simmonds' disease, one of dwarfism, five of malnutrition, and four under treatment with large doses of corticosteroid. In Simmonds' disease, GH stained normally in the pituitary and its secretion was normal, indicating that GH, although controlled by the hypothalamus, was not as dependent on the latter as are tropic hormones. In the suspected cases of anorexia nervosa, no GH was stained by this technique. Therefore, this disease, if it became cachectic, would also lead to hypofunction of the pituitary and to failure of GH formation and secretion. In dwarfism, sufficient GH generally was produced by a normal number of cells in pituitaries. However, the possibility that these cells appeared slightly hypotrophic and secretion might be insufficient could not be ruled out. Definite tendency of decrease in both number of GH-producing cells and amount of production per cell was observed in cases of infantile malnutrition; in severe cases, production appeared absent. In all patients treated with large doses of corticosteroid, GH-producing cells increased in numbers, and many of these cases showed low storage of GH within the cells. Pituitary acidophilic cell tumor in acromegaly was stained; GH was secreted actively.

VII. CELL FUNCTION

6918

Braun, W.; Nakano, M. 1965. Influence of oligodeoxyribonucleotides on early events in antibody formation. Proc. Soc. Exp. Biol. Med. 119:701-707.

The number of hemolysin-forming cells, assayed by Jerne's technique in spleens removed from AKR mice, 48 hours after immunization with heterologous red cells, is significantly higher when antigen is administered in conjunction with an enzymatic digest of calf thymus DNA. This stimulation by oligodeoxyribonucleotides is not matched by comparable effects of oligoribonucleotides; mixtures of monodeoxyribonucleotides or -sides are inactive. The stimulation appears to involve a stimulated multiplication of early appearing, or early activated, antibody-forming clones, and is more difficult to discern as the interval between immunization and assay of spleen cell population increases. Oligodeoxyribonucleotides do not stimulate early immune responses unless specific antigen is administered concurrently; possible reasons for this requirement of antigen, in contrast to a lack of such requirement in comparable stimulations produced by bacterial endotoxins, are considered. The influence of dosage and route of administration of DNA digest has been analyzed and an effect on secondary as well as primary responses has been demonstrated. Kinetin riboside abolishes the stimulatory effects of oligodeoxyribonucleotides without influencing the basic, nonstimulated immune response, whereas actinomycin D interferes with the immune response in the absence and presence of oligodeoxyribonucleotides. Possible relationships to problems of natural and adjuvant-elicited stimulations of antibody production are discussed.

6919

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1964. Cell wall replication: I. Cell wall growth of Bacillus cereus and Bacillus megaterium. Can. J. Microbiol. 10:43-48.

Cell wall replication of Bacillus cereus and Bacillus megaterium was studied by differential labeling with fluorescent and nonfluorescent antibody. Growth of new cell wall in B. cereus was initiated near the poles. In the old wall, additional new wall segments gradually developed to form an alternating pattern of new and old wall segments. Further growth elongated the new wall and pushed the old segments apart. Separation of daughter cells appeared to involve splitting of the transverse septa laid down at or near the old wall segments. Growth of new cell wall of B. megaterium was initiated either at one of the poles or at the central area of the cell. Multiple segments of new and old wall appeared along the cell length. Further elongation was followed by formation of transverse septa and separation of daughter cells incorporating either old or new wall segments. Our evidence clearly shows that growth and elongation of the two bacilli do not occur by diffuse intercalation of new cell wall into the old.

6920

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1964. Cell wall replication: II. Cell wall growth and cross wall formation of Escherichia coli and Streptococcus faecalis. Can. J. Microbiol. 10:473-482.

Cell wall replication in E. coli and S. faecalis was studied by differential labeling of living cells with FA and non-fluorescent antibody. In E. coli the initial step in cell division was the formation of a cross wall at the cell equator, followed by the appearance of new cell wall on either side of the cross wall. The process was repeated in sequence at subsequent sites in the polar, the subcentral, and the subpolar areas. Constriction occurred at random so that the divided parent cells were composed of several daughter cells. A polar type of unidirectional cell wall growth and elongation was also observed in E. coli. It was initiated by the synthesis of a ring of new cell wall material around the polar tip. A second ring was then formed at the subpolar area during the rapid enlargement of the first ring in a single direction. Evidence shows that cell wall synthesis is independent of cell division and that in E. coli, it is initiated at multiple but specific sites within the cell and not by diffuse intercalation of old and new walls. Contrary to the synthesis of cell wall at multiple sites in E. coli, S. faecalis replicated new cell wall at only one site per coccus. The new wall segment was initiated and enlarged at the coccal equator, and was followed by the formation of a cross wall, centripetal growth and constriction to separate the daughter cells.

6921

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1965. Cell wall replication in Saccharomyces cerevisiae. Bacteriol. Proc. G146:38.

The bud formation in Saccharomyces cerevisiae was studied by differential labeling of living cells with fluorescent and nonfluorescent antibody. Examination of smears at consecutive intervals of 15 minutes showed that the bud was nonfluorescent, but the mother yeast remained as discrete fluorescent areas. The bud formation was initiated as a small bulge that enlarged and gradually developed into a small bud. Further increase in size of the bud was accompanied by the formation of a cross wall and constriction at the base area to separate the bud from the mother yeast. It appears that the cell wall of the bud was newly synthesized first at the base area, and then added to the actively growing new bud in a direction away from the base. After the separation of the yeast cells, the birth and bud scars were clearly visible on the fluorescent cell wall.

6922

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1965. Cell wall replication in Saccharomyces cerevisiae. Can. J. Microbiol. 11:953-957.

Cell wall growth and bud formation in Saccharomyces cerevisiae were studied by FA. Labeled cells were grown in a glucose yeast extract broth and examined at 15-minute intervals. The new cell wall was largely non-fluorescent; the old wall showed no reduction of fluorescence during growth of the bud. Bud formation was initiated as a small bulge on the cell wall, and further increase in size was accompanied by the formation of a constriction around the basal end that led to the separation of the bud from the mother yeast cell. The actively growing area of the bud was an annular band close to the base. It appears that the cell wall of the bud was, almost entirely, newly synthesized and contained very little old cell wall material. The process of wall synthesis is compared with the pattern found in several bacteria and with what is known of the process in other fungi.

6923

Cole, R.M. 1963. Cell wall replication in Salmonella typhosa, followed by immunofluorescence. Bacteriol. Proc. G13:26.

To follow cell wall growth, O antigen (9 and 12) was labeled with fluorescein-conjugated homologous antibody globulin (FAG). Cells of Salmonella typhosa, first incubated 1 to 2 hours in FAG, were washed and transferred to Penassay broth. Alternatively, instead of washing, an excess of the same globulin, unlabeled, was added with a little broth. Samples were taken at zero time and at 60-minute intervals during incubation with shaking at 37 C. After a lag of 1 hour, growth was exponential. Samples were centrifuged and washed and smears made. Several strains of S. typhosa and appropriate antibody globulins gave similar results. Findings show progressive and generalized decrease of intensity of cell-wall fluorescence with time of incubation after removal or block of excess FAG at zero time. The reverse technique of prior growth in unlabeled antibody, wash, reincubation in broth, and FAG staining after smearing shows increasing cell-wall fluorescence with time. The lack of discrete and microscopically resolvable areas of old cell-wall fluorescence is opposed to previously reported findings for Streptococcus pyogenes, in which old labeled wall remains discrete, resolvable, and continuously distinguishable from new. The results are compatible with an hypothesis of cell-wall replication in S. typhosa by diffuse or generalized ultramicroscopic intercalation of new cell-wall material. At least two different modes of cell-wall replication have been distinguished among unrelated bacteria.

6924

Cole, R.M. 1964. Cell wall replication in Salmonella typhosa. Science 143:820-822.

Changes in the fluorescence of the cell wall of Salmonella typhosa were studied during growth after direct labeling with fluorescein-conjugated homologous or anti-O globulins. Fluorescence decreased evenly with culture growth and cell division, but the addition of chloramphenicol resulted in large, nondividing cells that showed increasing interruption of fluorescence of the wall marker. The process thus differs from the equatorial origin and discrete hemispherical addition of new wall previously described in Streptococcus pyogenes. These findings, in addition to demonstrating the formation of new wall in the presence of chloramphenicol, appear consistent only with the concept that wall replication in the salmonellas occurs by diffuse intercalation of new materials among old.

6925

Cole, R.M. 1965. Symposium on the fine structure and replication of bacteria and their parts: III. Bacterial cell wall replication followed by immunofluorescence. Bacteriol. Rev. 29:326-344.

This is an interpretive and critical report. The author urges further application of FA to the study of surface-antigen replication in walled microorganisms. Confirmation or denial of controversial points in this study area will follow only from such further study. FA has clear advantages over any other method for cell wall study. The chief advantage is the ability to apply a specific label to the wall of a living cell.

6926

Frank, G.M.; Samosudova, N.V.; Kryukova, M.Ye.; Kalamkarova, M.B.; Ogievetskaya, M.M. 1963. Localization of muscle protein in isolated myofibrils by fluorescent antibody techniques. Biofizika 8:5:569. In Russian.

Photographs of FA-labeled myofibrils indicate the redistribution of proteins of the actomyosin complex during extension and contraction. The change in the localization of antibodies corresponds to the sliding model of Huxley only to a certain degree. This conformity is expressed in the expansion and contraction of the central fluorescent bands of the A-disc, coincident with the H-zone, and in the expansion and contraction of the fluorescent bands of the I-disc. On the other hand, the behavior of the proteins under study during the stage of strong contraction does not conform to the picture of the actin filaments pushed into the anisotropic disc. The discrete distribution of antibodies in myosin and its components indicates the existence of heterogeneity of the myosin filaments along their length within the anisotropic disc.

6927

Friedman, M.E.; White, J.D. 1965. Immunofluorescent demonstration of cell-associated staphylococcal enterotoxin B. J. Bacteriol. 89:1155.

FA staining of cultured staphylococcal cells, strain S6, yielded brilliant peripheral staining. Washing the cells readily reduced fluorescence, indicating loose binding of enterotoxin that may be a cell-surface constituent.

6928

Geason, D.J.; Romano, A.H. 1964. Sheath formation in Sphaerotilus natans, followed by immunofluorescence. Bacteriol. Proc. G132:38.

Labeled homologous antisheath globulin was obtained by injecting rabbits with purified sheath material from S. natans, fractionating the serum with ammonium sulfate, and conjugating with fluorescein isothiocyanate. S. natans was grown on slides immersed in Stokes medium at 28 C for 9 hours and reacted with the homologous labeled globulin. The slide cultures were then washed with buffered saline, pH 7.2, and incubated further in fresh medium. Samples were removed at intervals and examined by fluorescence and phase microscopy. Old portions of the sheath remained discretely labeled with no diminution in intensity of fluorescence, but nonfluorescent new sheath material was observed at the tips of the filaments. New sheath material is not formed by intercalation or intussusception, but by linear extension of pre-existing sheath as new cells are formed at the ends of filaments. This interpretation was confirmed by an alternative procedure, in which old sheath was reacted with unlabeled homologous globulin and new sheath was identified by the addition of labeled globulin after incubation. Fluoresence was most intense at the growing tips of the filaments.

6929

Gill, F.A.; Cole, R.M. 1965. The fate of a bacterial antigen, streptococcal M protein, after phagocytosis by macrophages. J. Immunol. 94:898-915.

A method is described for studying the postphagocytic fate of a bacterial surface antigen during degradation of the organism by macrophages. Observations of Group A, Type 1 streptococci after phagocytosis by unstimulated mouse peritoneal macrophages demonstrate cessation of bacterial growth, early partial loss of an immunofluorescent complex from the bacterial surface, and later extrabacterial pooling of part of the complex in macrophage vacuoles. Evidence is presented that suggests that the observed changes in fluorescence reflect the fate of M protein still bound to its fluorescent label. The observations indicate, therefore, that M protein is removed from the bacterial cell wall and pooled within

the surrounding macrophage vacuole. The significance of a mechanism within the macrophage that modifies particulate antigen is discussed in relation to initiating antibody synthesis and explaining the action of agents that alter the immune response.

6930

Goos, R.D.; Summers, D.F. 1964. Use of fluorescent antibody techniques in observations on the morphogenesis of fungi. Mycologia 56:701-707.

Observations on cells of Candida albicans stained by FA indicate that wall material of the parent cell is incorporated into the wall of daughter cells or into hyphal walls when these are produced. A diminution in fluorescent intensity along the length of the newly formed hyphae was apparent, suggesting a dilution of parent wall material with continued growth. Fluorescence of newly formed elements was observed when the cells were grown in non-labeled immune serum ruling out the possibility that these cells became stained by possibly dissociated antibodies. Conidia of Fusarium oxysporum f. cubense stained intensely, but newly emerged germ tubes failed to do so. Sites of germ tube emergence were readily apparent as nonfluorescing areas of the conidium wall. When young colonies were treated with FITC labeled serum, the phialide tips, the microconidia, and macroconidia all stained intensely with antisera prepared against microconidia, although hyphal walls failed to react with the stain, suggesting an antigenic dissimilarity in the walls of the hyphae and conidia.

6931

Hahn, J.J.; Cole, R.M. 1963. Streptococcal M antigen location and synthesis, studied by immunofluorescence. J. Exp. Med. 118:659-666.

Streptococcal M protein has been studied directly in the intact streptococcal cell by specific immunofluorescence. By this method, it can be seen to be concentrated in or on the cell wall, but cannot be detected in the capsule. The lack of type-specific immunofluorescence after trypsinization, and the inhibition of group-specifc immunofluorescence by unlabeled type-specific antibody, are observations most compatible with a location of the M antigen determinants on the cell surface superficial to the group antigen. M antigen is not resynthesized after trypsinization of living cells, but appears anew only at sites of new cell-wall growth. A limited amount of such growth, leading sometimes to detectable amounts of M in the gross, can take place in deficient media without detectable increases in optical density of the cell population.

6932

Kent, S.P.; Evans, E.E.; Attleberger, M.H. 1964. Comparative immunology, lymph nodes and immunohistology of the amphibian, Bufo marinus. Federation Proc. 23:530:189.

There are a number of statements in the recent literature to the effect that amphibia do not have lymph nodes. In studying antibody formation to bovine serum albumin by Bufo marinus, encapsulated nodules of lymphoid tissue were noted along the great vessels of the upper thorax, neck, and axilla. Four to ten such nodules were noted on either side of the midline in the 53 animals dissected. Similar structures were not found in the lower thorax, abdomen, or inguinal areas. The nodules were composed of masses of lymphocytes associated with reticular fibers and thin-walled vascular spaces. The nodules phagocytized India ink and contained cells with pyroninophilic cytoplasm. Using the fluorescent antibody technique, antibody-forming cells were demonstrated in the lymph nodules, spleen, liver, kidneys, and thymus of animals appropriately stimulated with bovine serum albumin. The anatomical and functional characteristics suggest that these lymph nodules are comparable to the lymph nodes found in mammals. Complete article.

6933

Kent, S.P.; Evans, E.E.; Attleberger, M.H. 1964. Comparative immunology: Lymph nodes in the amphibian Bufo marinus. Proc. Soc. Exp. Biol. Med. 116:456-459.

Encapsulated nodes of lymphoid tissue have been demonstrated in the upper thorax, neck, and axilla of the amphibian Bufo marinus. The nodes phagocytize India ink and form antibody to bovine serum albumin. The anatomical and functional characteristics of these lymph nodes indicate that they are comparable to lymph nodes of mammals.

6934

Kriukova, I.N.; Obukh, I.B. 1964. Distribution of infectious virus Rous and viral antigen in infected rat and mouse organisms. Vop. Onkol. 10:3:3-8. In Russian.

The localization of infectious Rous virus and viral antigen was studied by fluorescent antibody in rats and mice infected with Rous virus when in newborn. In rats with hemorrhagic disease, infectious Rous virus was detected very rarely. Viral antigen was revealed in the newly formed cyst walls, liver, and lymph nodes. Hence, Rous virus seems to multiply in rats, but its maturing takes place very rarely. Data on our recent experiments on irradiation of pieces of rabbit fibrous nodes and rat

viscera and examination of affected animal sera for presence of virus-neutralizing antibody show a more complete form of integration of Rous virus with cells in rats than that in rabbits. In infected mice, viral antigen was detected very rarely.

6935

Litt, M. 1964. Studies in experimental eosinophilia: Uptake of immune complexes by eosinophils. J. Cell Biol. 23:355-361.

A method is described whereby immune complexes may be visualized in a single cell. Bovine serum albumin labeled with a red-fluorescing dye was joined to a rabbit antiserum labeled with a green-fluorescing dye to yield an immune complex that fluoresced yellow when illuminated by ultraviolet light. Such yellow-fluorescing immune complexes were injected into the peritoneal cavity of guinea pigs, and the peritoneal exudates were examined subsequently. Yellow fluorescent particles were seen in eosinophils obtained from guinea pigs sensitized to hemocyanin and from normal animals. Eosinophils of the blood and of the bone marrow could also take up the complexes in vitro. Neither antigen nor antibody alone was taken up by eosinophils, nor was a mixture of labeled antigen and labeled normal globulin. Similar observations were made with human blood eosinophils. These experiments suggest that eosinophils act as part of the defense against the pathogenic effects of certain immune complexes.

6936

Mikhailov, I.F.; Stanislavsky, E.S. 1963. Staining of isolated bacterial structures with fluorescent antibodies. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:6:74-79. In Russian.

Antigenic interrelationships between individual structural elements of intestinal bacteria were studied with the aid of FA. S. paratyphi B and E. coli 0111 were used. Staining peculiarities of isolated structural elements of the bacterial cell with fluorescent antibodies were also established. Antigens common to the two species were located in the cell membrane and cytoplasmic membrane. Cytoplasm contained no O antigen. H antigen was present only in flagellae. An even specific fluorescence of the stained structures was noted in studying the antigens of individual structural elements of the microbial cell. Isolated membranes were characterized by brighter fluorescence along the periphery.

6937

Okazaki, K.; Holtzer, H. 1965. An analysis of myogenesis in vitro using fluorescein-labeled antimyosin. J. Histochem. Cytochem. 13:726-739.

It is necessary to understand the intracellular activities of individual cells and the cross-talk between homologous and heterologous cells before one may understand the genetic controls of differentiation. FA is particularly suited to help in this understanding. The genetic control of multiplication functions in a greater variety of microenvironments than do the genes regulating myogenesis. This paper discusses the uses of FA for location of extra- and intracellular antigens and antibodies involved in myogenesis and related cell functions.

6938

Pierce, G.B., Jr.; Midgley, A.R., Jr. 1963. The origin and function of human syncytiotrophoblastic giant cells. Amer. J. Pathol. 43:153-173.

In an immunofluorescence and electron microscopic study of choriocarcinoma (approximating 14-day human trophoblast), evidence was obtained strongly supporting the concept that syncytiotrophoblast was derived by differentiation from cytotrophoblast. As the differentiated cell type of trophoblast, syncytiotrophoblast has as a principal function the secretion of chorionic gonodotropin.

6939

Romano, A.H.; Geason, D.J. 1964. Pattern of sheath synthesis in Sphaerotilus natans. J. Bacteriol. 88:1145-1150.

Formation of the characteristic sheath of Sphaerotilus natans was followed by immunofluorescence. Fluorescent antisheath antibody was obtained by injecting rabbits with purified sheath material from S. natans, fractionating the serum with ammonium sulfate, and conjugating the globulin fraction with fluorescein isothiocyanate. To follow sheath formation, S. natans was grown on slides immersed in Stokes medium at 28 C for 9 hours, and was reacted with labeled antibody. The slide cultures were then washed to remove unbound antibody, and were incubated further in fresh medium. Samples were removed at intervals and examined by fluorescence microscopy. Old portions of the sheath remained discretely labeled with no diminution in intensity of fluorescence, but nonfluorescent new sheath material appeared at the ends of the filaments. These results indicate that sheath synthesis does not take place by intussusception or diffuse intercalation, but by linear extension of pre-existing sheath. This interpretation was confirmed by a reverse procedure, whereby old sheath was reacted with unlabeled antibody, and new sheath was identified by addition of labeled antibody after further incubation. In this procedure, fluorescence was most intense at the growing tips of the filaments.

6940

Shockman, G.D. 1965. Symposium on the fine structure and replication of bacteria and their parts: IV. Unbalanced cell-wall synthesis; autolysis and cell-wall thickening. Bacteriol. Rev. 29:345-358.

Biochemical and morphological studies have indicated that unbalanced growth of S. faecalis can result in autolysis. There are quantitative and perhaps qualitative differences between specific nutritional and inhibitory situations that result in the same general effect. These differences, which may have considerable significance, have not been emphasized in this paper. Other cellular components, such as DNA, RNA, ribosomes, protein, and membrane, are affected also by a change in environment. Our present state of knowledge allows us only to speculate about the biochemical mechanisms that regulate either balanced growth and cell division or the more specialized unbalanced states. A hypothetical scheme is presented in an attempt to correlate observations on cell-wall thickening and autolysis with those by FA studies.

6941

Strachiloff, D.; Mohr, J. 1965. The use of the immunofluorescence method for antigen analysis of Salmonella. Zentrabl. Bakteriol. Parasitenk. Infektionskrankh. Hyg. 196:1:29-33. In German.

The application of the direct and indirect FA technique in the demonstration of the flagellum system of salmonellae is described. BA-46-99196.

6942

Takeuchi, Ikuo. 1963. Immunochemical and immunohistochemical studies on the development of the cellular slime mold Dictyostelium mucoroides. Develop. Biol. 8:1-26.

Hyperimmune sera were produced in rabbits against the spores of D. mucoroides. The heated antisera agglutinated spores as well as vegetative and interphase amebas, with no significant differences in titers. Precipitin ring tests of the antisera with extracts of cells at various stages of development indicated a marked increase in reactivity at the migrating pseudoplasmodium stages and a further increase at the spore stage. In order to study localization of antigens and combining groups at various stages of development, immunohistochemical studies were made by using fluorescein-conjugated antispore sera, both absorbed and unabsorbed. The staining of the cell surfaces indicated that there is a change in the surface in the interphase, this taking place some time after the amebas have finished feeding. Thereafter the cell surface remains unchanged until the time of spore formation. Antibody-stained cytoplasmic granules were observed at all

stages except the vegetative. During interphase, cells begin to synthesize a new combining group in or on certain granules in the cytoplasm. Location of differentiation and variation was studied. The significance of the cell sorting in the normal development of the cellular slime molds was discussed and compared with the development of animal embryos.

6943

Wagner, M. 1964. Studies with fluorescent antibodies on growing bacteria: I. The new formation of the cell wall of Diplococcus pneumoniae. Zentralbl. Bakteriol. 195:87-93. In German.

Living pneumococcus cells were stained with fluorescent antibodies and re-incubated after removal of excess antibody. The growing chains mostly divided simultaneously at all individual cells. The dark zones that appear during division are the new sections of cell wall. Growth of the pneumococcus cell takes place in an equatorial zone.

6944

Walker, P.D.; Batty, I. 1963. Facets of Clostridium sporogenes and Cl. botulinum as shown by fluorescent labeled antibodies. J. Appl. Bacteriol. 26:v.

Some of the antigenic changes occurring on the surface of Clostridium sporogenes during sporulation and germination were followed by means of spore antisera coupled with FITC and vegetative cell antisera coupled with RB 200. The first apparent change during germination was a loss in some areas of the spore antigens, probably synchronous with the splitting of the spore coat. Following this, vegetative antigens emerged, and the antigen of the spore gradually disappeared until only the vegetative cell antigens remained. During sporulation, spore antigens appeared on the surface of the vegetative cell only after the organism had become fully permeable to spore strains. Fluorescent labeled antisera to heat killed organisms were used to discriminate between the various types of C. botulinum. An antiserum prepared against a Type A organism stained all strains of Types A, B, and F, but none of Types C, D, and E. Antisera prepared against Type C and D gave complete cross-reaction with both these types and with no other type. An antiserum prepared against Type E stained organisms of Type E only. The antisera stained flagella as well as the cell wall. Considerable overlap of flagella staining was noted in the various types. Complete article.

6945

Walker, P.D.; Batty, I. 1964. Fluorescent studies in the genus Clostridium: I. The location of antigens on the surface of Clostridium sporogenes during sporulation and germination. J. Appl. Bacteriol. 27:137-139.

Fluorescent labeled specific antisera against spores and vegetative cells have been used as stains to follow the antigenic changes that occur on the surface of Clostridium sporogenes during sporulation and germination.

6946

Walker, P.D.; Batty, I. 1965. Surface antigenic changes in Bacillus cereus during germination and sporulation as shown by fluorescent staining. J. Appl. Bacteriol. 28:194-196.

Antigenic changes occurring on the surface of Bacillus cereus during sporulation and germination have been followed using FA.

VIII. NEOPLASMS

A. BASEMENT MEMBRANES

6947

Midgley, A.R., Jr.; Pierce, G.B., Jr. 1963. Immunohistochemical analysis of basement membranes of the mouse. Amer. J. Pathol. 43:929-943.

Suitably characterized fluorescein-labeled antiserum against a murine neoplastic hyalin has been shown to stain reticulin and all basement membranes of the mouse. Exhaustive absorption of this conjugated antiserum with splenic pulp resulted in a reagent that stained nearly all epithelial basement membranes of the mouse, including the pia-glial membrane on the surface of the brain and the extension of this membrane around vessels within the central nervous system, but did not stain reticulin or vascular basement membranes. Because antigens of epithelial basement membranes have been found in the cytoplasm of epithelial cells and not in connective tissue, it has been postulated that epithelial basement membranes in general are produced by the bordering epithelial cells.

6948

Midgley, A.R., Jr.; Pierce, G.B., Jr.; Sri Ram, J. 1963. Immunofluorescent studies on basement membranes of the mouse and other species. Amer. J. Pathol. 43:4a-5a.

FA against a murine neoplastic hyalin (fl-anti NH) was shown previously to react with reticulin and basement membranes of the mouse. After absorption with splenic pulp until the reaction with reticulin and vascular membranes was abolished, the conjugate stained a membrane of proven epithelial origin and was, therefore, epithelial-specific. Fl-anti NH specifically stained reticulin and all murine basement membranes studied. This conjugate stained nearly all epithelial basement membranes of the mouse but did not stain reticulin or vascular or endothelial basement membranes, including Descemet's. Because epithelial basement membranes are antigenically different from those of connective tissue origin, it was postulated that they were probably formed by the epithelial cells. The epithelial-specific conjugate also stained the cerebral pia-glial membrane and its investments around cerebral vessels. With the exception of rabbit, the species of origin of anti-NH, all vertebrates examined cross-reacted with fl-anti NH.

6949

Mukerjee, H.; Pierce, G.B.; Sri Ram, J. 1964. Chemical studies on epithelial basement membranes of the mouse. Federation Proc. 23:804:235.

A basement membrane antigen, neoplastic hyalin, NH, secreted by a parietal yolk sac carcinoma of the mouse was found by immunofluorescence and electron microscopy to be common to epithelial basement membranes but distinct from those in connective tissue. Chemical studies of NH also revealed significant differences from reticulin. NH contains 76 per cent protein, 13.8 per cent nitrogen, 2.6 per cent glucosamine, 2 per cent glucose, 2.2 per cent galactose, 2.8 per cent mannose, and 1.2 per cent lyxose. Representative values from amino acid analysis, grams per 100 grams, were glycine, 9.4; hydroxyproline, 7.0; and tyrosine, 2.4. NH can be solubilized by all proteolytic enzymes including collagenase and elastase or alkali and acid. In studies designed to obtain a soluble homogeneous fragment, treatment with acid or alkali yielded a single, electrophoretically pure nondialyzable fragment; tryptic digestion gave two nondialyzable fragments, with which was associated most of the carbohydrate of NH. Gel diffusion studies also revealed two antigenic fragments in the tryptic digest of NH. Complete article.

6950

Myers, J.; Frei, J.; Rose, B. 1964. An immunological investigation of basement membrane. Federation Proc. 23:2096:451.

Tan and Kaplan have shown fluorescent staining to be limited to basement membranes and reticulin with antimouse beta globulin. This pattern is identical to that we obtained with antiserum to neoplastic hyalin, NH, from a transplantable teratocarcinoma of mice. As an understanding of this phenomenon might help to further clarify the nature of basement membrane, the NH was compared immunologically with extracts of liver, kidney, and spleen and with serum or plasma. Absorptions sufficient to inhibit precipitin lines with all extracts do not inhibit the NH - anti-NH reaction, and the absorbed sera retain their specific fluorescent staining of basement membrane and reticulin. These findings support the theory of a dual nature of basement membrane. Complete article.

6951

Pierce, G.B., Jr. 1965. Basement membranes; VI. Synthesis by epithelial tumors of the mouse. Cancer Res. 25:656-669.

This is an extension of previous studies upon synthesis of basement membranes by epithelial cells. Two breast carcinomas and a granulosa cell carcinoma, when grown in vitro in the absence of connective tissue elements, synthesized basement membranes. Antigenically, by FA, these basement membranes were identical with each other and with the basement membranes adjacent to most epithelia in the mouse. Synthesis of basement membranes by carcinomas probably represents maintenance of a normal function after malignant transformation. Accordingly basement membranes should not be considered part of the defensive mechanism of the host designed to resist neoplastic invasion.

6952

Pierce, G.B., Jr.; Beals, T.F.; Sri Ram, J.; Midgley, A.R., Jr. 1964. Basement membranes: IV. Epithelial origin and immunologic cross-reactions. Amer. J. Pathol. 45:929-961.

Immunohistochemical techniques were employed to study the cross-reactions of Reichert's membrane, a basement membrane that is synthesized by parietal yolk sac epithelium. This epithelium basement membrane was antigenically distinct from connective tissues, yet it was present in the basement membranes of most epithelia. Reichert's membrane had no antigens in common with erythrocytes, beta globulins, or tissue components. Ferritin-labeled antibodies to this epithelial basement membrane antigen localized in the endoplasmic reticulum of parietal yolk sac epithelium and in extracellular basement membrane material. They also localized in the basement membranes of the testis, seminal vesicle, and visceral yolk sac, but failed to react with collagen or reticulin. Basement membranes of epithelia appear to be antigenically and chemically definable amorphous or finely fibrillar bands of mucoprotein synthesized by the adjacent epithelial cells. Antisera against the various tissues were used in direct FA studies.

6953

Pierce, G.B., Jr.; Beals, T.F.; Sri Ram, J.; Midgley, A.R., Jr. 1964. Immunohistochemical and immunoelectron microscopic study of basement membranes. Federation Proc. 23:803:234.

A basement membrane of the murine placenta, the Reichert membrane, is secreted by parietal yolk sac cells and its counterpart, neoplastic hyalin, NH, is secreted by a parietal yolk sac carcinoma. In order to demonstrate the specificity of these epithelial basement membrane

antigens, antibodies were obtained against NH, murine placentas, reticulin, collagen, serum proteins, and red blood cells; each antiserum was absorbed with a variety of tissue antigens and used immunohistochemically. It was concluded that NH and the Reichert membrane contain an antigen found in almost all epithelial basement membranes but not in connective tissues or red blood cells. Ferritin-labeled anti-NH, Fe anti-NH, when applied to cells secreting NH, localized in the endoplasmic reticulum and extracellular NH. Absorption of anti-NH with a microsomal fraction of these cells abolished staining of NH, suggesting that the epithelial basement membrane antigen is synthesized in the endoplasmic reticulum. When Fe anti-NH was applied to tissues neither reticulin nor collagen stained but the lamina densa of epithelial cells was heavily labeled. It appears that reticulin and collagen are not integral parts of the basement membrane. Complete article.

6954

Pierce, G.B., Jr.; Midgley, A.R., Jr.; Sri Ram, J. 1963. Histogenesis of basement membranes. Federation Proc. 22:575:256.

A parietal yolk sac carcinoma of the mouse that secretes large quantities of basement membrane - like material has been used to study the formation of basement membranes. Suitably characterized fluorescein-labeled antibodies prepared in rabbits against this material, fluorescent anti-NH, stained basement membranes of epithelial structures and vessels as well as reticulin. When absorbed with splenic pulp until neither vascular basement membranes nor reticulin fluoresced, fluorescent anti-NH still stained the basement membrane - like material of the tumor, its normal embryonic counterpart, the Reichert membrane, and the basement membranes at the bases of epithelial cells. This indicates that basement membranes of epithelia are formed independently of connective tissue or its ground substance. The epithelial specificity of anti-NH was confirmed electron microscopically. Epithelium-specific ferritin-labeled anti-NH localized in the basement membrane of epithelium but not in the adjacent connective tissue elements. Furthermore, epithelium-specific ferritin anti-NH localized in the endoplasmic reticulum of the parietal yolk sac cells, indicating this organelle as the intra-epithelial site of synthesis of basement membrane material. Complete article.

6955

Pierce, G.B., Jr.; Midgley, A.R., Jr.; Sri Ram, J. 1963. The histogenesis of basement membranes. J. Exp. Med. 117:339-348.

A parietal yolk sac carcinoma of the mouse that secretes large quantities of basement membrane - like material has been used to study the formation of basement membranes. Suitably characterized fluorescein-labeled antibodies against this material stained basement membranes of epithelial structures and vessels, as well as reticulin. When absorbed with reticulin and vascular basement membranes of the spleen until these structures no longer fluoresced, the antibody still stained the basement membrane - like material of the tumor, its normal embryonic counterpart, and the basement membranes at the bases of epithelial cells. Since a basement membrane, proved to be an epithelial secretion, is antigenically similar to basement membranes at the bases of all epithelial cells studied, but antigenically different from connective tissue elements, it is postulated that the basement membranes at the bases of epithelial cells in general are an epithelial secretion, and are not a condensation of ground substance as is commonly believed.

B. DIFFERENTIATION OF MALIGNANT FROM NORMAL TISSUE

6956

Carruthers, C.; Baumler, A. 1965. Immunochemical staining with fluorescein-labeled antibodies as an aid in the study of skin cancer formation. J. Nat. Cancer Inst. 34:191-200.

With fluorescein-labeled antibody as an immunochemical stain, differences have been demonstrated between the antigenic composition of mouse epidermis and carcinogen-induced squamous cell carcinomas. Antisera against epidermis after the hair was plucked, hyperplastic epidermis, and differentiated squamous cell carcinomas were used. After absorption of each of these antisera with the sediments of normal guinea pig liver, mouse lung, and kidney tissue, each antiserum strongly stained all of the tissues employed for antisera production. When the various antisera were absorbed with the sediments of the normal tissues and highly differentiated squamous cell carcinomas, only the squamous cell carcinoma antisera did not stain all the tissues. Normal and hyperplastic epidermis appears to have antigens that are not present in the carcinomas. When the various antisera were absorbed with the sediments of the normal tissues and epidermis, only the squamous cell carcinoma antisera stained the various tissues. Squamous cell carcinomas apparently contain antigens that are not present in the epidermis.

6957

Clarkson, B.; Katz, A.; Dann, M.A.; Karnofsky, D.A. 1964. Behavior of P388 mouse leukemia in the chick embryo and hatched chick. J. Nat. Cancer Inst. 32:471-505.

P388 and several other mouse leukemias grow poorly on the chorioallantoic membrane of the chick embryo, but may proliferate rapidly when introduced into the embryo by intravenous injection, or by metastasis from chorioallantoic explants. Proliferation of P388 cells in the embryo probably continues until shortly after it has hatched, and then stops; however, viable leukemic cells may persist in the chick brain for as long as 6 weeks after hatching. The brain appears to be the most favorable site for growth and long-term survival of P388 cells. P388 cells, identified by FA, injected intravenously or intracerebrally into 1-day-old hatched chicks will survive for long periods, but, when given to 7-day-old and older chicks, the cells fail to survive. This suggests the rapid maturation of immunological competence by the chick shortly after hatching. By using specific fluorescent antibodies or bioassay for leukemia in susceptible mice, or both, we generally observed persistent survival of some P388 cells in the organs studied (brain, liver, spleen, heart) until the 20th day of incubation.

6958

Gold, P.; Freedman, S.O. 1965. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J. Exp. Med. 121:439-462.

Two methods were used to demonstrate the presence of tumor-specific antigens in adenocarcinomata of the human colon. Rabbits were immunized with extracts of pooled colonic carcinomata, and the antitumor antisera thus produced were absorbed with a pooled extract of normal human colon and with human blood components. Newborn rabbits were made immunologically tolerant to normal colonic tissue at birth, and were then immunized with pooled tumor material in adult life. The antisera prepared by both methods were tested against normal and tumor antigens by agar gel diffusion, immunoelectrophoresis, hemagglutination, PCA, and immunofluorescence. Distinct antibody activity directed against at least two qualitatively tumor-specific antigens, or antigenic determinants, was detected in the antisera prepared by both methods. Two additional tumor antigens were detected exclusively in antisera prepared by the tolerance technique. Tumor-specific antibodies were not directed against bacterial contaminants or against the unusually high concentrations of fibrin found in many neoplastic tissues. The pooled tumor extracts contained tumor-specific antigens not present in normal colonic tissue. Identical tumor-specific antigens were demonstrated in a number of individual colonic carcinomata.

6959

Gold, P.; Freedman, S.O. 1965. Specific antigenic similarity between malignant adult and normal fetal tissues of the human digestive system. J. Clin. Invest. 44:1051-1052.

To demonstrate the existence of tumor-specific antigens in adenocarcinomata of the human colon, corresponding tumor-specific antisera were prepared by two techniques: (1) antiserum produced by rabbits immunized with an extract of pooled human colonic cancers was absorbed with an excess of pooled normal colonic tissue components and with pooled human plasma, and (2) rabbits made immunologically tolerant to pooled normal colonic tissue in neonatal life were immunized as adults with an extract of pooled colonic cancers. Antibodies against tumor-specific antigens were demonstrated in both types of antisera by various tests including FA. Tumor-specific antigens were also detected in individual specimens of human colonic cancers. Antigenic components identical to those found in colonic cancers were demonstrated by precipitin-inhibition in specimens of primary cancers of the human rectum, duodenum, stomach, esophagus, and pancreas. These antigenic components were absent from all other adult malignant or normal tissues tested. Human fetal gut, liver, and pancreas obtained between 2 and 6 months

of gestation contained the same antigenic components found in cancers of the digestive organs. Tumor-specific antigens may arise during malignant transformation as a consequence of a reversion of the entodermally derived epithelial cells of the human digestive system to a more primitive dedifferentiated state.

6960

Goudie, R.B.; McCallum, H.M. 1963. Loss of tissue-specific autoantigen in thyroid tumors. Lancet 2:1035-1038.

Twenty-two thyroids were studied by the fluorescent antibody technique, using cytotoxic Hashimoto's serum as a specific stain for the cytoplasmic autoantigen peculiar to thyroid epithelium. The cells of four thyroid carcinomata were found to be deficient in autoantigen, and less obvious autoantigen loss was readily demonstrated in some or all of the cells in three of six simple thyroid adenomata and in two of four adenomatous goiters. Autoantigen loss was trivial or absent in normal thyroid and in six of seven thyrotoxic thyroids. These immunological findings may have a bearing on invasive behavior of thyroid-carcinoma cells.

6961

Haberman, S.; Sanford, B.; Stapp, W.F.; Race, G.J. 1965. The use of fluorescent-dye-tagged anti-DNA-P in detection of certain cancers. Amer. J. Clin. Pathol. 44:573.

Specific anticancer DNA sera were prepared in rabbits, using undenatured DNA-bound protein extracts (DNA-P) and nuclear fragments obtained from several human cancers. The antisera were absorbed with normal tissue homogenates to remove antihuman and antiorgan reactivity. When these antisera were conjugated with FITC, they could be used on tissue for the detection of cancerous DNA-P within the nuclei of certain neoplastic cells. Considerable cross-reactions between cancers were found; e.g., the ar serum made with nuclear material obtained from a mesothelioma gave in- nuclear staining with mesothelioma, squamous cell carcinoma of lung, adenocarcinomas of the colon, stomach, pancreas, and liver, duct-cell carcinoma of breast, and an in situ squamous cell carcinoma of the cervix. These cross-reactions did not correlate with the embryologic development of the tissues, but they seemed to involve an antigen within the DNA-bound proteins of the nuclei found in these cancers. These findings offer a new approach for a diagnostic test for cancer.

6962

Herbeuval, R.; Duheille, J.; Goedert-Herbeuval, C. 1965. Diagnosis of unusual blood cells by immunofluorescence. Acta Cytol. 9:73-82.

Characterization of tumor cells by immunofluorescence still gives rise to great difficulties, but application of this technique seems likely to expand in the future. The indispensable requirement for progress in this field lies in a better knowledge of tissue antigenicity. The use of immunologic techniques allowing quantitation of these antigens, such as immunoelectrophoresis and double diffusion in gelose, is of the greatest significance. Such methods permit identification of some of the detected antigens, through enzymatic or histochemical reactions. One may hope, in a few cases, to isolate specific antigens in tumor cells, and consequently obtain specific labeling in immunofluorescence.

6963

Kyogoku, M.; Yagi, Y.; Planinsek, J.; Bernecky, J.; Pressman, D. 1964. Localizing properties of anti-rat hepatoma antibodies in vivo. Cancer Res. 24:268-279.

The localizing properties of the antibodies against the stroma sediments of N-2-fluorenylacetamide-induced hepatoma and normal liver were compared in tumor and normal rats. Radioautography with I-125-labeled antibodies and the fluorescent antibody technique were used. When the globulin fractions of both antisera were injected directly into the tumor-bearing rats as well as into the normal rat, antibody localized on the walls of sinusoids and in connective tissue parts of tumor as well as in normal liver. No difference has been found between the localization properties of both sera. After passage through normal rat there were some differences in the localization pattern of these two antisera in the tumor. The anti-hepatoma antibody still localized specifically in the dense connective tissue, but no localization was observed with the antinormal liver serum. There was no specific localization on the wall of sinusoid-like vascular channels with either serum. The possible reason for this difference was discussed, and the usefulness of the combination of radioautography and fluorescent antibody technique for the detection of the site of localization of antibody at a cellular level is stressed.

6964

Meyers, R.L.; Miller, J.N. 1965. Immunologic studies on the Walker 256 carcinosarcoma cultivated in vitro. Federation Proc. 24:278:177.

The Walker 256 carcinosarcoma was established in tissue culture and found to retain its capacity to produce tumors. An antiserum was prepared to the tissue culture cells and specific antibodies were demonstrated using solubilized cells. Gel diffusion studies with rabbit anti-tissue culture cell serum demonstrated that deoxycholate-solubilized cells contained at least three precipitating components; two components were detected with ultrasonicated cells and one with pulverized cells. Antisera prepared against sera from normal and tumor-bearing rats did not cross-react with tissue culture cells. The specific tissue culture cell antiserum did not react with saline extracts of normal rat liver, spleen, skeletal muscle, kidney fibrin, or red cell hemolysate, but rabbit antiserum to normal rat serum reacted with all extracts. The cytoplasmic cellular localization of the antigens in tissue culture cells was detected by the indirect fluorescent staining technique. The presence of antibody in the gamma globulin fraction of the specific tissue culture antiserum was demonstrated by the passive cutaneous anaphylaxis technique. Complete article.

6965

Perez-Cuadrado, S.; Haberman, S.; Race, G.J. 1964. Production of specific antisera to human cancer cell nuclear antigens. Federation Proc. 23:2095:451.

Rabbits were used to produce antisera to DNA-containing cancer antigens prepared from ground cell pulps either by differential centrifugation and nucleic acid enzyme treatment or by extraction with glycine-salt solutions. These antisera, when purified by absorption with noncancerous tissues, showed specificity for certain cancers when tested on more than 100 cancerous and noncancerous specimens. The methods used were agglutination of cell particles, agglutination of antibody-sensitized latex balls, and fluorescent antibody staining of tissue sections. The cancer-specific globulins, when separated from the purified antisera by saturated ammonium sulfate, gave up to five bands of precipitation with antirabbit globulin in agar double diffusion plates; only two bands were observed when these antisera were absorbed with cancerous tissues. The DNA nature of the cancer-specific antigens was revealed by histochemical studies and UV light spectrophotometry of the crude extracts from which sodium-DNA was obtained. Fluorescent antibody staining showed bright fluorescence only within cancer cell nuclei in which the DNA moiety was preserved. Complete article.

6966

Perez-Cuadrado, S.; Haberman, S.; Race, G.J. 1965. Fluorescent antibodies to human cancer-specific DNA and nuclear proteins. Cancer 18:193-200.

Direct and indirect FA tests were used. Fractions of cancerous tissue were used to prepare the antisera. Cancer-specific antigens were identified as rounded, intranuclear DNA bodies. They could be made immunologically unreactive by certain chemical treatments. Other characteristics of the antigens are described. The antigens were located by FA in tissue sections of adenocarcinomas.

6967

Tanigaki, N. 1963. Studies on the cell-surface antigens of rat ascites tumors by fluorescent antibody technique. Gann 54:137-148.

Immunological properties of the antiserum prepared against the insoluble fraction of GDT-2, a rat ascites hepatoma, have been investigated by the indirect fluorescent antibody technique. Rat tumor antigens were widely distributed in stromal and epithelial elements of normal rat tissues. The cell surface of rat ascites tumor contained some antigens distinct from that of rat red blood cells, serum, or liver, although the specificity of such antigens was not convincing because of the indication that the antigens were distributed in other organs. Immunological significance of the cell surface and interpretation of some phenomena observed by fluorescent antibody technique are also discussed.

C. GENERAL TUMOR STUDIES

6968

Bachmann, A.E.; Pedace, E.A.; Pavlovsky, A. 1965. Serum and immunological studies in lymphoma: II. Application of polyacrylamide electrophoresis techniques to antiglobulin consumption and immunofluorescence. Medicina 25:39-42. In Spanish.

The sera of 25 patients with lymphomas and 58 patients with other conditions were investigated by disk electrophoresis. No abnormal seric proteins were observed. The lymph nodes obtained from biopsy in 24 patients with lymphoadenopathies, together with the corresponding sera, were studied by the antiglobulin consumption and indirect immunofluorescence tests. All the results were negative with the first technique; by indirect immunofluorescence, a nonspecific fluorescence was observed in the lymph nodes of two patients with lymphomas and in another two with reactive adenitis.

6969

Back, N.; Hiramoto, R.; DeWitt, G.; Shields, R.; Branshaw, R.; Ambrus, J.L. 1963. Immunofluorescent staining technique as an aid in studying plasmin localization. Federation Proc. 22:2396:561.

Specific localization of plasmin onto fibrin has been established. With the aid of a sensitive immunofluorescent staining method, the character of this localization has been studied. The technique also has been adapted to the study of fibrin deposition and plasmin localization in rodent tumors. Unlabeled specific antisera were prepa in rabbits with the following antigens: streptokinase, SK, urokinase, UK, SK-, UK-, and spontaneously activated human plasmin, and $CHCl_3$-activated bovine plasmin. Experimental clots or tumors were removed at various time intervals following intravenous plasmin or activator injections. Frozen tissue sections were incubated with antisera and antibody-antigen complex visualized with fluorescein-labeled horse antirabbit gamma globulin reagent. Observations made by UV microscopy revealed plasmin localization in the periphery as well as central portions of clots and tumors. Complete article.

6970

Baumgarten, A.; Curtain, C.C.; Whiteside, M.G. 1965. The immunocytochemical localization of cryomacroglobulins. Australian Ann. Med. 14:125-129.

Cryomacroglobulin was localized by FA in the plasma cells of the bone marrow and spleen of a patient with malignant lymphoma of an unspecified type. Cryomacroglobulin was also localized in the plasma cells of the bone marrow of another patient with lymphoma. In neither case was the cryomacroglobulin detected in the small lymphocytes with scanty cytoplasm, which were observed in relatively large numbers in bone marrow and splenic smears from the two patients. Although there is no evidence that these lymphocytes are direct precursors of the cryomacroglobulin-containing plasma cells, there may be a transfer of information between the two cell lines analogous to that suggested in normal antibody production between macrophage and lymph node cell. BA-46-80323.

6971

Blaylock, W.K.; Scoggins, R.B.; Malmgren, R.A.; Van Scott, E.J. 1963. Characterization of antibodies produced in a horse following injections of mycosis fungoides lymphoma tissue. J. Invest. Dermatol. 41:429-438.

Preliminary investigations demonstrated that serum from a horse injected with mycosis fungoides tumor homogenates fixed complement in the presence of homogenates of similar tumors; fixation was absent or reduced with homogenates of normal skin, normal lymph node, and with human serum. A common precipitin band between this horse serum and homogenates of mycosis fungoides tumor and normal skin seemed identical to the bands occurring between the horse serum and normal human serum. Fluorescent antibody studies indicate a stromal antibody in Joe II horse serum that was reactive in all tissues exposed to serum. In addition, antibody against the cytoplasm of cells in mycosis fungoides tumors was demonstrable. Small amounts of Joe II serum injected into mycosis fungoides tumors produced necrosis in the first 24 hours. Necrosis was not seen when small amounts of normal horse serum were injected into similar tumors. Possible explanations of these findings are presented.

6972

Burtin, P.; Buffe, D. 1963. Immunofluorescent studies of human plasma cells in gamma and beta-2A myelomas. Proc. Soc. Exp. Biol. Med. 114:171-175.

Thirty-seven bone marrow aspirates of human gamma and beta-2A myelomas were smeared and labeled by fluorescent antibodies against gamma and beta-2A globulin. Never were all the observed plasma cells reactive with fluorescent antibodies, and in half of the cases, no fixation by any plasma cell was noted. Plasma cells of different types were fluorescent, most of them having a granular structure. They were found to fix fluorescent antibodies against gamma globulin or beta-2A globulin, but never bot'.

6973

Chibowski, D. 1964. Immunohistochemic studies on the cells of Ehrlich's ascites carcinoma. Ann. Univ. Mariae Curie-Sklodowska Sect. D Med. 19:52:429-439. In Polish.

Heterologic serum from rabbits against Ehrlich ascites tumor cells was found to contain antibodies reacting with normal glomerules, the mesothelia of the liver sinuses and the walls of the blood vessels, thus appearing to contain antigens of normal tissues besides their specific antigens. When cytotoxic changes occurred, antibodies directed against antigens of the cell membrane were most active. Fluorescence of the cytoplasm after the cell membrane was damaged suggested that the cell membrane and cytoplasmic stroma have features of the antigenic structure in common. BA-47-72784.

6974

Cox, M.T. 1964. Malignant lymphoma of the thyroid. J. Clin. Pathol. 17:591-601.

Nine cases of malignant lymphoma of the thyroid are described; the clinical features and histological patterns are recorded and the latter are related to results of serological tests for thyroid antibodies. The value of such tests in clinical assessment is discussed. The distribution of metastases and their predilection for the gastrointestinal tract are noted.

6975

Curtain, C.C. 1964. Immunocytochemical localization of two abnormal serum globulins in one bone-marrow smear. Australasian Ann. Med. 13:136-138.

With the use of a two-label fluorescent antibody technique, it was demonstrated that the bone marrow of a patient suffering from myeloma contained two groups of cells, each possessing a different protein antigen in its cytoplasm. The antigen corresponded to an 18S and a 7S globulin occurring in the patient's serum. This finding is discussed in relation to the current hypotheses concerning the origin and identity of the abnormal globulins of myeloma.

6976

Dumonde, D.C.; Bitensky, L.; Cunningham, G.J.; Chayen, J. 1965. The effects of antibodies on cells: I. Biochemical and histochemical effects of antibodies and complement on ascites tumor cells. Immunology 8:25-36.

Biochemical and histochemical methods were used to study the interaction of antibodies and complement with mouse Ehrlich ascites tumor cells. In the presence of complement, both iso- and hetero-antibodies caused cell lysis with penetration of antibodies into the damaged cells, as detected by immunofluorescence; the cells were then unable to support aerobic glycolysis, although they retained their ability to consume oxygen in the presence of succinate. Under these conditions there was unmasking of phospholipid, particularly at the cell surface, together with lysosomal changes resulting in diffuse staining for lysosomal acid phosphatase. In the absence of complement, antibodies did not appear to penetrate the cells, which respired normally and were not lysed.

6977

Eaton, M.D.; Levinthal, J.D.; Scala, A.R.; Jewell, M.L. 1965. Immunity and antibody formation induced by intraperitoneal or subcutaneous injection of Krebs-2 ascites tumor cells treated with influenza virus. J. Nat. Cancer Inst. 34:661-672.

Incubation of Krebs-2 ascites tumor cells with influenza virus at high multiplicity reduced the tumor-producing capacity of cell suspensions. These virus-treated cells could then be used as an antigen to stimulate resistance to the tumor when given intraperitoneally. An attempt was made to define the optimal conditions of virus treatment for a nonlethal but effective antigen. Since less than ten Krebs-2 cells given intraperitoneally produced lethal ascites tumors in most Swiss mice, immunization with fully viable cells by this route was virtually

impossible. Some mice could be given large numbers of cells subcutaneously, with production of immunity to intraperitoneal challenge. Virus-treated cells given subcutaneously failed to immunize. The serum from mice hyperimmunized with Krebs-2 cells gave specific reactions by fluorescein-antibody labeling of surface antigens. With other tumors a reaction was observed with 1 to 5 per cent of cells in a suspension. When complement was present, the labeling of surface antigens of Krebs-2 cells was followed by cytolysis.

6978

Eklund, A.-E.; Gullbring, B.; Lagerlof, B. 1963. Blood-group-specific substances in human gastric carcinoma: A study using the fluorescent antibody technique. Acta Pathol. Microbiol. Scand. 59:447-455.

The fluorescent antibody technique has been used in fresh tissue sections to study the occurrence of blood-group-specific substances in normal gastric mucosa and gastric carcinomas of varying degrees. These occur in glands, on the mucosal surface and on the cellular surface itself. The results were not dependent on the secretor status. With one exception - a scirrhous carcinoma poor in cells - all the carcinomas showed positive fluorescence.

6979

Emmart, E.W.; Bates, R.W.; Turner, W.A. 1965. Localization of prolactin in rat pituitary and in a transplantable mammotropic pituitary tumor using fluorescent antibody. J. Histochem. Cytochem. 13:182-190.

With fluorescent antiprolactin globulin and appropriate controls, lactogenic hormone has been localized in granules and mitochondria of acidophils of normal rat pituitary and in a transplantable rat pituitary tumor. Cells containing prolactin have been identified as acidophils by their reaction with orange G and by positive staining of their cytoplasmic granules with acid fuchsin in the Russell technique. Immunochemical data are presented reaffirming the heterogeneous character of purified ovine prolactin. Evidence that this is associated with polymerization of prolactin is discussed.

6980

Engelhardt, N.V.; Khramkova, N.I.; Postnikova, Z.A. 1963. Antigenic structure of mouse hepatomas: IV. Study of the liver, organospecific antigen in the liver, and hepatomas with fluorescent antibodies. Neoplasma 101:133-142.

The localization of one of the mouse liver organospecific antigens was studied in liver and four strains of transplantable hepatomas by means of indirect fluorescent antibodies. Monospecific antibodies to the studied

antigen were used for this purpose. The studied antigen was revealed in the cells of liver parenchyma, so the alterations in its concentration in tumors were caused by changes of the hepatic cells when transformed into tumor cells.

6981

Forrester, J.A.; Dumonde, D.C.; Ambrose, E.J. 1965. The effects of antibodies on cells: II. Changes in the electrophoretic mobility of ascites tumor cells treated with antibodies and complement. Immunology 8:37-48.

This communication describes the use of micro-electrophoresis in studying the changes in ascites tumor cells exposed to antibodies and complement. Treatment of the cells with rabbit antibody led to a change in electrophoretic mobility consistent with a surface adsorption of gamma globulin. The addition of complement led to a reduction in this electrophoretic effect of antibody. Treatment of the cells with neuraminidase, which produced a marked fall in their electrophoretic mobility, did not alter the effect of rabbit antibody and complement on the cells. Incubation with iso-antibody, in the presence or absence of complement, did not alter the mobility of the ascites cells measured at pH 7.0. The possibility is discussed that, during immune cytolysis, unmasking of phosphate groups of phospholipids might take place in the cell surface. FA demonstrated surface antigens.

6982

Ghose, T.; Tso, S.C. 1964. Uptake of protein by regenerating liver cells. Nature 204:1210-1211.

Regenerating hepatic tissue in vivo shares with malignant cells the capacity to take up intact protein molecules. Hepatic parenchymal cells, even after minor injury, are known to be permeable to serum proteins, and further studies must be made on other rapidly growing in vivo systems before any generalization can be drawn. We have yet to learn whether the uptake of intact protein molecules is attributable to increased permeability of the cell membranes of rapidly growing tissues or to a more active process, and also how such intracellular serum proteins contribute to the increased metabolic demands of rapidly growing systems.

6983

Gluck, E. 1963. Immunohistochemical investigations of human neoplasia. Acta Unio Int. Contra Cancr. 19:154-157.

The immunological activity of Bjorklund's antihuman cancer serum (AHCS) in sections from normal and neoplastic tissues has been investigated by indirect FA. Both normal and neoplastic tissues show specific fluorescence with AHCS. Absorption of AHCS with antigens from normal and neoplastic tissues abolishes the fluorescence in both the structures, indicating that the antigens in both structures are identical. Absorption of AHCS with organ-specific antigens seems to indicate that AHCS contains organ-specific antibodies. Tumor-specific antibodies have not been demonstrated with the FA technique.

6984

Greenspan, I.; Brown, E.R.; Schwartz, S.O. 1963. Immunologically specific antigens in leukemic tissues. Blood 21:717-728.

The injection of extracts of leukemic and Hodgkin's disease tissues of man elicit an antibody reaction in both man and rabbit. This response is similar to that elicited by leukemic mouse tissues when injected into rabbits. The antibody reaction may be demonstrated by passive cutaneous anaphylaxis, immunodiffusion, microprecipitin, and FA. Tissue extracts from non-leukemic individuals do not elicit a similar response. Rabbits immune-tolerant to normal human tissues produce antibodies specific to leukemic human antigens. Antibodies develop in those individuals who are exposed for a long time to either human or mouse leukemia. These studies demonstrate specific antigenic differences between normal and leukemic tissue extracts. The difference between normal and leukemic extracts may be the consequence of the presence of viruses or the alterations caused by them.

6985

Herbeuval, R.; Duheille, J.; Bellut, P. 1964. Immunofluorescence study of the antigenic properties of the atypical epithelioma of Guerin's T 8 rat. Compt. Rend. 258:2938-2941. In French.

A fluorescent immune serum prepared against a microsomial fraction of the atypical epithelioma of Guerin's T 8 rat cells demonstrated the existence of a specific antigen that was more abundant as the cells' maturation state was advanced. The nature of this antigen is still unknown, but it does not seem to be of viral origin. Electron microscope study of the tumor showed only rare and variable particles having viral appearance.

6986

Hiramoto, R.; Jurand, J.; Bernecky, J.; Pressman, D. 1963. Immunohistochemical differences among N-2-fluorenylacetamide-induced rat hepatomas. Cancer Res. 23:109-111.

Selected antihepatoma microsome antisera and a pool of antinormal rat liver microsome sera were studied by the indirect fluorescent antibody technique. Some of these sera were able to detect antigenic differences among several induced rat hepatomas from individual rats. Two selected antihepatoma sera out of 11 showed marked specificity in their reaction with hepatoma. After rat liver absorption each serum was able to react only with the hepatoma against which it was prepared and did not react with other hepatomas or with normal liver.

6987

Hobbs, J.R. 1963. Amyloid and the lymphocyte. Lancet 1:1217.

As a portion of this report, reference is made to positive FA staining of PAS-positive cells in distorted lymph nodes from malignant lymphoma using an anti-beta-2 macroglobulin serum.

6988

Holmes, B.; Weiser, R.S. 1964. The mechanism of rejection of sarcoma I ascites tumor in the C57Bl-6K mouse. Federation Proc. 23:1514:353.

Our previous phase-cinematography studies demonstrating that immune peritoneal macrophages possess specific affinity for tumor cells, TC, were confirmed. Although the fluorescent antibody method failed to demonstrate antibody on sarcoma I, SAI, cells during rejection, its presence was indicated by cytotoxicity effects induced by rabbit complement, both in vivo and in vitro. Attempts to increase the low rate of phagocytosis of TC by in vitro treatment with hemagglutinating antibody, or with antibody plus complement prior to injection into immune animals, failed. On the 5th day of tumor, before a significant amount of hemagglutinating antibody was present, the injection of either antibody or rabbit complement had no effect on the tumor. In contrast, the injection of both antibody and complement at this time caused a marked increase in the per cent of nonviable tumor cells. Decomplementation by intraperitoneal injection of BSA - anti-BSA precipitate failed to influence the course of tumor rejection. These findings indicate that the limiting factor in SAI rejection by humoral antibody in the C57 black mouse is a deficiency in complement that may be either quantitative or qualitative. Complete article.

6989

Isojima, S.; Bernecky, J.; Planinsek, J.; Yagi, Y.; Pressman, D. 1965. Differences between antibodies against the dense sediment and microsome fractions of the N-2-fluorenylacetamide-induced rat hepatoma. Cancer Res. 25:968-975.

Rabbit antibodies against the microsome and dense sediment fractions of N-2-fluorenylacetamide-induced rat hepatoma were compared in vivo and in vitro. Although both antibodies localized in the hepatomas and normal livers when injected into tumor-bearing or normal rats, clear differences in their localizing properties could be shown by various techniques. Staining tissue sections in vitro by FA showed that the anti-microsome antibodies were directed against the components of the hepatic cells and the anti-sediment antibodies against the components of the connective tissues.

6990

Jarett, L.; Lacy, P.E.; Kipnis, D.M. 1963. Characterization by immunofluorescence of an 'ACTH-like' substance produced by non-pituitary tumors. Amer. J. Pathol. 43:11a.

Porcine ACTH (25 mu per mg) served as an antigen for the production of antibodies in rabbits. The antibodies were conjugated to FITC. Normal human pituitary glands obtained at necropsy stained positively by direct and indirect methods. The staining reaction could be blocked by ACTH or by pretreatment of sections with nonfluorescent antiserum. Approximately 1.0 mu ACTH was inactivated by 1.0 ml of antiserum as determined by neutralization techniques. Patient E.D. had an undifferentiated carcinoma of the parotid gland. Another, M.J., had an islet cell tumor of the pancreas with symptoms of the Zollinger-Ellison syndrome. Previous bioassays of the serum and tumor of these patients for ACTH revealed elevated serum levels of ACTH-like activity. FA studies were performed on frozen-dried tumor of both patients and the pituitary of M.J. Positive staining was obtained on both tumors. In the pituitary of M.J., the cells that stained were fewer in number and less intense than in normal human pituitaries. Various non-pituitary tumors may be capable of producing and storing a substance immunologically similar to ACTH.

6991

Jarett, L.; Lacy, P.E.; Kipnis, D.M. 1964. Characterization by immunofluorescence of an ACTH-like substance in nonpituitary tumors from patients with hyperadrenocorticism. J. Clin. Endocrinol. Metab. 24:543-549.

Antiserum specific to porcine ACTH was used to study nonpituitary tumors from patients with hyperadrenocorticism by FA in order to characterize further a substance in the tumors that is biologically similar to ACTH. These tumors stained specifically for ACTH by this technique. The pituitaries of two of the patients contained lower than normal amounts of ACTH and the plasma of the patients contained increased levels of ACTH-like activity by bioassay. One of the tumors with biological activity did not stain. The histopathology of these tumors and its relation to the ACTH content of the tumors is discussed. It would seem that certain tumors are capable of producing a substance biologically and immunologically similar to ACTH that suppresses the normal ACTH-producing mechanism in the pituitary.

6992

Johnson, W.; Jurand, J.; Hiramoto, R. 1965. Immunohistologic studies of tumors containing myosin. Amer. J. Pathol. 47:1139-1155.

Antibodies to myosin, a protein present in embryonal rhabdomyosarcoma, were developed by injecting into rabbits myosin extracted from normal human skeletal muscle. Fluorescein-tagged goat anti-rabbit-globulin globulin was obtained and used in the indirect FA method on frozen sections to study four embryonal rhabdomyosarcomas. Three of these exhibited positive immunofluorescence. Two of four Wilms's tumors also revealed groups of FA positive cells. Skeletal muscle cells were found in the same two tumors by light microscopy. Mouse rhabdomyosarcoma exhibited positive immunofluorescence by this technique. Positive controls consisted of normal human skeletal muscle. Normal human liver and spleen were used as negative controls.

6993

Krivenkov, G.N.; Khan, N.T. 1965. Diagnostic and prognostic significance of examining the body phagocytic reaction in leukemia and lymphogranulomatosis: I. A new method for observing the digestive capacity of leukocytes in dynamics. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:10: 84-89. In Russian.

The authors suggest a method of prolonged observation over the phagocytic activity of individual cellular elements incubated with the test microbes in a microchamber, i.e., in conditions of combined bacteriologic and

bactericscopic investigations. The method is not complicated, is demonstrative, and permits recording various stages of phagocytosis. With its aid, it is possible to characterize the intensity of completed phagocytosis both qualitatively and quantitatively. During the observation period in the microchamber, optimal conditions for leukocytes and test microbes were maintained. Observations were carried out by phase-contrast or fluorescence microscopy. The method may be used in the study and assessment of various functional states both of phagocytes and microbes under the effect of various factors of external and internal environment.

6994

Lejneva, O.M.; Zilber, L.A.; Ievleva, E.S. 1965. Humoral antibodies to methylcholanthrene sarcomata detected by a fluorescent technique. Nature 206:1163-1164.

The best results were obtained in experiments with living cells. In all experiments a green-yellow fluorescence of three types was observed: fluorescence of all the dots on the whole cell surface, fluorescence of single dots, fluorescence of vacuoles due to pinocytosis of fluorescent dye. Living cells pre-treated with mouse antiserum and afterwards exposed to the fluorescent rabbit anti-mouse serum usually demonstrated the first type of fluorescence considered specific. The proportion of fluorescent cells in the experiment was about 85 per cent. To estimate the results of the experiments, a fluorescence index was calculated. Various reactions of two antitumor sera against three types of tumors are recorded.

6995

Leznoff, A.; Davis, B.A. 1963. The cytological localization of human chorionic gonadotropin. Can. J. Biochem. Physiol. 41:2517-2521.

The indirect fluorescent antibody technique was used to determine the cellular site of human chorionic gonadotropin (HCG) in normal and toxemic placentas and in choriocarcinomas. In placental tissue specific fluorescence was located in the syncytial cells of the chorionic villi but not in the cytotrophoblast cells. In choriocarcinomas specific fluorescence was seen in the syncytial giant cells. No distinct difference could be demonstrated between normal and toxic placentas. Differences in the content of HCG in placentas at various stages of pregnancy were noted. Maximum amounts were demonstrated in tissue of less than 14 weeks' gestation. Lesser quantities could be seen in more mature placentas and some specific fluorescence could be seen in most full-term placentas.

6996

Miller, D.G. 1963. Abnormal antibody production, autoimmune disease, and allergic disease in patients with lymphosarcoma and chronic lymphocytic leukemia. J. Clin. Invest. 42:957.

In studying the course of patients with lymphosarcoma and chronic lymphocytic leukemia for infectious complications, it became apparent that autoimmune and allergic diseases were also prominent. The autoimmune diseases included rheumatoid arthritis and other connective tissue diseases, hemolytic anemia, and thrombocytopenic purpura. The allergic processes included dermatitis, allergic purpura, and anaphylactic reactions. The gamma globulins, antibodies, and related phenomena in these patients are outlined. The leukocytes of four patients were coated with globulin, as demonstrated by fluorescein-labeled antihuman globulin. The presence of abnormal antibodies did not always correlate with the clinical findings.

6997

Möller, G. 1963. Studies on the mechanism of immunological enhancement of tumor homografts: II. Effect of isoantibodies on various tumor cells. J. Nat. Cancer Inst. 30:1177-1203.

Attempts were made to induce enhancement with tumors showing partial or full sensitivity to the cytotoxic action of H-2 antibodies. Partially sensitive tumors could be enhanced in H-2 systems. A completely sensitive leukemia could be enhanced in non-H-2 systems. Hypotheses concerning immunological enhancement were studied. It could not be demonstrated that antibodies stimulated tumor growth. On the contrary, inhibition was constant with several tumors and different test systems. Tumor cells exposed to antibodies for a short period in vitro, or a long time in vivo, did not change their transplantation characteristics to increased homotransplantability. Enhancement was obtained with tumor cells coated with antibodies in the absence of circulating antibodies in the hosts. Such antibody-coated cells were inferior to untreated cells in their ability to elicit the homograft reaction. It was concluded that antibodies did not change tumor cell characteristics, but probably exerted their effect by an inhibition of transplantation immunity and not by a central mechanism.

6998

Möller, G. 1964. Effect on tumor growth in syngeneic recipients of antibodies against tumor-specific antigens in methylcholanthrene-induced mouse sarcomas. Nature 204:846-847.

Attempts were made to demonstrate the presence of humoral antibodies against the tumor-specific antigens in the sera used for these experiments by in vitro techniques. The cytotoxic test was negative with all three sarcomas studied. The indirect fluorescent antibody technique performed on living tumor cells in suspension gave positive staining reactions with MALA sarcoma cells treated with specific antiserum prepared in syngeneic hosts, but MDAY and MC57G cells gave negative reactions. The positive staining reaction with MALA cells was similar to that seen with anti-H2 sera: the membrane of living cells was clearly outlined by a fluorescent ring, but controls were unstained or stained with the homogeneous reaction characterizing dead cells. The positive staining reaction with MALA cells in the fluorescent antibody technique was caused by the presence of antibodies directed against tumor-specific antigens.

6999

Nairn, R.C.; Philip, J.; Ghose, T.; Porteous, I.B.; Fothergill, J.E. 1963. Production of a precipitin against renal cancer. Brit. Med. J. 1:1702-1704.

A patient with advanced renal cancer was immunized with a microsomal fraction in Freund's adjuvant of a homologous tumor with similar morphology. A precipitin against human renal cancer was first demonstrated by gel-diffusion studies of the patient's serum 4 weeks after immunization. Immunohistological examination of the tumor postmortem showed localization of globulin on the surface of the cancer cells. There was no indication that the immunization procedure had any effect on the tumor growth or the clinical course.

7000

Osserman, E.F.; Rifkind, R.A.; Takatsuki, K.; Lawlor, D.P. 1964. Studies of morphogenesis and protein synthesis in three mouse plasma cell tumors. Ann. N.Y. Acad. Sci. 113:627-641.

A group of related studies of the morphology of three murine plasma-cell tumors have been presented, and an attempt has been made to correlate the observed morphological features with the protein-synthesizing activities of these neoplastic plasma cells. The effect of progressive loss of differentiation of the X5563 neoplasm on the production of protein has been noted. Preliminary observations on the in vitro behavior of

X5563 tumor has been described. The intracellular distribution of globulin in the X5563 neoplasm, as delineated by electron microscopic study of cells stained with ferritin-conjugated anti-X5563 globulin antiserum, has been reviewed. By immunohistochemical techniques it has been shown that the Bence Jones protein products of the two adjuvant tumor-cell lines are present in these cells during mitosis.

7001

Shapiro, J.; Rosenblatt, M.; Czajkowski, N.; Cushing, F.; Morris, A.; Wolf, P. 1965. An autoimmune approach to experimental and clinical cancer. J. Lab. Clin. Med. 66:125:1021.

The immunological destruction of cancers in animals and man by active immunization has been under study. To avoid the confusion of cross-reacting isologous antigens, such as blood group substances, each animal was immunized with its own tumor only. C-3-H mice with methylcholanthrene-induced squamous carcinoma and spontaneous mammary carcinoma showed significant decreases in tumor volumes. Distinctive histological evidence of regression occurred, consisting of hydropic degeneration of the cytoplasm, pyknosis of nuclei, cellular fragmentations, and lymphocytic infiltration. Circulating antibody to tumor extracts was demonstrated by Ouchterlony plate, immunofluorescence, and precipitin techniques. A similar technique was used to treat a small number of human neoplasms. Three of the treated patients showed histological and clinical evidence of regression. Circulating antibody to tumor extracts was demonstrated, after treatment, by Ouchterlony plate and immunofluorescence. Unfavorable clinical response was associated with large tumor masses, immunological unresponsive states, and reticulum cell sarcoma. A favorable response was associated with immediate positive skin reactions to saline extracts of tumor. Patients with negative or weakly positive skin tests had unaltered or accelerated downhill courses.

7002

Solomon, A.; Fahey, J.L.; Malmgren, R.A. 1963. Immunohistologic localization of gamma-1 macroglobulins, beta-2A myeloma proteins, 6.6S gamma myeloma proteins, and Bence Jones proteins. Blood 21:403-423.

The cellular localization of 6.6S gamma globulins, beta-2A globulins, gamma-1 macroglobulins, and Bence Jones proteins was studied by immunohistochemical procedures in 21 patients with multiple myeloma or macroglobulinemia. Each type of protein was identified by FA in a variety of morphologic forms of malignant cells. Some cells were typically plasmasitic, some were lymphoid, and others were immature forms. It was clear that these serum proteins were not associated exclusively with a single morphologic form of malignant cell. The variety of FA positive cells in each patient was more restricted, however, than in a group

of patients with a specific protein abnormality. In patients with anomalous protein, all or almost all of the malignant cells contained the specific anomalous protein. In patients with Bence Jones proteins as the sole anomalous protein, all the malignant cells appeared to have Bence Jones protein. Where an anomalous serum globulin coexisted with Bence Jones proteins, indirect evidence indicated that the Bence Jones proteins and the larger globulin were formed in the same cells.

7003

Stirling, G.A.; Daoud, E.H.; Hughes, L.E. 1964. Human breast carcinoma examined by the fluorescent antibody technique. Nature 201:1235-1236.

No specific fluorescence of tumor cells was observed in 24 breast carcinomas. The addition of complement, increasing the incubation times or temperature, or using unfixed sections of tissue did not affect the results. Specific antibodies, fixed to tumor cells or free in the serum, were either absent or were of too weak a titer to be demonstrated. Double-layer FA staining was used.

7004

Tellem, M.; Plotkin, H.R.; Meranze, D.R. 1963. Studies of blood group antigens in benign and malignant human breast tissue. Cancer Res. 23:1528-1531.

A series of benign and malignant breast tissues was studied by fluorescent antibody and hemagglutination inhibition for the presence of A, B, and D blood group antigens. No differences were found between these two groups of tissues for any of these antigens. The benign or atypical tissue immediately adjacent to the malignant area more often showed the same loss or retention of antigen as the carcinoma. There was no correlation of the loss of blood-group antigen in the primary cancer site with that of axillary lymph node metastasis. An absence of staining mixed with areas of specific staining was noted only in the malignant and adjacent tissue sections.

7005

Uehara, Y. 1965. Studies on the antigenicity of tumor tissues by fluorescent antibody technique. Jap. J. Allergy 14:351-384. In Japanese.

Fluorescent antibody technique is useful for the study of the antigenicity of Ehrlich ascites cells. The antiserum against Ehrlich cell homegenate cross-reacts strongly with mouse liver, yet the serum absorbed with mouse liver can still stain the cytoplasm. Different fractions of the cell homogenate extracted with saline were tested for antigenicity. Antibodies against ascitic fluid and supernatant failed to stain Ehrlich cells specifically,

due to a strong background. On the other hand, antibodies against mitochondria, microsomes and nuclei stained the cells clearly. After the absorption by mouse liver, antiserum of microsome and nucleus were still useful. Cells from rat AH130 were fractionated and the nuclei purified. CF test showed that antibodies against nucleus, acid protein component, insoluble fraction and microsomes were specifically reactive with the components of AH130 cells. Specificity was highest with antimicrosome serum. In order to detect tumor specific antigen, even with soluble antigen, it is necessary to absorb with insoluble fraction of other main organs. Fluorescent antibody staining failed in most of human gastric cancers. Only two cases showed significant results.

7006

Wanebo, H.J.; Clarkson, B.D. 1965. Essential macroglobulinemia: Report of a case, including immunofluorescent and electron microscopic studies. Ann. Intern. Med. 62:1025-1045.

A patient with essential macroglobulinemia relapsed, developing anemia and splenomegaly. Disease was found only in the spleen. This responded to splenic radiation, after which he had a remission lasting more than a year. He then developed rapidly progressive reticulum-cell sarcoma and died. During the first relapse, immunofluorescent studies demonstrated cytoplasmic macroglobulin in a small percentage of the atypical lymphocytes in the spleen, and it was concluded that they were producing the macroglobulin. Electron microscopic studies of these cells are described, with special attention to the ultrastructure of their protein-synthesizing apparatus. These findings are discussed in relation to the findings in other patients with macroglobulinemia and to the nature and origin of the cells that produce the macroglobulins. It is considered likely that essential macroglobulinemia is caused by a neoplastic transformation of globulin-producing cells belonging to the lymphocyte-plasma cell series. Excessive production of macroglobulin is a direct consequence of an overabundant, neoplastic proliferation of a clone of cells with the capacity to synthesize this protein.

7007

Whitehouse, F., Jr.; Kilduff, J.T. 1963. Loss of cytotoxicity of rabbit anti-Ehrlich ascites tumor antibody following pepsin digestion. Federation Proc. 22:2005:496.

Rabbit globulin, precipitated from Ehrlich ascites tumor antisera at 33 per cent saturation of ammonium sulfate, was digested with pepsin. Digested globulin was precipitated between 40 and 60 per cent saturation, as with S. typhosa H agglutinins. Globulin and digested globulin had sedimentation coefficients of approximately 7S and 5S respectively. 5S fractions are currently designated as linked Porter Fragments I and II.

In vitro preparations assayed in mice demonstrated cytotoxicity of globulin at a concentration of 0.078 mg per ml in the presence of guinea pig serum; digested globulin was not cytotoxic. Similar results were obtained with a modified Shreck test, although during this procedure there was more pronounced agglutination with the digested globulin. Fluorescein-tagged globulin and digested globulin caused tumor cells to fluoresce with approximately equal intensity. These findings suggest that Porter Fragment III, shown to be required for complement binding by others in different systems, must be present to bind a component in fresh guinea pig serum necessary for cytotoxic activity of antibody. Complete article.

7008

Wilkinson, P.C.; Zeromski, J. 1965. Immunofluorescent detection of antibodies against neurones in sensory carcinomatous neuropathy. Brain 88:529-538.

The sera from patients with carcinomatous neuromyopathy, which had previously been shown to contain brain-specific complement fixing antibodies, were investigated by a double-layer fluorescent artibody technique. After treatment of guinea pig brain sections with a first layer of the serum under test, followed by a second layer of fluorescein-conjugated antihuman gamma globulin, specific granular fluorescence of the cytoplasm of neurones from all parts of the central nervous system was seen. With one exception, the sera that gave this reaction were from patients with the sensory type of carcinomatous neuropathy. The neuronal antigen involved was organ-specific but not species-specific and was unrelated to the Forssman antigen system. Sera from normal controls failed to give granular fluorescence of neurones when layered on to sections of guinea pig brain.

7009

Yoshida, T.O.; Takahashi, M.; Okada, H.; Kawashima, H. 1965. Application of fluorescein isothiocyanate (FITC) anti-beta-1C antibody technique to tumor immunology. Jap. J. Exp. Med. 35:53P.

FA was used to determine the role of complement in damaged tumor cells in vivo and to see the localization of tumor specific antigens. After the specificity and sensitivity of anti-beta-1C antibody to C'3C were determined with EAC'1423C, the bound C'3C of MM-2 ascites carcinoma cells (in isologous C3H/He mouse), Ehrlich ascites tumor cells and sarcoma 180 ascites tumor cells (in homologous albino mouse) was examined by FITC anti-beta-1C antibody. Homologous tumor cells and MM-2 cells placed in the peritoneal cavity of the highly immunized C3H/He for two hours were strongly stained with it. By contrast, the fluorescence of isologous MM-2 cells was weak.

7010

Young, S.; Cowan, D.M. 1965. Immunofluorescence of mast cells. Nature 205:713.

The evidence from these experiments does not favor an immunological explanation for the cause of spontaneous tumor regression. It suggests that the fluorescence found in mast cells is nonspecific.

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This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume IV contains 869 annotated literature citations, arranged according to major subject areas, and a complete author index.

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