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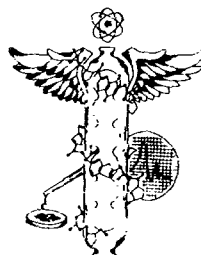
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ONR REPORT ACR-70

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RESEARCH HIGHLIGHTS



MEDICINE AND DENTISTRY BRANCH

OFFICE OF NAVAL RESEARCH



JANUARY 1962

Department of the Navy
Washington, D.C.

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FOREWORD

Research Highlights is primarily a compendium of progress report abstracts prepared by the investigators whose research is currently supported by the Medicine and Dentistry Branch of the Office of Naval Research. The major research areas of interest are mentioned in the NOTES, and the abstracts are categorized accordingly. The abstracts in each category are arranged alphabetically by principal investigator.

This publication is presented in the interest of coordinating the results of research by investigators whose programs are interrelated. Further, it is hoped that this presentation of data will stimulate a broader exchange of scientific information among investigators of the Medicine and Dentistry Branch.

The reports which this publication describes are not to be considered as published literature. These abstracts have been transmitted at the request of this Office and were done in a personal and cooperative way. The data presented in the investigators' reports, in general, will be published in the open literature at a later date. We, therefore, ask you to regard these reports as PRIVILEGED PERSONAL COMMUNICATIONS and request that no reference be made to this information without the written consent of a given investigator.

We appreciate greatly the cooperation of our investigators in preparing these report abstracts. Credit for this volume belongs to the investigators whose research is described in these Research Highlights.

Joseph F. Saunders
JOSEPH F. SAUNDERS, Ph.D.
Head, Medicine and Dentistry Branch
Office of Naval Research

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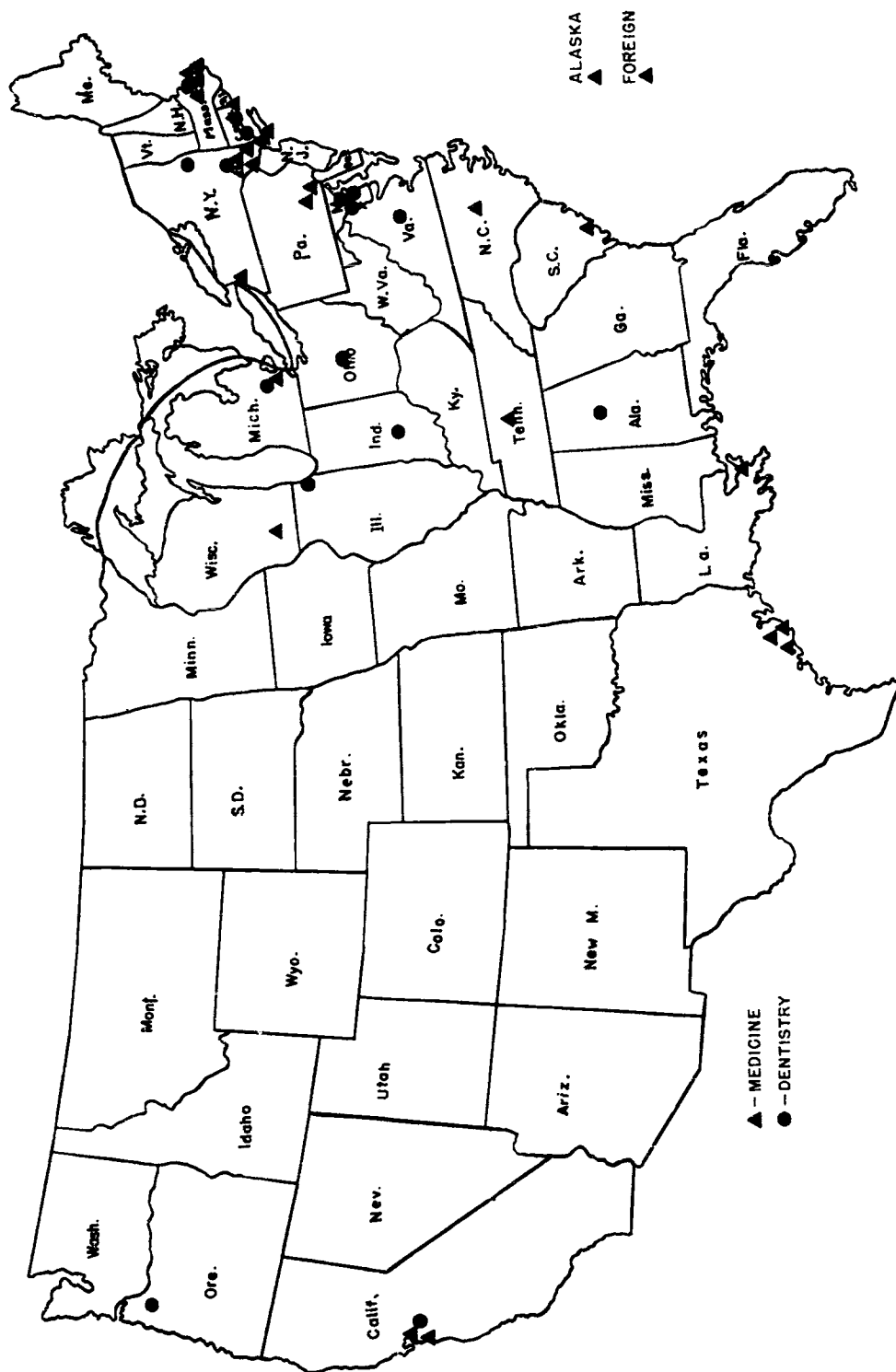
The contract research program of the Medicine and Dentistry Branch of the Office of Naval Research emphasizes four major areas of fundamental interest to the Navy. These are (1) tissue transplantation with emphasis on mechanisms of the "transplantation reaction"; (2) low temperature biology which includes studies dealing with the preservation of blood and blood components, and cold injury, for example; (3) metabolic chemistry attending traumatic injury; and (4) dental disorders with primary attention to studies related to the correlation of systemic disorders and oral diseases. These areas are interrelated and represent a concerted effort to solve some of the mysteries attending some of the exotic diseases and traumatic injuries to which military personnel may be exposed.

Of the 43 tasks currently supported by the Medicine and Dentistry Branch, 28 are in the category of medicine and 15 encompass dentistry. Of these, four are sponsored by the Bureau of Medicine and Surgery. These are the contracts of Drs. Carbone and Harper, Mills, Latham, and Tullis. The contract of Dr. Darby is sponsored by the National Institutes of Health and the Bureau of Medicine and Surgery.

Late in the year, an agreement was reached between the Government of the United Kingdom, the Linde Company, and this Office for a collaborative research program on the low temperature preservation of blood using the liquid nitrogen process. The British phase of this research program is being undertaken by the British Army Medical Services and coordinated by a steering committee of British civilian and military scientists.

Three scientific exhibits describing various programs of the Branch have been displayed at various scientific meetings and to lay public audiences. These exhibits depict research in Low Temperature Preservation of Blood, Infrared Electronics in Ophthalmology, and Tissue Transplantation.

Finally, several investigators have been recognized through honors, awards, and higher appointments by their institutions during 1961. Dr. Albert E. Sobel of the Jewish Hospital of Brooklyn was awarded the Claude Bernard Medal and the Claude Bernard Visiting Professorship by the University of Montreal. Dr. Mark A. Hayes of Yale University was promoted to the rank of Professor of Surgery. Dr. W. W. Nowinski of the University of Texas Medical Branch was elected to Fellowship in the New York Academy of Sciences.



Geographic Distribution of Research Contracts

Transplantation of Tissues

This program encompasses studies dealing with the preservation and transplantation of tissues, host-donor interactions, suppression of the "immune response" (induced tolerance), intermediary metabolism of tissues, transplantation mechanisms, and the utility of biologic polymers as tissue substitutes. In essence, the program represents a concerted effort of the various disciplines in the fundamental biologic and clinical sciences. Although they are not categorized in this program, the basic contributions of scientists in the dentistry program are considered fundamental to tissue transplantation. These are the studies of Drs. Ellison, Horner, Kruger, and Sobel.

EFFECT OF β -RADIATION ON THE INTERMEDIARY METABOLISM OF
MAMMALIAN SKIN

I. A. Bernstein
The University of Michigan

ASSISTED BY P. Foster

TASK NO. NR 105-223

CONTRACT Nonr-1224(35)

OBJECTIVES

(a) To determine what metabolic aberration, if any, is associated with the inhibition of mitosis in the epidermis and hair follicles of newborn rats exposed to low levels of β -radiation and (b) to identify the compounds in young rat skin which may be directly involved in the mitotic process and determine their stability toward low levels of β -radiation.

ABSTRACT

An essentially complete - but temporary - cessation of mitosis in the epidermis and hair follicles occurs when newborn rats are exposed to as little as 20 rads of whole body beta radiation. The number of mitotic figures which can be observed (without the use of mitotic poisons) begins to decrease immediately after irradiation until, by two hours post-radiation, the level is less than 10 per cent of normal. The normal rate of mitosis is restored by 4 hours post-radiation.

A study of the metabolism of ribo- and deoxyribonucleic acid indicated that no significant decrease in the rate of incorporation of sodium phosphate- P^{32} into either compound occurs after exposure to 25 rads. Neither the levels nor the specific radioactivities of the constituent nucleotides in both polymers were altered by this level of radiation. These data suggest that a dosage of 25 rads causes no gross change in either nucleic acid. Autoradiographic studies with thymidine- H^3 , confirm the lack of gross interference by such low levels of radiation with the synthesis of deoxyribonucleic acid in the basal cells of the epidermis. There is no convenient technique available, however, to determine whether this radiation produces small changes in specific molecules of either nucleic acid which interferes with the mitotic process.

When the radiation dosage is increased to 50 rads, there is a decrease of 30-40 per cent in the specific radioactivities of each of the various nucleotides in both nucleic acids although no significant change occurs in the content of these components. A similar decrease occurs in the incorporation of thymidine- H^3 into the thymidylic acid of deoxyribonucleic acid. As this inhibition occurs in both nucleic acids to the same extent it seems possible that the primary effect is on another system which influences the synthesis of ribo- and deoxy-ribonucleic acid.

Since radiation may cause mitotic inhibition by interfering with the availability of chemical energy for the process, an investigation of the effect of radiation on the "phosphate" containing compounds in the "acid soluble" fraction was started. A dosage of 20 or 60 rads was found not to alter the level of adenosine triphosphate in the skin. Isotope dilution experiments using P-³² labelled adenosine triphosphate, indicated a level of 0.22-0.25 μ moles/g. of skin in normal as well as irradiated tissue. However, differences have been obtained in the incorporation of inorganic phosphate-P³² into the "diphosphate" and "triphosphate" fractions. When the "acid soluble" components of normal and irradiated (50 rads) skin were separated on columns of Dowex 1 (HCOO⁻), the P³² content of the "diphosphate" and "triphosphate" fractions were abnormal in the irradiated tissues. It does not appear that adenosine diphosphate or adenosine triphosphate are responsible for these differences and an effort is being made to identify other phosphorylated components in these fractions in order to see if radiation affects their synthesis. Adenosine di- and triphosphate are by far the most prevalent in their respective fractions while uridine derivatives are next - but much lower - in concentration.

The level and biosynthesis of thioacyl derivatives will also be investigated to look for radiation effects. It is also planned to begin a simultaneous study on metabolic aspects of cell division in a suitable microorganism in the hope of identifying metabolites which are involved in mitosis and which can, therefore, be studied in normal and irradiated skin.

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(b) I. A. Bernstein, P. Foster, and K. Fukuyama (1961, "Metabolic effects of β -radiation, in vivo, in the skin of young rats." Abstract, Fifth International Congress of Biochemistry, Moscow, USSR.

(c) I. A. Bernstein and P. Foster (1962)." A biochemical study of the mitotic inhibition in rat skin resulting from exposure to beta radiation." Abstract, Twenty-Third Meeting, Society for Investigative Dermatology, Chicago, Ill.

THE ROLE OF ENERGY PRODUCTION AND ENERGY REQUIREMENTS
IN THE PROCESS OF WOUND HEALING BY GRANULATION

T. G. Blocker, Jr. and W. W. Nowinski
University of Texas Medical Branch

ASSISTED BY T. Ohkubo and A. Worcel

TASK NO. NR 105-198

CONTRACT Nonr-1598(05)

OBJECTIVES

To study the sources, synthesis and use of energy (ATP) during the process of wound healing by granulation.

ABSTRACT

Studies on 10-day granulation tissue, obtained by extirpation of a rabbit skin from an area of approximately 5 x 7 inches, showed a partial block in the Embden-Meyerhof pathway at the level of Phosphofructokinase: very small amounts of pyruvic acid were formed when glucose, glucose-6-phosphate, or fructose-6-phosphate were added as substrates. However, when fructose-1, 6-P₂, or 3-phosphoglycerate were added, the amounts of pyruvic acid formed increased considerably. Further studies showed that the phosphofructokinase works at similar rate in other tissues and that the "block" is, in fact, a substrate competition between the two alternate pathways of metabolism: formation of pyruvate and formation of glucosamine. In the presence of the glutamine, glucose-6-phosphate is transaminated to glucosamine-6-phosphate, which is the preferred reaction. However, when glutamine is withdrawn from the medium, more glucose is broken down to pyruvate. The substrate competition is less manifested in later stages (20 days), i.e., larger amounts of pyruvate are formed, even in the presence of glutamine.

CURRENT REPORTS AND PUBLICATIONS

(a) T. Ohkubo, W. W. Nowinski and T. G. Blocker, Jr. (1961), "Existence of a partial metabolic block in the Embden-Meyerhof pathway during the process of granulation". Biochim. Biophys. Acta, 53, 613-614.

SPONTANEOUS POTENTIALS FROM
EXPLANTS OF BRAIN TISSUE IN VITRO

A.W.B. Cunningham, M. B.
The University of Texas - Medical Branch

TASK NO. NR 105-189

CONTRACT Nonr-1598(04)

OBJECTIVES

To explore the embryonic brain of various animals at various stages of development for foci of spontaneous electrical activity and to investigate the cultural techniques necessary for this.

ABSTRACT

In July 1959, a technique, apparatus and materials was finally developed which allowed explants of brain tissue (chick embryo cerebellum) to be maintained in vitro in such a manner that they produced spontaneous potentials. Since this time this technique has been expanded and spontaneous potentials have been obtained from explants of telencephalon, thalamus, corpus striatum, medulla oblongata, pons and spinal cord and from explants of adult human cerebellum. The technique has been improved and observations made on the changes in the spontaneous potentials from chick embryo cerebellum over 120 hours in vitro and on the effects of changes in the environment and the administration of anesthetics, analeptics and barbiturates on spontaneous potentials from explants of telencephalon.

The form and pattern of these potentials seem to be characteristic for each anatomical area of the brain and to be produced as the result of a series of cells acting in concert in a network. It is therefore now possible for the first time to study the "community-behavior" of cells bound in their normal histological relationships and acting as natural co-ordinating groups. This should provide information on the manner in which the various areas of the brain handle the potentials previously observed in neurones. Also this new technique makes previously unattainable areas of the brain available for investigation by the traditional method of intracellular electrodes.

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(b) A.W.B. Cunningham and B.J. Rylander (1961), "Behavior of Spontaneous Potentials from Chick Cerebellar Explant During 120 Hours in Culture." *J. of Neurophysiology* 24, 141

(c) A.W.B. Cunningham (1961), "Spontaneous Potentials from Explants of Chick Embryo Medulla Oblongata in Culture." *Naturwissenschaften*, 48, 104

(d) A.W.B. Cunningham (1961), "Spontaneous Potentials from Explants of Human Adult Cerebellum in Culture." *Nature (London)*, 190, 918

(e) A.W.B. Cunningham (1961), "Spontaneous Potentials from Explants of Chick Cerebral Hemispheres (Telencephalon) in Tissue Culture." *Nature (London)*, 190, 816

(f) A.W.B. Cunningham (1961), "Spontaneous Potentials from Explants of Chick Embryo Spinal Cord in Tissue Culture." Accepted by *Naturwissenschaften*

(g) A.W.B. Cunningham (1961), "Spontaneous Potentials from Explants of Chick Embryo Pons in Tissue Culture." *Experientia*, 17, 233

(h) A.W.B. Cunningham and S.G. Stephens (1961), "The Qualitative Effect of Anesthetic Gases on Spontaneous Potentials from Brain Tissue in Culture." *Experientia*, 17, 409

(i) A.W.B. Cunningham and S.G. Stephens (1961), "Qualitative Effect of Strychnine & Brucine on Spontaneous Potentials from Explants of Telencephalon." Accepted by *Experientia*

(j) A.W.B. Cunningham and S.G. Stephens (1961), "The Effect of Temperature Variation on Spontaneous Potential Production from Explants of Brain Tissue in Culture." Accepted by *Experientia*

ON THE NATURE OF TOLERANCE

Prof. G. Dogo, University of Padua
School of Medicine, Padua, Italy

ASSISTED BY A. Gasparatto, P. A. Visentini, G. F. Girardi, F. Celleghini

TASK NO. NR 105-245

CONTRACT Nonr-(G)0007-60

OBJECTIVES

(a) Ascertaining whether artificially induced tolerance is a hereditarily transmissible phenomenon; (b) studying whether-by the method of hibernation-it is possible to induce into the cells a certain amount of damage consistent with their survival, though apt to modify the antigenic power of the tissues they belong to.

ABSTRACT

The research is carried out by using two breeds of animals (Albany strain and Fischer 344 Albino).

Controls (skin grafts) were carried out on both groups in order to ascertain the genetic uniformity of the individual animals of each group.

The animals of each single group, perfectly identical to one another as far as their quantity and quality and the disposition of their genes were concerned, were crossed among them so as to obtain a perfectly uniform generation.

A male rat of the Albany strain was coupled with a female Fischer 344 Albino and we are now shortly expecting the hybrids of the first generation.

We are carrying on researches along this line. We have adopted a method (which we call "hibernation"), by which we assume we shall succeed in dosing-by varying the temperature and the time of hibernation-the amount of the damage we wish to cause to the cellular elements of human and animal full-thickness skin, after it has been detached from the donor organism and made tubular.

In order to identify the nature of the damage caused to the cellular elements, we adopted histological and histochemical controls, tissue culture, the observation of the phlogogenic power and, finally, of the behavior of epidermal and dermal grafts obtained from the hibernated tissues transferred onto homologous hosts.

On the basis of the incomplete evidence of the clinical and laboratory tests reported here, it is possible to consider the following facts as sufficiently demonstrated:

- 1) hibernation is a simple and inexpensive method for preserving human tissues;
- 2) tissues submitted to hibernation survive for a period not yet well identified, but which can be calculated as some weeks or months;
- 3) during hibernation tissues show vital facts;
- 4) the single tissues composing a tubular skin fragment (epidermis, derm and fat) are affected in a different way by the results of hibernation;
- 5) such results seem to be more important when affecting the fate tissue and appear with more visible histochemically appreciable alterations;
- 6) that the hibernated dermal grafts on homologous hosts have a duration stay (14,7 days) definitely longer than the one of:
 - a) hibernated epidermal grafts (18,5 days)
 - b) dermal-epidermal hibernated grafts (18,5 days)
 - c) fresh dermal grafts (14,5 days)
 - d) freeze-dried epidermal grafts (13,0 days).

CURRENT REPORTS AND PUBLICATIONS

- (a) G. Dogo, P. A. Visentini (1958), "Ricerche sulla tolleranza artificialmente indotta. Nota Preliminare", Minerva Dermatologica
- (b) G. Dogo (1961), "Comportamento, su ospite omologo, di tessuti cutanei umani ibernati. Nota Preliminare", Boll. Soc. Ital. Biol. Sper., Vol. XXXVII, Fasc. 19
- (c) G. Dogo (1961), "Omoinnesti, tolleranza e ibernazione", Atti Soc. Emil. Romagn. Triv. di Ortopedia e Traumatologia, Vol. VI, Fasc. 3

DIAGNOSTIC APPLICATIONS OF INFRARED
ELECTRONICS IN OPHTHALMOLOGY

Joel Friedman, D.D.S.
Columbia University

TASK NO. NR 105-193

CONTRACT Nonr-2648(00)

OBJECTIVES

Techniques have been developed to define the routine and pre-surgical diagnostic potential of the near infrared image converter in the examination of the eyes of patients with corneal and lenticular opacifications.

ABSTRACT

The infrared image converter has been successfully applied alone and in combination with the slit lamp microscope for the visualization and diagnosis of a variety of anterior segment pathoses. Information useful in the determination of a favorable prognosis for surgical intervention can be disclosed by this means in the presence of ocular opacities impenetrable to routine visible light ophthalmology.

A similar standardization of procedures for utilization of the infrared instrument combined with retinal diagnostic instruments in the visualization of retinal structures obscured by opacified corneas and lenses is the next phase in these studies.

CURRENT REPORTS AND PUBLICATIONS

(a) J. Friedman (1960), "Penetration of corneal opacities by infrared electronics." IRE Transactions on Med. Elect., ME-7, 182-183.

(b) J. Friedman (1960), "Diagnostic applications of infrared electronics in ophthalmology." Proc. Third Int. Conf. on Med. Elect., 345-347

**A Study of the Prolonged Preservation of Human Skin for Use as
a Temporary Covering in Extensive Burns with Associated Clinical
and Experimental Evaluation of the Burned Patient.**

**N. G. Georgiade
Duke University Medical Center**

ASSISTED BY I. Amigo, R. Georgiade, A. Eiring

TASK NO. NR 105-071

CONTRACT Nonr-1537 (00)

OBJECTIVES

(a) To determine whether a satisfactory method of long term preservation of skin in a viable state can be obtained, (b) to evaluate the efficacy of freeze-dried skin with preserved viable skin, (c) to evaluate the role of homografting as a life-saving temporary covering for the extensively burned patient, (d) evaluation of the serum of burn patients as to the presence or absence of toxins.

ABSTRACT

Previous experimental work established the basal respiration of human skin. Skin stored in 20% glycerine with a balanced salt solution can be kept viable for varying lengths of time up to 1480 days. The temperatures used to maintain viability were -45°C and -79°C . The length of survival of skin both post mortem and during preservation has been evaluated utilizing tissue culture techniques with the criteria for viability of skin being increased in cell population, change in skin from alkaline to acid, repeated subcultures and presence of cells in mitosis.

The use of viable homografts preserved in a 20% glycerol solution and the use of freeze-dried skin in the over-all treatment of severely burned patients is being continued and compared.

Tissue culture methods have been used for evaluation of a toxic factor in burn serum, however, to date this technique has not been conclusive. Stock cultures of strain HeLa and Chang's liver cells have been used and maintained in T30 and T60 flasks. The media is composed of human serum 10%, Eagle's basal media for HeLa cells 90%, penicillin, streptomycin and mycortatin in concentrations of 100 units, 100 micrograms, and 50 units respectively per ml. of media added. Suspensions of these cells are obtained by adding trypsin. Following centrifuging, the pellet is resuspended in Eagle's media and a count is made using a hemocytometer. Aliquots are removed so as to give a final concentration of cells of 400,000 per ml. and these are added to the test tubes containing the burn serum to be tested. We have observed that in normal serum control flasks the cells settle out and attach to the glass in 1-2 hours, however, in the burn serum flasks the cells remain floating and do not attach in a short time and those that have settled out show evidence of injury.

A method is being developed to isolate if at all possible the toxic fraction in serum of burned patients. This serum is being obtained as early as possible post burn and at weekly intervals during the course of their convalescence. Analysis and isolation of the serum fractions are being carried out in attempt to isolate the toxic fraction.

CURRENT REPORTS AND PUBLICATIONS

Evaluation of Post Mortem Survival of Skin by Tissue Culture Methods- Pl. & Rec. Surgery 21:483, June, 1958

Evaluation of Viability of Preserved Rabbit Corneas by Tissue Culture Methods-Am. J. Ophth. 47:772, June, 1959

Long Term Preservation of Donor Tissue for Corneal Grafting - Am. J. Ophth 49:729, 1960

The Prolonged Preservation of Tissues in a Viable State- Trans. Int. Cong. Pl. Surgery, 2:456, July 1959

Long Term Storage of Skin and Corneas for Grafting after Burns- Trans. Int. Cong. Burns, Sept. 1960

The Prolonged Preservation of Tissues in a Viable State- The Amer. Surgeon, 28:6, 1962

Changing Concepts in the Management of the Burned Patient- Southern Med. Journal (In press)

THE STORAGE AND TRANSPLANTATION OF
HUMAN COSTAL CARTILAGE

R. F. Hagerty
Medical College of South Carolina

ASSISTED BY T. B. Calhoun and H. L. Braid

TASK NO. NR 105-088

CONTRACT Nonr-434(02)

OBJECTIVES

(a) To study human costal cartilage in regard to its adequacy as a supporting material in homologous transplantation, and (b) to determine the factors necessary for maintaining the integrity of this cartilage after long periods of storage and subsequent transplantation into a human host.

ABSTRACT

Several methods of storage have been investigated in an effort to maintain the viability of the cartilage cells and the integrity of the matrix. These methods of storage are: 1:10,000 merthiolate-saline solution, human plasma and air (storage in sterile containers at 3 to 5° C.). Cartilage stored under these three conditions was transplanted into human volunteer hosts for long periods of time, and upon removal from the host was evaluated and results compared to those obtained prior to implantation. From this study two conclusions were drawn: (1) the viability of cartilage stored in air is maintained for longer periods of time than cartilage stored in the other two media studied, and (2) viable homogenous cartilage maintained its viability to a considerable extent after long periods of transplantation and was associated with the least invasion by the host, and is, therefore, superior to nonviable cartilage for surgical use.

In order to prove conclusively the value of viable homografts as compared to nonviable grafts, a method of killing the cartilage cells with minimal effects on matrix integrity was developed. Cartilage treated by this method (exposure to anhydrous ammonia gas) is being transplanted with viable, air stored cartilage for purposes of comparison.

In an attempt to prolong the viability of cartilage in storage, a solution containing plasmalyte, glucose and a plasma extract (to provide the osmotic colloid fraction of plasma) buffered by tris-hydroxy-methylamine (THAM) is being investigated as a storage medium. Results obtained in a previous study of human plasma as a storage medium indicated that the base-binding capacity may be modified by the pH of the storage medium. The pH of the human plasma decreased progressively over a two week period. By maintaining the pH of the plasmalyte solution it may be possible to avoid the electrolyte shifts and loss of viability previously experienced, as well as avoiding the subsequent dehydration of the cartilage encountered in long term "dry" air storage.

In addition to our standard methods of analyzing stored and transplanted cartilage (neutral red supra vital stains, radioautographs, metabolism studies and histological stains) for cellular viability and condition of the matrix, we are setting up techniques for an evaluation of the antigenic properties of cartilage homografts. These techniques are: fluorescein-antibody staining, gel diffusion and immunoelectrophoresis. Though these techniques will be applied to human grafts, the antigen-antibody response of cartilage grafts will be investigated primarily by use of animal homografts and autografts, because of the availability in animals of larger quantities of transplantable cartilage and the elimination of the difficulty of finding recipients for the implants.

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- (b) R. F. Hagerty, T. B. Calhoon, W. H. Lee, Jr., and J. T. Cuttino (1960), "Human Cartilage Grafts Stored in Merthiolate". Surg. Gyn. Obst., 110:229-233
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- (d) R. F. Hagerty, T. B. Calhoon, W. H. Lee, Jr., and J. T. Cuttino (1960), "Human Cartilage Grafts Stored in Air". Surg. Gyn. Obst., 110:433-436
- (e) W. H. Lee, Jr., R. F. Hagerty, and H. L. Braid (1960), "Measurements of Cellular Viability. A Comparative Study of Neutral Red and Radioactive Sulfate in the Examination of the Viability of Cartilage". J. Plastic & Reconstr. Surg., 26:280-285

THE IMPLANTATION OF ENDOCRINE TISSUE AS A THERAPEUTIC
MEASURE IN THE TREATMENT OF GLANDULAR DEFICIENCY

E. L. House, B. Pansky and M. S. Jacobs
New York Medical College

ASSISTED BY G. M. House and K. Miller

TASK NO. NR 105-199

CONTRACT Nonr-2754(01)

OBJECTIVES

(a) To establish the ideal age of both donor and host for the transplantation of pancreas into diabetic animals of the same strain
(b) to investigate ways and means to promote the growth and prolong the life of the graft until it can "take over" the endocrine function of the host or to substitute for it until spontaneous recovery occurs.

ABSTRACT

The following results have been obtained and are already published: (a) a revision and improvement in the orbital sinus method either for securing frequent blood samples or for introducing substances intravenously; (b) the establishment of norms for many blood parameters in several age groups of hamsters, with the notation that some of these change with age; (c) the effect of alloxan diabetes on the blood picture of the hamster of various ages; (d) the effect of homoplastic transplants on the diabetic condition, in which it was shown that in many instances, where the graft had persisted, the diabetic condition improved; (e) the establishment of the "ideal" donor age at 14 to 15 days, fetal with the indication that tissue taken within 24 hours after birth was very nearly as good; (f) the determination that the age of the host is not especially important in the survival of the transplant.

Work has been completed and the manuscripts of the following articles are now in the hands of the editors: (a) the effects of two types of insulin on the spontaneous recovery of hamsters from alloxan diabetes in which it was shown that, if the diabetes is well-controlled for as much as two full weeks, the rapidity and percentages of occurrence of spontaneous recovery were greatly increased, at least among the low and moderate cases; (b) the effect of such hormones as cortisone, insulin and growth hormone, used alone or in combination, on the growth and survival of pancreatic homografts - here it was found that cortisone alone was quite effective in enhancing the persistence of the graft; that a cortisone-insulin regime gave the best results; that growth hormone lead to an earlier deterioration of the graft.

Work on the following is complete and the manuscripts are being prepared: (a) the establishment of the blood volume, total protein and total cholesterol in normal and diabetic hamsters in which it was noted that, among young animals, a greater blood volume is found in those of lighter weight whereas, in adult animals, the blood volume varies in proportion to the body weight; that diabetes does not effect

blood volume; that total protein is not affected by diabetes; that there is a marked shift in the lipoprotein electrophoretic patterns, showing a fall in the albumin fraction and a rise in the beta-gamma fraction, especially among the high diabetics; that in severe diabetes there is a significant elevation in serum cholesterol; (b) an investigation to determine whether homografts of this size actually produce any shift in the total blood picture of the host and, if so to note whether such changes were as marked in those series where the graft grew larger and persisted longer than in those where the graft was rejected early; (c) an attempt to confirm the fact that the acinar tissue of the transplant and to determine the nature of the secretion - here the transplant was placed in a special "diverticulum" which, in a few days, was seen to be enormously distended with fluid; analysis of the latter showed traces of lipase.

Work now in progress. Since transplantation of tissue through a series of hosts has been shown to be effective in enhancing certain types of transplant growth, this was tried on the hamster, with tissues being taken from the primary host after variable lengths of time and placed in the pouches of another animal; thus far, none of these have shown as good a growth as that observed in the primary host and none have shown any islet differentiation at all.

Since we have evidence that pancreatic acinar tissue does secrete, sometimes producing cysts or vesicles of some size, it was felt that, if only from the point of view of pressure, this might have a deleterious effect on the growth and differentiation of the islets. Thus, means are being tested to destroy the exocrine portion. Duct ligation is effective but this necessarily makes the donor age far beyond the limits of transplantability. It is known that D-L ethionine will, in 2 or 3 weeks, destroy the acinar tissue. Again, even if tried on weanlings, the age becomes an insurmountable obstacle. Therefore, this substance is being tried on pregnant and lactating animals.

Procedures still to be tried: (a) provided that we are unable to destroy the exocrine tissue in the fetus or newborn, to transplant standard donor tissue into the cheek pouches of hosts under ethionine treatment; (b) the transplantation, under aseptic conditions, of pancreatic tissue directly into the normal pancreatic bed of cortisone and insulin treated diabetic hosts.

CURRENT REPORTS AND PUBLICATIONS

- (a) B. Pansky, M. S. Jacobs, E. L. House and J. P. Tassoni (1961), "The Orbital Region as a Source of Blood Samples in the Golden Hamster". Anat. Rec., 139, 409-412
- (b) E. L. House, B. Pansky and M. S. Jacobs (1961), "Age Changes in the Blood of the Golden Hamster". Am. J. Physiol., 200, 1018-1022
- (c) E. L. House, B. Pansky and M. S. Jacobs (1961) "The Effect of Alloxan Diabetes on the Blood Picture of the Hamster". (1961), J. Cell. and Comp. Physiol., 57, 203-209

- (d) E. L. House, B. Pansky and M. S. Jacobs (1961) "The Effect of Pancreatic Homografts on the Diabetic State of Alloxan-treated Hamsters". Anat. Rec., 140, 341-343
- (e) E. L. House, M. S. Jacobs and B. Pansky (1961) "Homoplastic Transplantation of Pancreas in Diabetic Hamsters". Trans. Bulletin, 28, 55-61

INDUCED TOLERANCE TOWARD SKIN HOMOGRAFTS

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TASK NO. NR 105-183

CONTRACT Nonr-1349(02)

OBJECTIVES

To establish immunological tolerance a) by means of hibernation and b) through parabiosis, established in immunologically unresponsive periods.

ABSTRACT

a) Plants can be grafted with success in the dormant season (equivalent to hibernation). Because basic biological phenomena are not isolated to one form of life, we made the assumption that if grafting can be successful in dormant plants this same might be true in hibernating animals. Bats were chosen for our experimentation, which readily undergo hibernation. Our findings were: 1) Homotransplants in bats were invariably rejected, indicating a heterogeneity of bat populations comparable to that of human populations. The inflammatory cellular reaction during rejection followed the same pattern as in other species. 2) Skin homografts in bats kept in hibernation at 6-8°C were not rejected. The graft tissue remained live during hibernation, though it did not show any vascularisation and there was no epithelial proliferation at the periphery of the graft. Following hibernation such grafts were rejected at the same pace as normal controls. 3) Skin homografts which have been already vascularised (4 days old) at the onset of hibernation and were examined after two months of hibernation appeared well vascularised, free of inflammatory cells and the graft epidermis showed fusion with the host epidermis. Subsequently, such transplants were rejected in the dehydrated animal but at a later date than in comparable controls. 4) Homografts made subsequent to hibernation are tolerated for a much longer period than in non-hibernated controls. From above findings the conclusion is drawn that a temporary immunological tolerance exists during and following hibernation.

b) The basic difference between parabiosis and immunization with tissue cells (i.e. graft) or tissue extracts is that in parabiosis there is a continuous interchange of blood and its hormonal and other constituents between two surgically united individuals whereas no such exchange exists from a graft.

If successful parabiosis can be established, immunological tolerance to subsequent homografts persists even after separation of the animals. Such parabiosis can be established during the embryonal age. We were curious to find out whether the same is true in the immunologically unresponsive post-hibernation period. 1) When we tested hamsters (another hibernating animal) for parabiosis rejection, without the use of hibernation we observed that, when golden (Quackenbusch) and albino (PD) hamsters were parabiosed none of the parabionts were rejected. Similarly, acceptance occurred when female albinos and male golden hamsters were sutured together. The area of operation became well vascularised. That cross circulation existed could be determined by injecting nembutal in one animal and observe its effects on the other. 2) When skin cross transplants were performed on the parabionts they did take and remained live even after separation of the animals. 3) In contrast when skin from these two strains were cross transplanted 90% were rejected. 4) When skin within each line was iso-transplanted there were approximately 60% takes and 40% rejections. The significance of these observations is being studied.

CURRENT REPORTS AND PUBLICATIONS

a) B. Konieczna-Marczynska, "The Induction of Tolerance in Hamster to Transplants of Mouse Sarcoma by Means of Heteroparabiosis". *Experientia*, 17, 370-1, 1961.

b) W.H. Hildemann and R.L. Walford, "Chronic Skin Homograft Rejection in the Syrian Hamster". *Annals N.Y. Academy Sci.* 87, 56-77, 1960.

THE EFFECT OF THERMAL BURNS ON PITUITARY CYTOLOGY AS STUDIED
WITH THE ELECTRON MICROSCOPE AND SELECTIVE STAINING METHODS

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The University of Texas Medical Branch

ASSISTED BY C. A. Waldron, and M. G. Williams

TASK NO. NR NR 105-167

CONTRACT Nonr-1598(03)

OBJECTIVES

(a) To study the acute effects of a severe scald on pituitary cytology in the rat and (b) to correlate the observed structural changes with known physiological alterations, with particular reference to secretion of ACTH.

ABSTRACT

Previous studies in our laboratory have shown that within 12 hours after subjecting rats to a standardized scald (while under nembutal anesthesia) there is a loss of approximately one-half of the pituitary store of ACTH. During this same time interval there is a significant decrease in the weight of the pituitary gland, although the microscopic appearance of the gland as studied with the light microscope is not dramatically changed. The major changes which have been noted are (a) a marked but transient increase, at 30 minutes after scalding, in the number of cells in mitosis (b) a general reduction in the size of all parenchymal cells which becomes most marked at 12 hours after scalding and (c) an increase, at 12 and 24 hours after scalding, in cytoplasmic basophilia of certain acidophiles. Electron microscopic changes have been noted both in the parenchymal cells and in the blood elements within the pituitary vessels. An increased number of reticulocytes and blood platelets were seen at 30 minutes after scalding. At this same time many acidophiles showed rows of secretory granules at the cell membranes and the process of granule discharge was readily visualized. By 12 and 24 hours after scalding the acidophiles showed evidence of increased synthetic activity. Thus, an increase in RNP particles in association with endoplasmic membranes was seen in many cells. At no time was widespread degranulation of any of the chromophiles seen.

Further evidence on the physiology of the pituitary in response to scalding was obtained by studying plasma levels of corticosterone. It was found that an elevated plasma level of this adrenal hormone could be sustained for at least 24 hours after scalding. This was taken to indicate a continued hypersecretion of pituitary ACTH during this time period. It seems likely that this hypersecretion is attended by an increased rate of synthesis of new hormone. Attempts have been made to label the newly synthesized ACTH with H-tryptophane and to visualize this radioactive hormone by radioautographic methods. Thus far, these experiments have met with only limited success. It has not yet been

possible to clearly delineate those cells responsible for ACTH production by this method.

In summary, while no changes in the cytology of the pituitary gland have been found which can be related specifically to ACTH secretion, several important alterations have been observed after scalding. These include (a) a clear visualization of the mode of granule discharge (b) changes in fine structure of certain cells which indicate an increased synthetic activity and (c) an increase in the number of reticulocytes and blood platelets seen in the pituitary vessels.

CURRENT REPORTS AND PUBLICATIONS

(a) E. G. Rennels (1960), "The effect of a severe scald on pituitary cytology in the albino rat". First International Congress of Endocrinology, Copenhagen. Acta endocrinol., 35, 53.

(b) R. F. Timmer and E. G. Rennels (1961), "The effect of scalding on plasma levels of corticosterone". In Press. (The text of this paper will appear in book form with other papers presented at the First International Congress on Research in Burns).

(c) E. G. Rennels (1961) "Effects of scalding on the fine structure of the rat pituitary gland". Fifth Pan American Congress of Endocrinology. Abstracts. pp. 213-214.

REPLACEMENT OF CONSTITUENTS OF GRAFTED TISSUE AND ITS RELATION TO HOST-DONOR INTERACTION

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ASSISTED BY F. M. Polack

TASK NO. NR 105-234

CONTRACT Nonr-266(71)

OBJECTIVES

- (a) To determine the degree of persistence of donor constituents of corneal transplants. This requires, in addition, that corneal constituents (cells and fibers) be labeled with biologically and physiologically stable isotopes and the demonstration that such labeled constituents persist for long periods of time in a normal cornea,
- (b) to investigate the early pathology of the graft rejection process.

ABSTRACT

The cellular elements of rabbit corneas have been "permanently" labeled with tritiated thymidine. The thymidine was incorporated in the corneal cells during the period of DNA synthesis and has been shown in this study to be retained by them for at least one year. Such labeled corneas have been grafted to non-labeled normal host rabbits. These grafts have remained transparent for long periods and are, therefore, clinically successful. Radioautographic investigation of such grafts recovered after one year show that the original donor cells are retained for at least that length of time. Similar studies are in progress in which the collagen fibers are labeled with long lived isotopes. This type of label has been shown to persist in normal corneas for at least one year. Successful grafts have been made with this material, but to date these experiments are not yet concluded. All of the experiments are interpreted to demonstrate that donor corneal tissue retains its identity when transplanted.

Immunological graft rejection phenomena may be evoked in corneal grafts. When such experiments were done the initial stages of graft rejection were characterized by damage to the endothelial cells rapidly followed by loss of metachromatically stained corneal ground substances. The ability of the corneal stroma at this stage to synthesize sulfated mucopolysaccharides (as tested by the administration of S^{35} labeled sulfate) became deficient. Pathology of the connective tissue elements appears to be secondary to lesions in the endothelium.

CURRENT REPORTS AND PUBLICATIONS

(a) G. K. Smelser and F. M. Polack (1962), "Incorporation of radioactive thymidine by corneal connective tissue cells following injury." On the program of the Association for Research in Ophthalmology, Boston, February. To be submitted at that time for publication in Investigative Ophthalmology.

(b) F. M. Polack (1962), "Early pathology of experimental corneal graft rejection." For presentation, June. To be submitted at that time for publication in Investigative Ophthalmology.

(c) G. K. Smelser and F. M. Polack (1962), "Survival of isotopically labeled cells in corneal homografts." To be presented at the American Association of Anatomists in Minneapolis, March. Abstract in the Anatomical Record and is submitted for publication in the Proceedings Society of Experimental Biology and Medicine.

(d) G. K. Smelser and F. M. Polack (1962), "Survival of isotopically labeled cells in corneal homografts." Presented at the Pan American Congress of Ophthalmology at Lima, Peru, February.

RETENTION OF FOREIGN BODIES IN THE CORNEA OF THE EYE
(PLASTIC ARTIFICIAL CORNEA)

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Massachusetts Eye and Ear Infirmary

ASSISTED BY S. Ore, F. Saad, and S. Zigman

TASK NO. NR 105-163

CONTRACT Nonr-1173(01)

OBJECTIVES

The scope of research under this task is fundamental research in biologic polymers and their behavior when implanted in the organism. Of prime interest is the use of methyl methacrylate and its retention in the eye as a "plastic artificial cornea". The ultimate objective is to fabricate an artificial cornea of this substance which will be retained in the human eye without untoward (immune response) reactions.

ABSTRACT

PLASTIC ARTIFICIAL CORNEA

a. Monkey Experiments: A series of methyl methacrylate plastic artificial corneas have been placed in rhesus monkeys. Forty per cent of these have remained in place. The remainder eroded through. It was found that the curvature of the plastic had to be more acute than in the rabbit corneas. As was noted previously, the doracouli monkey did not tolerate either a lamellar transplant or a plastic cornea. All of the lamellar transplants placed in the rhesus monkey in this laboratory have been successful. Some unknown factor prevents successful lamellar transplants as well as creates intolerance to plastic discs in the doracouli.

Work in silicone rubber discs was temporarily suspended because of discoloration of the material after fabrication. Newer material has recently eliminated this defect. Experimentation with this material has commenced again.

b. Human Experimentation: Three patients have been prepared with preliminary lamellar grafts and will be the first cases to have the plastic cornea. These are people who have been blind for years, have good entoptic phenomena and because of the extent of their scarring and vascularization, are unsatisfactory for eye-bank corneal transplantation. It is expected that discs will be placed in these patients within the next three to four months.

The culling for suitable cases of the Massachusetts Eye and Ear Infirmary records of the past ten years has been completed. Letters are in the process of going to selected patients suggesting that a new experimental procedure might be of benefit to them. These patients will be examined and discs will be placed in the most suitable.

c. Standardization and Purification of Plastic Monomers: The problem of purification and standardization of the plastics used has concerned us for many years. It has been observed that plastic not only varies in chemical and physical properties in samples from the same manufacturer, but approximating parts of the same cylinder demonstrated considerable variation.

It is of importance to control the types and amounts of accelerators, inhibitors, copolymers, fillers, etc., which go into the fabrication of the unfinished material. The ultimate tissue reaction is dependent upon the individual constituents, the process of fabrication, hydrolyses when imbedded, and the degradation products at a later date.

In order to examine this problem, Dr. Svein Ore, Head of the High Polymer Chemistry Department of the University of Oslo, is working on the purification, standardization and alteration of properties, of methyl methacrylate, silicone rubber and silicone resins. Tissue reaction to the constituents and products are a major part of this study.

d. Work on Collagens and Nucleic Acid Metabolism of the Cornea: Farida Saad, Ph.D., working on collagens of the cornea, and Seymour Zigman, Ph.D., studying nucleic acid metabolism of the cornea are both, as yet, in the stage of study of the collagens and nucleic acid metabolism of the normal cornea, before continuing to the study of changes in wound healing and in relation to the plastic discs. Dr. Zigman has demonstrated the relative amounts of phosphorus containing substances, the nucleotides by paper electrophoresis and chromatography, and is studying the P³² (as phosphate) uptake in vivo and in vitro in the various fractions.

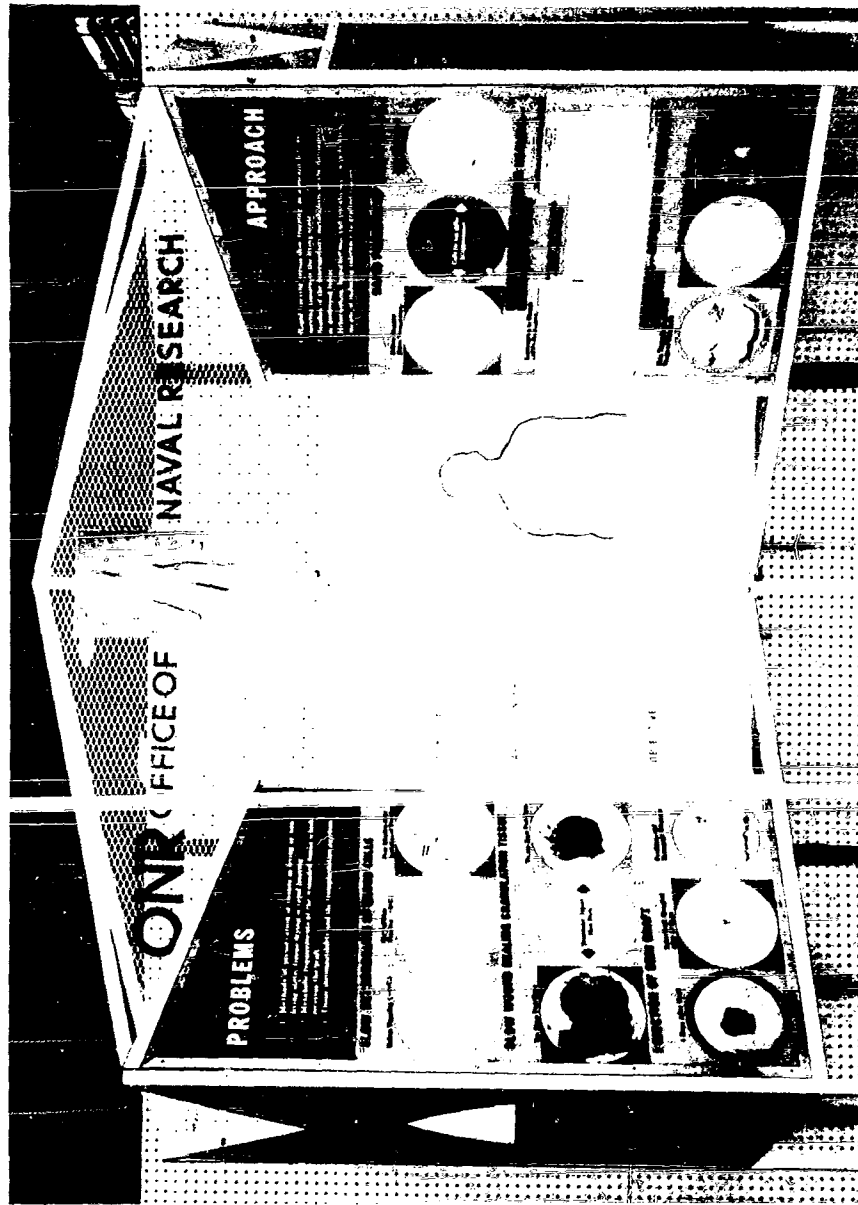
FLUID TRANSPORT STUDIES

Our findings in elasmobranchs reported last year, were corroborated and it was demonstrated that the same osmolarity differences existed in teleosts, i.e., a marked hypertonicity of plasma over aqueous--the plasma being approximately 35-40 milliosmoles higher than the aqueous. Further, the total osmolarity of the teleost is in the mammal range. It was also demonstrated that changes in the environmental fluid to hypotonicity, did not lower the osmolarity of the aqueous. This, along with other evidence, indicates that water does not cross the cornea.

Based on the assumption that water diffuses freely and equally in both directions through a semipermeable membrane, the energy expended for its active transport would have to be considerable. Nevertheless, the evidence from this work over the past four years continues to substantiate the hypothesis that water is actively transported.

CURRENT REPORTS AND PUBLICATIONS

R. F. Doolittle, C. Thomas, W. Stone, Jr., Osmotic Pressure and Aqueous Humor Formation in Dogfish, Science 132:3418, 1 July 1960, pp 36-37.



The ONR Tissue Transplantation Exhibit highlights: (1) the purpose for and some areas of research interest; (2) examples of some of the current problems which must be overcome in transplantation research; and (3) the research approach for solving these problems. The objective is to typify the need for concerted fundamental and clinical research to solve a vital biologic problem.

Low Temperature Biology

The scope of this program is directed along three principal lines: studies of the use of low temperatures for the preservation of individual cells, lower organisms, and tissues of higher animals; the mechanism of ice crystal formation in tissue and the mechanics of cold injury; and the effects of temperature reduction in warm-blooded animals. The low temperature biology studies are coordinated with the program in tissue transplantation in that the former complements and implements the latter.

**A PRACTICAL FACILITY FOR PROCESSING
RED BLOOD CELLS FOR LONG-TERM STORAGE**

A. Latham, Jr. and L. E. Steimen
Arthur D. Little, Inc.

ASSISTED BY A. H. Buckley and G. C. Kekopoulos

TASK NO. NR 105-262

CONTRACT Nonr-3414(00)

OBJECTIVES

To provide research leading to the study, design, and development of a practical facility for the processing of red blood cells for long-term storage.

ABSTRACT

A practical system for the processing of red blood cells for long-term storage is being developed. An experimental test unit of this system has been constructed and delivered to Chelsea Naval Hospital and is currently being tested and evaluated at Chelsea Naval Hospital. The principle that was utilized in this system was developed originally by the Protein Foundation. Basically, it consists of first centrifugal separation of the plasma from the red cells and then glycerolizing the red cells by perfusion with glycerol solutions while they are held in a centrifugal field. The glycerolized cells may then be stored for extended periods at -80°C. When the red cells are needed for transfusion they are retrieved from deep freeze storage, reprocessed (deglycerolization cycle) with solutions of sodium lactate and saline to remove the glycerine and resuspended in natural plasma, serum albumin, or other selected media.

The development under this contract can be divided into two parts. The first was to replace the existing ADL-Cohn Blood Fractionator, which requires disassembly, washing, reassembly and sterilization of many non-expendable parts after each glycerolization or deglycerolization cycle, with an expendable sterile plastic liner. The red blood cells will be glycerolized, stored, and deglycerolized within this plastic liner after which the liner will be discarded. Some of the special problems encountered in the design and fabrication of the expendable plastic liner have been the requirement for a small sterile rotary seal, the stationary internal disk pump, the control of turbulence of fluids at contact with the disk, the shape factors involved in adequate mixing with the liner, and a complete bench technique for the vacuum molding and assembly of the plastic liner and its components. The second was to provide an automatic system of controls and pumps which could be programmed to proportion bulk sterile solutions used in the processing of red cells, thereby eliminating the need for a skilled operator. The special

problems here were concerned with timing circuits which could be preset to insure duplication of a predetermined solution cycle and yet be flexible enough to accommodate changes as continuing testing dictates optimum values.

The delivery of the experimental test unit to Chelsea Naval Hospital on January 15, 1962, and the success of the initial experimental trials constitutes major progress in the development of a practical facility for the processing of red blood cells for long-term storage. Evaluation and refinement of the equipment is expected to continue during the remainder of 1962 and, concurrently, problems connected with the quantity manufacture of the expendable plastic liner kit will be further investigated with one or more major producers of pharmaceutical plastic goods. Following this evaluation phase, it will be desirable to finalize the design and fabricate a complete facility for full-scale routine processing of red blood cells for long-term storage.

CURRENT REPORTS AND PUBLICATIONS

A. Latham, Jr. and L. E. Steimen, "Development of an Expendable Liner and Automated Solution System for Red Cell Glycerolization", Protein Foundation, Annual Scientific Meetings, Cambridge, November 20, 1961.

CLINICAL AND EXPERIMENTAL RESEARCH
IN COLD INJURY

William J. Mills, Jr., M.D.
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ASSISTED BY R. Whaley, M.D. and W. Fish, M.D.

TASK NO. NR NR 105-249

CONTRACT Nonr-3183 (00)

OBJECTIVES

(a) To evaluate all methods and results of treatment of human cold injury, especially rapid re-warming. (b) To determine a method for early evaluation of depth of injury and ultimate prognosis, utilizing available enzymes. (c) To investigate circulatory response by radio-isotope tracer techniques. (d) To evaluate clinical findings by application of sterile thermocouples in frozen and thawed tissue.

ABSTRACT

Despite the volume of literature on cold injury, little agreement in the past has been demonstrated regarding its pathogenesis, treatment, and prophylaxis. Annually, large numbers of cold injury cases in the Alaskan area make a study of this problem possible, and provide constant evaluation and follow-up throughout the year. The accelerated military and civilian activity in the Arctic has caused increased exposure to severe low temperatures, with further increase in the number of cases hospitalized for deep frostbite.

Experience with fifty-two cases of cold injury, reported in Alaska Medicine, has demonstrated that regardless of depth of penetration, or duration of freezing, best results are obtained by rapid re-warming the frozen extremity in a water bath of 110-118 degrees Fahrenheit. The duration of immersion in the bath has not been determined to be a fixed time, but is instead, that time required to cause complete thawing and return of vascular supply to the terminal digits. Poorest results are obtained by thawing in excessive dry heat (150-180 degrees Fahrenheit) and results almost as poor are found after slow thawing with ice, ice and snow water, or snow packs with friction massage. Variable results are obtained from thawing by exposure to room temperatures.

These studies, combined now with unpublished data for over one hundred cases, demonstrate no effective response in the early stages of frostbite (injury to sixty days) by the use of anticoagulants, vasodilators or sympathectomy. It appears from clinical and isotope studies that after thawing there has been a "local sympathectomy", proximal to the injury, resulting from response to injury, and not improved by chemical or surgical attack.

Further clinical evidence is accumulating that indicates immediate rapid re-warming yields much better results and preserves tissues far exceeding that of other methods of thawing utilized at present. Enzyme studies (serum transaminase, aldolase, lacto-dehydrogenase) appear to demonstrate that an early rise in enzyme level with an early return to normal values, indicate a good result with little or no tissue loss while a delayed enzyme rise (four to twelve days) signals the advent of considerable tissue loss. These studies are continuing, and are being subjected to careful scrutiny because of other factors present in body tissues that influence enzyme levels.

Radio-isotope determinations have been made on patients permitting these studies, to further our understanding of the vascular response in the injured area. Insufficient data has prevented any definite conclusion in the acute frozen or immediately thawed stage of frostbite using radioactive isotopes. However, these studies have confirmed that in the patient post injury of over one year, thawed by means other than rapid re-warming, with peripheral vascular residual deficit, that late sympathectomy may be quite helpful. This conclusion has been arrived at as a result of simultaneous sympathetic block in the area involved, with radio-isotope injection, and measurement of the results by radioactive tracer techniques.

Further studies of these tissues are planned in the near future, utilizing thermocouples during the frozen and immediately post thawed state, to determine the temperature level in the area frozen and it's rise after various means of thawing, including rapid re-warming.

In all cases regardless of thawing methods, infection has been limited or prevented by whirlpool bath daily with Hexachlorophene^R. Debridement has been discouraged. Function has been maintained in these extremities by active physiotherapy from onset of injury.

CURRENT REPORTS AND PUBLICATIONS

(a) W.J.Mills and R.Whaley (1960) "Experience with Rapid Re-warming and Ultrasonic Therapy" Alaska Medicine 2, 1-4, (part 1).

(b) W.J.Mills, R.Whaley and W.Fish (1960) (1962) "Experience with Rapid Re-warming and Ultrasonic Therapy" Alaska Medicine 2, 114-122; 3, 28-36.

(c) W.J.Mills, (1962) "Frostbite: Problem for the Nurse", January, 1962 - Alaska Nurse.

PHYSIOPATHOLOGIC STUDIES OF EXPERIMENTAL LOCAL COLD INJURY

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ASSISTED BY Ann Sayen, Research Assistant

TASK NO. NR 105-018

CONTRACT Nonr-551(03)

OBJECTIVES

Functional and histologic studies of residual neuromuscular block resulting from immersion of the rabbit's hind limb in water at temperatures just above freezing.

ABSTRACT

In earlier studies under ONR contract a method was developed for chilling one hind leg of the rabbit in water at 2 to 3 degrees C in a manner that permits mobility of both hind limbs and reduces masking effects of postural edema. During the first hour of chilling a decrease in temperature of skin and muscle, and in volume blood flow as measured by the strain gauge plethysmograph, is followed by transient increases of temperature and blood flow upon motion of the chilled limb. The latter recur throughout periods of from 4 to 120 hours of chilling. Platinum electrodes developed to make amperometric measures of oxygen tension in skin and muscle show some reduction of oxygen tension in skin and muscle during chilling. This is reversed if the animals breathe pure oxygen. However subsequent dysfunction of muscle is little benefitted by oxygen inhalation during chilling and not at all by administration of cortisone during immersion and recovery.

Residual oxygen uptake of muscle (Warburg apparatus) is not depressed unless the leg has been chilled for 24 hours or longer. A week after 72 hours of chilling, during periods of inflammation and regeneration (studied histologically) oxygen uptake increases to above that of the unchilled control limb.

Following immersion, skin and muscle temperatures return to pre-exposure levels even after 120 hours of chilling. A residual loss of contraction strength of muscle is accompanied by edema of connective tissues in skin, and in tissue surrounding muscle and nerve. There is histologic evidence of arterial patency with capillary and venous engorgement, focal vacuolization and cellular infiltration of muscle fibers (H & E stains). There is degeneration of myelin sheath of nerve (Marchi stain). All of these changes are first seen after chilling for 4 hours and increase as immersion is prolonged.

Electrophysiologic measures of neuromuscular dysfunction made with D. S. Scott, Jr. suggest that myoneural junction is primarily impaired since after 4 hours of chilling muscle fails to respond to indirect stimulus and response to direct stimulus and action potential

of nerve are only moderately reduced.

Recent studies of nerve endings in muscle stained by a gold chloride technique at various times during recovery from 4 hours of chilling show a marked reduction in number of myoneural junctions (motor end-plates) and less frequent retrograde changes along the last few hundred microns of terminal axons. Damage is minimal at 0 hours, marked at 24 hours, and returning toward control values 72 hours after immersion. 168 hours after chilling intact myoneural junctions and terminal axons are comparable in number to those of the unchilled leg. Damage to myoneural junctions and axons is increased and recovery is prolonged if chilling is extended. Muscle spindles and tendon organs are not damaged unless the leg is chilled for 8 and 24 hours respectively.

An aurophilic "mast-like" connective tissue cell is seen in muscle stained by the gold chloride. The number of these cells is increased in muscle excised during regeneration of nerve endings 72 hours after 4 hours of chilling. Since mast cells contain phospholipids similar to those of the myelin sheath of nerve it became important to know whether a known source of mast cells (the mouse's ear) is stained by the gold chloride technique. These, and additional stains of mouse and rabbit skeletal muscle by a toluidine blue technique, modified to prevent hardening and shrinking of muscle, confirm our impression that the unidentified cells are mast cells. It is of interest that the mast cell is known to be degranulated by cortisone and that earlier studies showed that a similar local cold injury is not benefitted by cortisone therapy.

Histologic studies of nerve endings in chilled muscle suggest that some of the residual damage is incurred during return of metabolism in the rewarmed limb. As rewarming may be accompanied by some oxygen debt we are making amperometric measures of oxygen tension in rewarmed muscle to see whether such a debt exists and if it can be modified by inhaling pure oxygen. To overcome the peripheral vasoconstriction induced by breathing high concentrations of oxygen, as well as to increase dissociation, and diffusion of oxygen throughout the previously chilled muscle, the leg is rapidly rewarmed in water at arterial temperature while the animal is breathing oxygen. To determine whether the residual neuromuscular injury is modified by oxygen inhalation and/or rapid rewarming, histologic studies of nerve endings in muscle are being made in these animals.

CURRENT REPORTS AND PUBLICATIONS

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BONE MARROW

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ASSISTED BY Maxim Persidsky, M.A.

TASK NO. NR NR 105-235

CONTRACT Nonr-3168(00)

OBJECTIVES

(a) To study diverse low temperature techniques for the preservation of bone marrow, (b) to develop techniques for assessing in a quantitative manner the viability of preserved bone marrow either by (1) phase contrast microscopy, or (2) reinjection into lethally irradiated animals, (c) to explore the long-term culturing of bone marrow, (d) to compare various preservatives in low-temperature preservation of bone marrow and (e) to develop methods for preservation of bone marrow by freeze-drying.

ABSTRACT

In our first year of research we discovered that polyvinylpyrrolidone in 10% concentration was a much better preservative of marrow than 15% glycerol. We worked out also the technique for determining cell viability employing phase contrast microscopy. We initiated studies in biological tests of viability, re-injecting the marrow into lethally irradiated mice.

This year we have carried out extensive studies trying to demonstrate the mechanism of protection of the cell from freezing by polyvinylpyrrolidone. Our studies have shown that a certain amount of polyvinylpyrrolidone reaches the intracellular space, thereby contributing to its protective effects during the freezing process, but the majority of polyvinylpyrrolidone acts as a surface coating agent on the cell membrane. We have increased the entry of polyvinylpyrrolidone into the cell by adding phosphate ion to the incubating medium. The permeability of PVP has been studied with Iodine-131 labelled PVP. Phosphate ion is known to enhance pinocytosis and this probably explains the increased penetration of PVP into the cell after incubation with phosphate ions.

A simplified culturing technique has been worked out to enable us to assess cell viability within a 6-hour period. This, in essence, is a suspension type tissue culture technique. After 4 hours of culturing in a rotating flask, the marrow cells are placed in Kimax bottles to permit them to become attached to the cover slips. After two additional hours the preparations can be removed and examined under the phase contrast microscope to determine viability.

The initial advantages of this culturing technique are being explored to study long-term culturing and preservation of bone marrow. A double flask rotating tissue culture method which enables changing of the tissue culture medium without disturbing the cells has been worked out.

We have succeeded thusfar in growing marrow for an 8 day period without morphological changes in the cells.

The details of the freezing process have been observed under low temperature microscopy. Polyvinylpyrrolidone has been demonstrated to permit super cooling of the cell so that freezing of many cells does not take place even at -79°C .

We have worked on the development of a freeze drying apparatus. We plan to study the viability of frozen dried marrow within the next year.

We have also studied the comparative effects of carbowax and dimethylsulfoxide on the preservation of marrow cells. These latter two preservatives equal polyvinylpyrrolidone in their preserving effects on marrow under low-temperature preservation.

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RESEARCH ON PROCEDURES FOR THE LOW-TEMPERATURE
PRESERVATION OF BLOOD

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ASSISTED BY C.W. Cowley, G.F. Doebbler, R.F. Dwyer, C. Ho, J.A. Lawrie, C.H. Nuermberger, H.D. Robbins, R.R. Sakaida, J.A. Sawdye, H.R. Schreiner, A.J. Short
TASK NO. NR 105-208 **CONTRACT** Nonr-3003(00)

OBJECTIVES

(a) To investigate the biochemical, biophysical, and physiological processes which occur during the freezing and thawing of blood, (b) to devise procedures for the intact recovery, after freezing and thawing, of erythrocytes and other formed elements of the blood, (c) to develop a simple, economical, and speedy process for the low temperature preservation, storage, and subsequent transfusion of human blood with a minimum of intermediate steps in the process from donor to patient.

ABSTRACT

Red cells appear to hemolyze during freezing and thawing by a sequence of events involving a loss of enzymes associated with cation transport, an increase in electrolyte permeability, and a net uptake of water under an osmotic pressure gradient dependent on intracellular hemoglobin concentration. Many compounds, including sugars, glycols, and polymers inhibit freeze-thaw damage to the erythrocyte at concentrations of 5 to 15% when rates of cooling and warming of the order of several degrees per second are employed. The mechanism of this protection is likely to involve interaction of these compounds with the red cell. Protective activity of solutes correlates with the concentration of potential hydrogen bonding groups provided, and is influenced by steric and electrostatic effects.

Freezing and thawing of unprotected red cells releases and activates adenosine triphosphatase and releases cholinesterase. Mechanical, but not osmotic hemolysis also leads to adenosine triphosphatase release and activation. Less enzyme release occurs when red cells are partially protected from hemolysis during freezing and thawing. Red cells recovered intact after freezing and thawing contain less potassium and more sodium than unfrozen control cells, and exhibit additional hemolysis upon resuspension in electrolyte solutions. This is largely inhibited in sucrose solution and completely prevented in polymer solutions of sufficient concentrations.

Experimental evidence indicates that intracellular ice as well as the dehydration conditions prevailing during freezing and thawing should be considered as possible agents of red cell injury. Calorimetric evidence clearly establishes, however, that the freeze-thaw destruction of human erythrocytes is not a direct consequence of ice crystal formation per se. On cooling whole blood containing ACD anticoagulant, erythrocyte damage does not set in until the freezing process has reached about 90% completion. Further progress of this phase change leads to immediate and massive hemolysis. While protective solutes such as polyvinylpyrrolidone,

dextran, lactose or glucose effectively limit the extent of ice formation on cooling as a function of their concentration in blood, other related solutes which do not afford protection retard the growth of ice in aqueous systems equally or better.

The viability of human erythrocytes preserved by rapid freezing and thawing in the presence of glucose or lactose-glucose has been studied in man and rabbits. These preliminary transfusion experiments show that the viability of glucose-treated erythrocytes appears to be related to the amount of additive used and the time it remains in contact with the cells prior to freezing. Longer periods of equilibration, e.g. with 12.5% glucose at 32° for four hours yield higher red cell recovery values after thawing but result in heavy osmotic destruction of the cells on reaching the recipient's circulation. Blood containing lactose (7.5%) and glucose (5%) equilibrated for one hour at 32° gave in vivo survival values at 24 hours ranging from 39 to 68% in six experimental transfusions. No unfavorable reactions were observed in any of ten subjects receiving blood containing lactose. Rabbit erythrocytes treated with glucose additive showed poorer recovery following thawing than did human red cells. Under processing conditions favorable for human blood, red cell survival averaged 43% in five animals. Rabbit erythrocytes treated with lactose-glucose additive showed somewhat poorer recovery following thawing than did human red cells under comparable processing conditions. Red cell survival, however, averaged 76% for the rabbit cells. Clinical studies of the in vivo survival of frozen and thawed blood containing polyvinylpyrrolidone (Plasdone-C) have been initiated recently. Preliminary results indicate a remarkable degree of stability of transfused cells which have been preserved in this manner.

Existing procedures for the determination of in vivo survival of red cells have been critically evaluated and found to possess limitations in terms of routine application in animal studies or potentially large errors when applied to frozen and thawed blood. A new method which meets both theoretical and practical criteria for assaying red cell survival of frozen and thawed blood has been developed. This method involves a comparison of the viability of preserved red cells to autologous normal red cells in terms of proportional isotope dilution. An injection of chromium-51 labeled autologous red cells is made into a test animal followed by sampling of the peripheral blood for specific red cell radioactivity. A subsequent injection of preserved red cells, also labeled with chromium-51 but at a 10-fold higher level of radioactivity, is made into the same animal followed again by sampling of the peripheral blood for specific red cell radioactivity. The degrees of dilution of the two injections of chromium-51 are compared. Deviation from proportionality allows calculation of per cent relative survival of the test cells.

The consecutive Cr-51 procedure has been evaluated extensively and shown to be capable of a precision comparable to that obtainable with the widely employed simultaneous P-32-Cr-51 method.

A basic engineering study of freezing and thawing procedures for whole blood has been carried out. Agitation by wrist-action shaking was found to improve heat transfer and consequently yielded higher recoveries of processed cells. A detailed description can now be given of the physical events occurring during the freezing and thawing of blood inside a

specially designed corrugated aluminum container. Agitation during freezing by immersion in liquid nitrogen causes the container to be completely filled with a turbulent mass of liquid containing dispersed air bubbles. The bulk temperature of this turbulent mass drops very rapidly to the freezing point where it remains while the blood freezes progressively in the form of a shell on the container wall, leaving a central cavity. With a suitable combination of container geometry and degree of agitation, the thickness of this frozen shell is essentially constant. This procedure permits a given volume of liquid to be frozen in a container of smaller linear dimensions than would be the case if quiescent freezing were employed.

At the beginning of the thawing operation melting starts at the container wall, and the resulting annular layer of water reduces heat transfer. Convection currents induced in the liquid film by shaking tend to reduce temperature gradients and decrease the insulating effect of the liquid film. Later, the frozen blood becomes detached from the container and oscillates back and forth as it melts while suspended in turbulent liquid. This information is providing the basis for a mathematical analysis of the heat transfer problems involved and has guided the development of blood processing equipment and techniques.

An automated, heavy-duty machine for the processing of pint and half-pint quantities of blood for frozen storage and for their subsequent thawing was constructed. While designed as a research tool the operation of this Blood Processing Unit ("BPU-1") was automated so as to permit fool-proof operation by semi-skilled technicians in clinical applications. All phases of the processing may be varied to allow for changes in blood preservation technique that may develop in the future. Shaking is provided by a heavy-duty, custom-made, wrist-action agitator. Injection and withdrawal of blood containers from the freezing and thawing baths is accomplished pneumatically. Containers are held on the agitator arms with simple, spring-loaded clamps. All phases of the processing - equilibration, coating of containers for improved heat transfer, freezing, and thawing - are integrated into BPU-1. The machine is self-contained, simple to operate, flexible and reliable. Since its construction it has been in continuous daily service processing blood in many types of containers over a wide range of processing conditions. Two additional BPU-1 machines have been constructed and placed into service at the American National Red Cross Laboratories, Washington, D.C., and at the Royal Army Medical College in London, England.

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PRESERVATION OF HUMAN RED CELLS
IN THE FROZEN STATE

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ASSISTED BY P. V. Strumia, L.S. Colwell and A. Dugan

TASK NO. NR 105-182

CONTRACT Nonr-2466 (00)

OBJECTIVES

The purpose of this study is to investigate basic principles related to the protection of red cells during the process of freezing and thawing, for the ultimate purpose of establishing a practical technique for prolonged storage of red cells at low temperatures.

ABSTRACT

The use of solutions of dextrose-lactose as an additive to whole ACD blood has been shown to have a good protective effect when the blood is rapidly frozen, stored at -93°C ., rapidly thawed and transfused without further modification within one hour of thawing.

ACD blood with a hematocrit of 64 - 70 has been modified by addition of varying amounts of human albumin solution to obtain a concentration of 4 - 19% of albumin.

Albumin in a concentration of 11% or better has a protective effect as good as or better than that obtained with the addition of sugars, previously reported. The recovery of red cells after freezing and thawing with the aid of albumin is better than obtained with other macromolecular solutions tested, including P.V.P., concentrated plasma and modified globin.

Addition of varying amounts of dextrose to obtain a final concentration of 2 - 7 gm. %, improves slightly the recovery of red cells, especially when the concentration of albumin is below optimal.

Motion during freezing and thawing, as previously reported, greatly enhances the recovery rate of red cells.

Under optimal conditions, with addition of albumin, the recovery of red cells is between 92 and 95 %.

CURRENT REPORTS AND PUBLICATIONS

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STUDIES ON THE PREPARATION AND PRESERVATION OF HUMAN RED CELLS
AND MARROW IN THE FROZEN STATE USING THE COHN-ADL FRACTIONATOR

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Protein Foundation

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TASK NO. NR 105-112

CONTRACT Nonr-1852(00)

OBJECTIVES

To evaluate the application of the Cohn-ADL Fractionator to the glycerolization and deglycerolization of human erythrocytes, and to determine the ability of such glycerolized red cells to survive storage in the frozen state.

ABSTRACT

Previous work on this project established the methodology of Tullis and collaborators as practical for the glycerolization and deglycerolization of human erythrocytes in the Cohn-ADL centrifuge. The equipment proved reliable, adaptable to a standard service hospital and capable of being operated by non-professional personnel. Human erythrocytes processed in this manner and subsequently frozen and stored up to five years showed sufficient post-storage yield and transfusion survival as to justify the technical requirements of adding glycerol before freezing and removing glycerol after thawing. Several wholly unanticipated outgrowths of these early studies with such processed cells proved sufficiently important one year ago to require a redefinition and a broadening of the scope of this project. These findings included: (a) the capability of "tailoring" the Transfusion unit at the time of thawing and deglycerolization by reincorporating the red cells into their native plasma as whole blood, or into any media adjusted to fit the exact recipient requirements; (b) a reduction in the non-hemolytic recipient reaction rate of red cells in albumin to 20-fold less than for fresh ACD blood.

During the past year, the collection, glycerolization, storage, deglycerolization, resuspension and transfusion of human red cells as whole blood or packed cells has become sufficiently standardized to permit: (1) commercial preparation of the solutions necessary for the processing; (2) collaboration on the development of a simplified apparatus embodying the same principals, in a disposable plastic bag centrifuge liner. The former already has led to significant improvement in the quality and quantity of red cell yield. The latter has passed through preliminary testing and will be ready shortly for large scale study; (3) the clinical studies have been extended on the physiologic side effects of multiple transfusions of deglycerolized red cells in albumin, in diverse medical and surgical states. Two unexpected findings have

been elucidated: (A) the dilutional thrombocytopenia which is a regular accompaniment of multiple transfusions of fresh or banked ACD blood, does not follow the transfusion of deglycerolized red cells in albumin; (B) the ability of the thawed, deglycerolized red cells to take up oxygen and release it to the tissues is comparable to freshly collected (ACD) red cells, and is greater than ordinary "banked" blood stored one week at $+4^{\circ}\text{C}$. Irrespective of duration of storage, the deglycerolized cells on the day of thawing do not show the deleterious left-shift in oxyhemoglobin dissociation described by Kennedy & Valtis for ACD red cells after short periods of storage.

During the past year, studies on the extension of the glycerol method to other tissues, especially bone marrow, has shown that other additives such as dimethyl sulfoxide possess merit. The latter agent leads to highly satisfactory preservation of human marrow at -80°C and possesses technical advantages over polyhydric alcohols such as glycerol. It cannot be removed from non-nucleated cells, however, and thus it cannot be used for red cells despite its satisfactory adaptability to marrow preservation.

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(b) U. G. Bethel and T. G. O'Brien (1961), "Use of Cohn-ADL Blood Fractionator in Continuous Flow Dialysis." Vox Sang., 6, 195

(c) T. G. O'Brien, M. T. Sproul, J. W. Zemp, J. A. Cavins and E. Watkins, Jr., "Clinical Use of Frozen Red Cells Suspended in Stored Heparinized Plasma." Ibid, 196

(d) R. J. Tinch and J. L. Tullis, "Studies on Removal of Plasma Components During Continuous Centrifugation." Ibid, 197

(e) A. Latham, Jr., "A Disposable Liner for Centrifuge Bowl of the Cohn-ADL Fractionator." Ibid, 198

(f) L. L. Haynes and J. L. Tullis, "Complete and Partial Exchange Transfusion with Glycerolized Stored Red Cells Suspended in Albumin." Ibid, 199

(g) H. M. Pyle and H. F. Boyer, "Effects of Dimethyl-Sulfoxide on Blood Cells and Bone Marrow." Ibid, 199

(h) J. W. Zemp, "Chemical Studies of Stored Red Blood Cells, I. Phosphate Partition Characteristics." Ibid, 211

(i) T. G. O'Brien, O. M. Frost, E. Watkins, Jr. and J. W. Zemp, "Gas Exchange Studies on Stored Blood." Ibid, 212

(j) T. G. O'Brien, L. L. Haynes, A. L. Hering, J. L. Tullis and E. Watkins (1961), "Use of Glycerolized Frozen Blood in Vascular Surgery and Extracorporeal Circulation." Surgery 49, 109

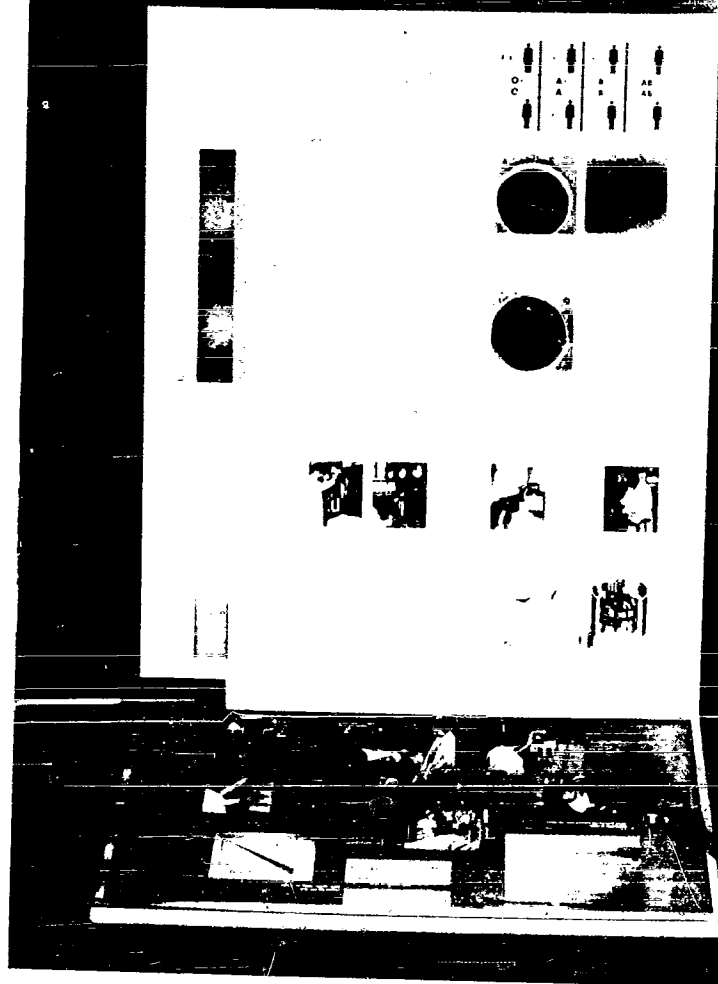
(k) J. L. Tullis, L. L. Haynes, J. A. Cavins, M. T. Sproul, "Effects of Exchange Transfusion of Frozen Red Cells in Albumin on Blood Cells and Coagulation Proteins." (in press)

(l) J. L. Tullis, "Minimum Requirements for Blood Bank Operation." (in press)

U.S. NAVY Office of Naval Research

BIOLOGICAL SCIENCES

BLOOD PRESERVATION



The ONR Blood Preservation Exhibit exemplifies some of the basic, interdisciplinary research being sponsored by the Biological Sciences Division of ONR. Illustrated and summarized are two processes of long-term blood preservation (the liquid nitrogen and glycerol methods) currently under study.

Trauma and Metabolism

Research in this program is concerned principally with the interrelationships of metabolic derangements subsequent to traumatic conditions. This section of the report should be correlated with the section on transplantation as many of those studies deal with the trauma of burns. Dr. Hoff's work, cited in the section on dentistry, should be integrated into this category.

COMBAT HEAD INJURY PROJECT,
FOLLOW-UP PHASE

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TASK NO. NR 105-086

CONTRACT Nonr 2690(00)

OBJECTIVES

To better understand the relation between craniocerebral trauma and its sequelae.

ABSTRACT

A study has been conducted of 407 U.S. Marine and Navy personnel from the Korean campaign who received head injuries in combat or supporting activities between 1951 and 1953. Small arms fire, mortar fragments, land mines and other missiles accounted for 214 of the injuries, blast for 52 and trauma unrelated to missiles for 141. Seen at the time of injury by Drs. Henry R. Liss, John S. Meyer or myself, these men were followed for the first five years (Nonr 266(26)) by a review of the original field and hospital records in 100 per cent of the cases, questionnaires in 90.6 per cent, personal correspondence in 37.5 per cent, periodic physical examination in 25.5 per cent, additional interviews in 24.5 per cent, American Red Cross field study in 69.0 per cent and Veterans Administration records in 66.5 per cent. Attention was directed to the stabilized neurologic deficit, posttraumatic epilepsy, posttraumatic syndrome and social and economic factors, as these appeared in this interval.

Now, some ten years after injury, additional data are being sought for each of the parameters. During the past several months new information concerning post-traumatic epilepsy has been acquired through registered mail, a review of recent entries in Veterans Administration records and, in special instances, by personal telephone calls. Of assistance in this has been the part-time aid of Dr. John H. Evans, a research associate, and Dr. James MacD. Watson, a former associate, now a staff member of the Veterans Administration Hospital in Syracuse, New York. Contact has been reestablished with 301 men, 167 of whom had received missile wounds, 134 blast and non-missile wounds. The present overall incidence of posttraumatic epilepsy is 27.4 per cent. In the missile injured incidence is 38.7 per cent, in the blast and non-missile injured, 12.7 per cent. Further analysis of these data is in progress.

To provide greater depth to the Korean material, the present status of former U.S. Army personnel will be sought in conjunction with their surgeon at the time of injury, Dr. Arnold Meirowsky. To better evaluate the significance of changing factors, i. e., the character of wounds, complications and therapeutic effort, a

comparative study has begun of sequelae of head injuries received in World War I, World War II and Korea. The first part of this has included a reappraisal of the British material from World War I by Dr. Peter B. Ascroft and of that from the U. S. Army in World War II by Dr. A. Earl Walker. It is hoped that this may continue, with the addition of German material from World War I and the collaboration of Drs. Joachim E. Meyer and Karl-Heinz Leuchs. The feasibility of this was determined by an ONR-sponsored pilot study of the German records at the Hirnverletztenheim in Munich during August and September of 1961.

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(b) William F. Caveness and Henry R. Liss (1961), "Incidence of post-traumatic epilepsy." Epilepsia, 2, 123-129

(c) William F. Caveness, A. Earl Walker and Peter B. Ascroft (in press), "Incidence of posttraumatic epilepsy in Korean veterans as compared with those from World War I and World War II." J. Neurosurg.

DESIGN AND TEST OF A NEW
PUMP-OXYGENATOR.

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ASSISTED BY E. M. Barsamian

TASK NO. NR NR 105-211

CONTRACT Nonr-1841(62)

OBJECTIVES

To develop a pump-oxygenator that will (a) be safe and simple to operate, clean and assemble (b) will have a low blood priming volume, to overcome the difficulties of matching and cross-matching a great number of donors prior to each use of the pump-oxygenator, (c) will sell at low cost, e.g. in the neighborhood of \$1000, (d) can be autoclaved in the assembled state, to permit its use in emergencies as well as in elective cases.

ABSTRACT

Although many of the existing variants of the screen, disc, bubble and membrane oxygenators are fairly satisfactory, they are all far from perfect and there is a need for a pump-oxygenating unit with the above mentioned features. This is especially true (1) if pump-oxygenators are to be made available to a great number of research as well as clinical institutions. (2) if the pump-oxygenators are to be put into their widest possible application, which lies not only in elective cardiac surgery but also in many cardiovascular and pulmonary medical problems and emergencies.

The unit described below substantially meets all the objectives we set out to achieve.

In Fig. 1 the machine is shown ready for use. The rectangular structure at the left contains the oxygen-driven engine and all of its associated parts: namely the piston, cylinder, crankshaft, cam shaft and six ball valves which control the engine itself, the blood pumps and the coronary suckers.

The cylindrical element at the center of the picture is the oxygenator. Its inner construction is shown in Fig. 3. An external stationary glass cylinder houses a rotatable stainless steel cylinder only slightly smaller in diameter than the glass cylinder. Within the rotating cylinder is a nest of several open-ended stainless steel cylinders and finally a cylinder with closed ends. When the outermost stainless steel cylinder rotates, those inside roll on each other as they tend to remain in the lowest possible position in response to gravity. The advantage of this arrangement is that all surfaces become filmed with blood even though the level of blood may

be quite low. The pump parts are also shown in Fig. 3.

The pulsations of the blood stream caused by the pump action are softened by the action of a surge chamber. A gauge on the engine block indicates the rise and fall in pressure within the surge chamber. The maximum and minimum pressures are related to the arterial pressure, systolic and diastolic, but larger in absolute value by the pressure drop across the cannula which delivers the blood into the artery.

After use the oxygenator, pumps, surge chamber and piping (everything which comes in contact with the blood) are cleaned and completely assembled. The resulting package is shown in hand in Fig. 2. This package is wrapped, autoclaved and kept in a sterile condition until needed again. It is then unwrapped and attached to the engine block by adjusting two screws. Three short plastic tubes are then slipped into place for supplying oxygen pressure to the pumps and to the surge chamber. The oxygen flowing in these tubes does not come in contact with the blood, hence these tubes do not require sterilization.

Since the unit operates on a very low priming volume (less than 1000 ml), it is essential to have a fool-proof mechanism to prevent air passing from the oxygenator into the pump. This is accomplished with (1) a float valve at the base of the oxygenator (2) a special arrangement whereby the weight of the oxygenator (and therefore the amount of blood in it) regulates the air exhaust valves of the pumps. When the level of blood in the oxygenator starts to fall, the exhaust valve of the pump is partially shut off so that the pump output diminishes. When the blood level in the oxygenator falls below a critical level, the exhaust valve is completely shut off and the pumps cease functioning.

The coronary suckers, not visible in the photograph, are small air pumps within the body of the engine block whose function is to establish siphons which withdraw blood from the site of operation and deliver it to the oxygenator.

The unit is gentle on the blood causing minimal hemolysis. The pumps have a capacity of more than 6 liters per minute and oxygenation is adequate. The whole unit weighs about 50 lbs. and occupies a cubic foot of space so that in an emergency it is easily portable to where it is required.

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BASIC AND APPLIED
CLINICAL INVESTIGATION

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ASSISTED BY P. D. Doolan, R. H. Watten, G. B. Theil, A. J. Draper

TASK NO. NR 105-156

CONTRACT Nonr-222(51)

OBJECTIVES

The conducting of clinical investigations of a broad and fundamental nature with particular reference to the special clinical material available at the Naval Hospital and the interests of the professional staff of the Center and other Services of the Hospital.

ABSTRACT

The research currently in progress at the CIC comprises four general activities.

1. Clinical and Experimental Studies in Renal Disease.

Peritoneal dialysis as a method for treating acute and chronic renal disease and various acute poisonings has been thoroughly evaluated. Further studies on the kinetic aspects of peritoneal dialysis are in progress. The suitabilities of various diagnostic technics for assessment of the significance of proteinuria in patients without evidence of renal disease are under study. A detailed analysis of the significance of "undetermined nitrogen" in normal and uremic subjects has been completed. There has also been completed a clinical appraisal of the relation between plasma concentrations and endogenous clearance of creatinine in patients with renal disease.

2. Cardiopulmonary Studies in Relation to Disease.

Complete studies on at least 100 cases of chronic pulmonary emphysema are projected. To date approximately 60 cases have been studied. In a particularly suitable experimental animal (the horse) studies are in progress utilizing occlusion of the nutrient pulmonary vessels to determine whether or not compromise of the systemic (bronchial) supply to the lung will produce emphysema. In some of the animals, experimentally sclerosing the bronchial artery has led to pathologic changes in the lungs identical to those observed in horses spontaneously developing emphysema.

3. Development and Evaluation of Diagnostic Technics.

(a) In a series of patients the value of the vectorcardiogram as compared to cardiac catheterization in estimating pulmonary vascular resistance in cardiac disease is being studied. (b) The excretion into the urine of hydroxyproline after ingestion of a test dose of gelatin is being measured as a possible clinical test of the proteolytic activity of the gastrointestinal secretions. (c) Excretion of 3-methoxy-4-hydroxy mandelic acid and of catecholamines is being measured prior and subsequent to histamine stimulation in control subjects and in patients with a diagnosis of essential hypertension.

4. Studies on Metabolism in Health and Disease.

(a) A generalized aminoaciduria has previously been observed in this laboratory in association with the nephrotic syndrome. During the past year it has again been detected in 2 adult patients with clinical renal disease. (b) Further studies on myophosphorylase deficiency glycogenesis are projected if additional patients become available. A screening test to detect such patients has been developed. (c) Earlier reference was made to studies of experimental production of emphysema. Studies are now in progress on the chemical composition of connective tissues of the lung in various animal species in an effort to relate this chemical parameter to functional changes, particularly in relation to emphysema.

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FUNCTIONAL ENDOCRINOLOGICAL INTERRELATIONS OPERATING
IN THE METABOLIC RESPONSE OF MAN TO TRAUMA

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TASK NO. NR 105057

CONTRACT NONR-609 (10)

OBJECTIVES

(a) To evaluate the various endocrine responses in man as they relate to changes in convalescent metabolism, (b) To study the specific alterations that occur in the response to trauma and determine how they may be beneficially altered, (c) To evaluate the metabolic changes existing after injury beyond the period of endocrinologic response to trauma.

ABSTRACT

It is important to indicate that current laboratory investigation is preliminary in the experimental animal. As these mechanisms are clarified more completely, the investigation can be applied in man in comparable clinical situations.

A study has been completed of the renal response to acidosis during anesthesia and operation. The study was composed of three parts: (a) The effect of acute dilutional hyponatremia on hydrogen ion and free water excretion during metabolic acidosis in anesthetized dogs. (b) The effect of operative trauma on hydrogen ion and free water excretion during metabolic acidosis. (c) Maintenance of homeostasis in acute respiratory acidosis during intravenous infusion of Ringer's Lactate solution and 5% glucose in water solution. Conclusions obtained from this study revealed that hyponatremia interferes with normal hydrogen ion excretion and free water clearance by decreasing the filtered sodium load (and perhaps also increasing proximal sodium absorption); this under-utilizes the distal sodium reabsorptive mechanism by which hydrogen ions are secreted and osmotically free water produced. It is further concluded that operation may interfere further with hydrogen ion excretion in the hyponatremic animal. Furthermore, by decreasing the rate of solute excretion (chiefly the result of decreased glomerular filtration rate) in the presence of a strong anti-diuretic tendency, it interferes with the ability of the organism to excrete free water. During acute respiratory acidosis, Ringer's Lactate solution permits greater hydrogen ion secretion by the kidney, and therefore greater concentration of bicarbonate bound base than does the administration of 5% glucose in water.

Further evaluation of the thyroid response to trauma was made. A significant increase in erythrocyte uptake of I^{131} was found in patients in whom chronic disease or recent acute stress was absent. Thyroid binding prealbumin decreased during the operative procedure. These changes are consistent with altered thyroid hormone transport mechanisms during operation. There was an increased concentration of free and conjugated 17-hydroxycorticosteroids in the plasma at this time. An inverse relationship between thyroid and adrenocortical activity has been suggested.

The conversion ratio of I^{131} changed as much under spinal as under ether anesthesia suggesting that the blood levels of thyroid hormones are controlled by factors other than those controlling blood levels of certain other hormones during operation.

Studies of calcium and magnesium metabolism in the preoperative and postoperative period revealed that there was little change, if any, in parathyroid function relating to operative trauma. There is, however, a negative calcium balance in the postoperative period, principally due to fecal calcium loss. Magnesium metabolism is not altered significantly in the postoperative period. There is no effect on lipid absorption from increased adrenocortical activity as produced by ACTH administration. Operative alteration of the gastrointestinal tract of dogs, diverting bile or pancreatic secretion from the duodenum or performance of small bowel resection, eliminated the decreased post-lobectomy triolein uptake observed in the intact animal. Mechanisms involved in lipid absorption after non-gastrointestinal operative stress still have not been clarified.

Further work in progress on thyroid adrenocortical relationships indicated that in animals with intact adrenal glands undergoing pulmonary lobectomy an increase in conversion ratio of I^{131} is noted. A much greater conversion ratio was present during the same operation when the adrenal glands are absent. This suggests adrenocortical antagonism to thyroid hormone.

Further studies are being done on improving and clarifying a method for the plasma determination of pressor amines.

Work has been completed on the effect of hypertonic solutions on renal function, intracellular water and electrolyte concentrations after various solutions.

Further work in progress is a study of renal blood flow and glomerular filtration rate and tubular function as a result of atraumatic cross clamping of the aorta versus extensive mobilization of the mesentery in cross clamping of the aorta.

Data is available from studies of patients during a long term convalescent study after trauma. In patients receiving no supplementary nutrition after operation, or before and after operation, caloric expenditures are exactly what would be expected in a starved person. The activation of the adrenal cortex influences very little the metabolic changes. If parenteral alimentation, isocaloric in value and composed of the same distribution as the general diet taken orally, is provided before and after operation, little or no negative nitrogen balance is found.

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STUDIES ON PULMONARY ANATOMY, PATHOLOGY AND TECHNIQUES
FOR TREATMENT OF ANOMALIES AND INJURIES

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ASSISTED BY Bloor, C.M., Tsai, L.S., Stern, H., Nyman, A.

TASK NO. NR 105-090

CONTRACT Nonr-609(21)

OBJECTIVES

To obtain an understanding of the cardiopulmonary system in health and under various circumstances of disease by a combined approach employing the techniques of anatomy, physiology and surgery.

ABSTRACT

1. Observations on the genesis of collateral circulation:

a. Effects of hormones: The existence of chemical factors in the initiation and control of collateral circulation has been established by previous observations, including those published in four reports from this laboratory (Bul. Internat. Assoc. Med. Mus., 31:1, 1950; Arch. Path., 57: 39, 1954; Am. J. Path., 33:539-571, 1957; Circ. Res., 8:353-376, 1950). Few qualitative and less quantitative data have, however, been available. In the current report period an experiment has been designed in collaboration with W. Meffert, to test the effects of hormones on the growth of pulmonary collaterals in the rat. In planning, account was taken of the expected final weights of the animals, and their near identity was assured by paired feeding. Rats treated with growth hormone and cortisone were compared with controls at four weeks, and in another series at eight weeks after ligation of the left pulmonary artery. Vinylite casts were prepared and the material from both series ranked according to the magnitude of the collateral circulation as established visually. This was done independently twice by each of three observers and the observations were analyzed by White's modification of Wilcoxon's ranking method. At four weeks cortisone resulted in a significant inhibition and at eight weeks growth hormone in a significant stimulation of collateral circulation ($p < 0.05$ in each instance). There was remarkable congruence between the two successive observations on the part of each observer, and among the three observers, and the findings were confirmed by weighing the casts of the vessels.

b. Chemical correlates: Previous observations in this laboratory have established two phases in the development of collateral circulation in the lung of the rat, the first a mechanical expansion that predominates in the first five days, and the second one of active growth thereafter. Chemical correlates of the latter are being sought. In the phase of active growth, the vascular buds are surrounded by large numbers of mast cells

and other non-granulated derivatives of the reticuloendothelial system. A start has been made by analysis of the lungs for histamine by the fluorophotometric method of Shore et al which has now been successfully applied. A definite rise in the content of this material has been found 24 hours after ligation of the left pulmonary artery, and this was not present in mock-operated animals. Further work is in progress.

c. Pulmonary collateral circulation in congenital heart disease: The lungs of approximately 100 patients with congenital heart disease have been studied, using vinylite casting and gelatin injection techniques and an extensive report is in preparation.

2. Study of the collateral circulation to the heart: For background an angiographic study of the coronary arteries in the normal dog has been completed. A new mechanical constricting clamp for the coronary arteries has been designed for use in the attempt to transplant the circulation of the heart to a collateral source induced by cardiopneumonopexy after ligation of the pulmonary artery.

3. Study of the effects of controllable arterial desaturation: A controllable method of producing chronic cyanosis in the dog has been devised, by gradually shunting the inferior vena caval blood into the left atrium through the stump of the right lower lobe pulmonary vein after removal of this lobe. Erythropoietin production has been found to increase rapidly but temporarily when systemic arterial desaturation is induced. A method has been devised for repeated study of urine oxygen tensions, by placing an indwelling minute polyethylene catheter into the ureter. This serves as a measure of renal oxygen tension, which is being investigated in correlation with glomerular clearance rate at various levels of oxygen tension. The development of osteoarthritis, and other possible changes, is being watched and application of this shunting procedure, combined with septal deviation, as a method for the treatment of transposition of the great vessels is being tested.

4. Fixation of the lung: A method has been devised for fixing the lung in an inflated state. The lung is inflated by controlled negative pressure within a vacuum jar. Fixation is accomplished by means of steam produced by boiling 10% formalin solution. This method permits a closer approximation of the lung in its fixed state to its size in the thorax. Histological detail after this method of fixation is excellent.

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A STUDY OF PULMONARY FUNCTION AFTER THORACIC SURGERY

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TASK NO. NR 105-230

CONTRACT Nonr-3079 (00)

OBJECTIVES

To determine the type and degree of alterations of pulmonary function after thoracic surgery. The over-all parameters of blood gas exchange, mechanical properties, distribution and ventilation and volumes were examined for this purpose.

ABSTRACT

Preoperative and postoperative studies of pulmonary function were performed on 62 patients who underwent thoracic surgery for the purpose of finding the alterations resulting from surgery. The types of surgery performed included pneumonectomy, segmentectomy, lobectomy, and thoracoplasty or thoracotomy alone. The pulmonary function tests included determinations of total lung capacity, and its subdivisions, distribution of inspired air, dynamic ventilatory tests, and arterial blood gases, and measurements of pulmonary compliance. Preoperative studies were made no longer than two weeks prior to surgery. Three interval studies were used in the postoperative period, within the first month, at six months, and again at twelve months. All parameters of pulmonary function were found lowered, no matter which operative procedure was undergone. The lowest values were observed whenever a postoperative complication occurred. A significant finding at the twelve month period of study was increase in residual volume, an increase in the Fowler Index (a measurement of the distribution of inspired air), and decrease in the dynamic ventilatory studies. Compliance studies were reduced with some change of end-expiratory pressure levels. The results suggest, when compared to the pre-operative determinations, the development of hyperinflation and a probable element of bronchial obstruction as the result of thoracic surgery. Except for cases presenting with postoperative complications, no complete correlation with the degree of resultant changes and the type of operation could be established.

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POSTTRAUMATIC SODIUM AND WATER RETENTION

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TASK NO. NR Nr 105-063

CONTRACT Nonr-475(07)

OBJECTIVES

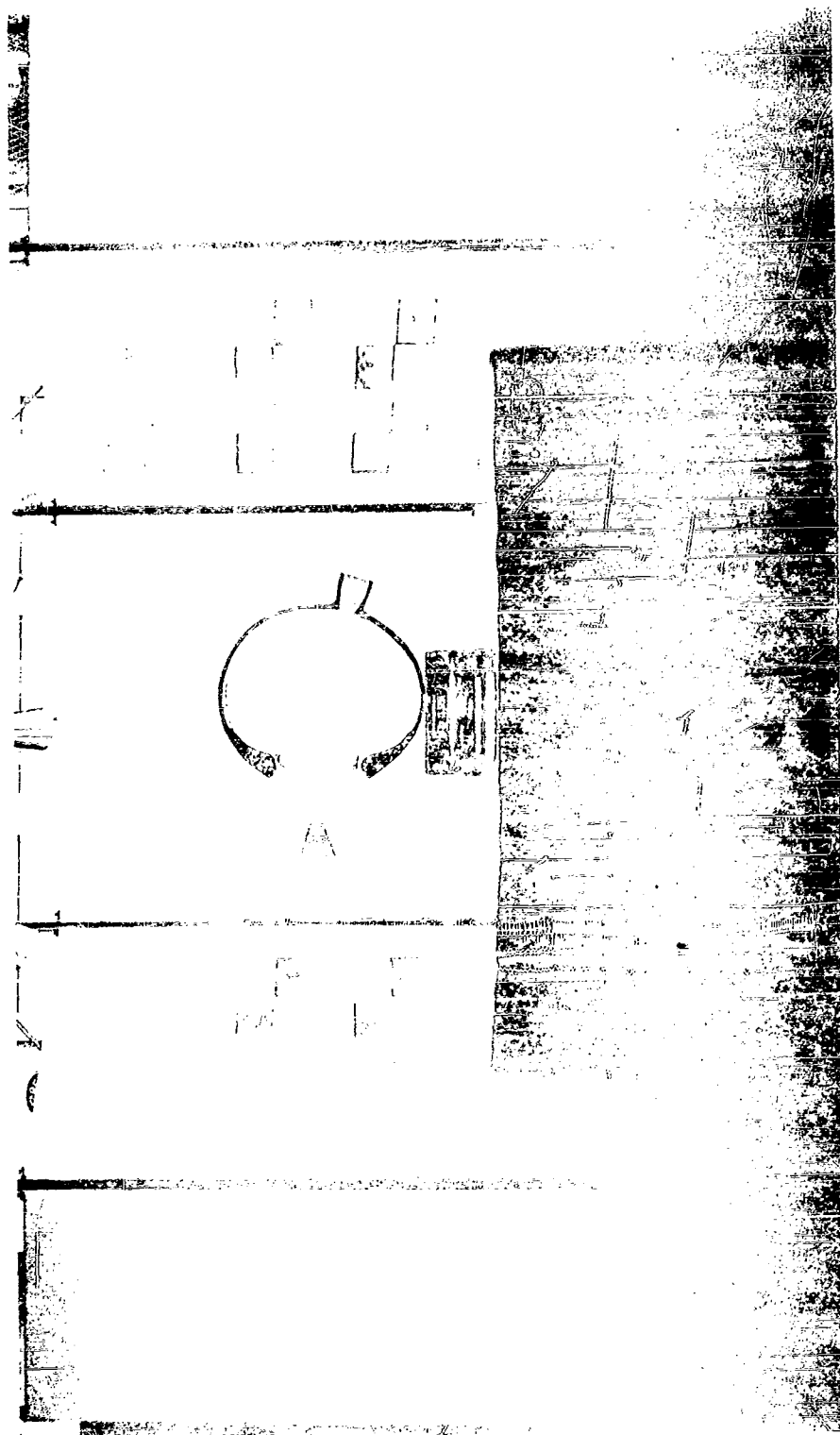
To study the effects of trauma on sodium and water metabolism. In particular the effect of osmotic diuresis as a protective mechanism on renal function in the face of the presence of nephrotoxic substances.

ABSTRACT

In the past year work has been in progress showing that the administration of 4% urea intravenously will increase renal blood flow if it is depressed by an acute traumatic situation. This increase in renal blood flow appears to be secondary to an increase in cardiac output and not to direct renal effect. This increase can be achieved with rapid administration of any intravenous fluid, however urea will be excreted when antidiuresis is present and consequently it is safe inasmuch as overhydration can be prevented. It has likewise been shown that administration of 4% urea intravenously can overcome traumatic edema of the ureters as produced by thermal injury or by ureteral catheterization. It appears that this edema is insignificant in terms of resulting obstruction when an osmotic diuresis is initiated. The mechanism is possibly due to an increase in ureteral peristalsis and possibly to a decrease in the traumatic edema or a combination of both. It has likewise been shown that urea in a 4% concentration which is present in concentrated urine is bactericidal, under experimental in vitro conditions, to the most common gram negative bacteria. Further work is in progress regarding the value of osmotic diuresis in preventing renal damage from various nephrotoxic substances. We have also attempted to evaluate the usefulness of sodium diuresis as an osmotic diuresis initiated by the administration of hydrodiuril under conditions of antidiuresis. Such appears effective in overcoming antidiuresis resulting from trauma.

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Infrared Electronics in Ophthalmology portrays the application of the technology of the physical sciences to biologic problems. Illustrated is the beneficial use of infrared as an instrument which aids diagnoses of structures of the eye despite corneal opacities.

Dentistry

The research activities in dentistry are directed principally toward learning more about the relationship between oral and systemic disorders. For example, the study dealing with the cerebral regulation of salivary function and the mechanism of calcification represent this approach. In addition, the fundamental basis of periodontal disease is emphasized whereas caries research is confined to rather unique studies such as those of Drs. Harris, Orland, and Pigman.

THE COMPOSITION AND METABOLISM OF GINGIVAL TISSUE

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TASK NO. NR NR 105-256

CONTRACT Nonr-3432(00)

OBJECTIVES

This investigation is directed toward a series of experiments designed to elucidate some of the changes in the gingival tissue associated with inflammation and aging.

ABSTRACT

Histological Studies: The biopsies for this portion of the study were classified into two groups dependent upon whether the gingival area had undergone pre-operative treatment or not. Whenever possible normal gingival tissue was obtained from the patient from whom the pathologic specimen was removed. Normal gingiva was also obtained from orthodontic patients requiring tooth extractions. Patient history, series of mouth x-rays, Kodachrome transparencies were recorded. All tissues were fixed in 10 per cent neutral formalin, and imbedded in paraffin. Serial sections, cut at 6 microns thickness, were stained with H and E, Masson Trichrome, PAS, and Mowry's modified reaction for mucopolysaccharides. Microscopic observations on inflamed gingiva indicated a marked PAS positive reaction in the cytoplasm of surface epithelium. The control sections gave no such positive reactions. Crevicular epithelium showed accentuated deposition of PAS stained material. The underlying connective tissue demonstrated decreased PAS reactivity. Diminution of iron binding material in the intercellular spaces of the stratum germinativum and the corium was noted. Histologically these tissues showed fewer fibrous elements, and a larger number of inflammatory infiltrates.

Chemical Analyses: Considerable difficulty was encountered in this phase of study. One of the major problems was to obtain a sample of sufficient size for analysis. The analytical methods utilized for the determination of hexosamine, hydroxyproline, and nitrogen were essentially those which have been developed for the analysis of skin and blood. These methods had to be modified and standardized for use in this study.

Patients, with periodontal disease where gingivectomy was indicated, yielded 80 samples. An average of 300 mg of tissue was used for duplicate analyses. Total hexosamine was determined by a modification of the Elson-Morgan method, hydroxyproline by the Martin-Axelrod technique, and total

nitrogen was estimated by the micro-method of Koch and McMeekin. The data was tabulated on the basis of age and sex of the patients. The results show that there was a trend toward an increase in each of the measured components with increasing age. The number of tissues completely analyzed is not large enough to permit the statement that these results are statistically significant. It appeared that the gingival tissue from male patients gave slightly higher values when compared to that from female patients. Some data were obtained from the analyses of normal gingival tissue, these are, as yet, too few to permit any valid comparisons.

The data also indicates an alteration in the collagen content with increasing age. This is in agreement with the histological observation of increased fibrosis in gingiva with aging. Whether this represents an increase in the soluble, neutral, or insoluble collagen remains for future investigations.

IMMUNOCHEMICAL STUDIES OF HUMAN SALIVA

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ASSISTED BY M. Solomon and F. Egan

TASK NO. NR 105-201

CONTRACT Nonr-266(63)

OBJECTIVES

(a) To identify, separate, and characterize the components in human saliva, and (b) to study the relation between variation in these substances and oral disease.

ABSTRACT

By using immunologic gel-diffusion methods, paper electrophoresis and other procedures, the major constituents of human parotid saliva were identified. These comprise serum proteins (albumin, and α -, β -, and γ -globulins) as well as intrinsic proteins and glycoproteins. The intrinsic proteins included amylase and an unidentified basic protein; the glycoproteins (accounting for 35% of the total protein) were cationic, contained fucose and hexosamines in a 1:1 ratio, hexoses, and little if any sialic acid. The application of column chromatography with several adsorbents to the separation and purification of these components was studied. Salivary amylase preparations with specific activities comparable to crystalline enzyme were obtained in this way, and observations were made of the chromatographic heterogeneity of this protein. Hydroxyapatites containing Sr or Ba were compared with Ca-apatite and proved useful in chromatography.

Comparison was made of parotid saliva from caries-free and caries-susceptible adults. Measurements were made of fucose, hexosamine, hexose, and protein, and electrophoretic analyses were made as well. The fucose and hexosamine concentrations in saliva from caries-free females was 3.04 and 3.63 mg/100 mg protein, respectively. This was significantly less than the concentration of these substances in saliva from caries-active females. Saliva from caries-active females, and from caries-active or caries-susceptible males contained similar amounts of fucose and hexosamine, viz. 4.09 and 5.04 mg/100 mg protein, respectively. The electrophoretic analyses did not show any differences among the several groups in the distribution or number of components.

In the course of extending our observations to include studies of the effect of latent virus infection with salivary gland virus on the composition of saliva, it was noted that herpes simplex virus infection of cultured Chinese hamster cells resulted in the production of a

carrier culture. Karyological studies of infected cells disclosed that chromosome aberrations similar to those produced by mutagenic agents could be seen following infection. These consisted of chromosome and chromatid breaks, accentuation of the secondary constrictions, and an increase in the proportion of cells showing translocations. These changes were restricted to the first cell division following infection even though virus was present during subsequent cell passages.

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PHOSPHORUS AND TOOTH DECAY

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ASSISTED BY Miss Norma J. Baker

TASK NO. NR NR 105-094

CONTRACT Nonr-1841 (33)

OBJECTIVES

(a) To determine whether the cariostatic effects of phosphates are related to the structure of the phosphate ion, (b) to determine the level at which phosphate salts can be added to diets without systemic side effects, and with optimal cariostatic properties and (c) to study the mechanism of phosphate action.

ABSTRACT

In a previous progress report (Naval Research Review, Feb. 1960), it was reported that: (1) doubling the total mineral content of an otherwise cariostatic diet by the use of a natural ash supplement reduced dental decay in hamsters by 60%; (2) a salt mixture prepared with 12 spectroscopically measurable elements to duplicate the natural ash, reduced caries by 95%; (3) this cariostatic action of the synthetic salt was completely lost when the phosphate compound was omitted; and (4) when metaphosphoric acid was used as a source of phosphorus and added as a supplement to an otherwise cariogenic diet, caries was significantly reduced in direct proportion to the amount of phosphate added.

Recently, an experiment was designed in order to estimate the level at which a phosphorus compound commonly used in foods, namely di-sodium-hydrogen phosphate, could be fed to rats without interfering with growth and development, and yet giving maximal caries resistance. The test diets contained 1.81, 3.62, and 5.43 per cent Na_2HPO_4 , which doubled, trebled and quadrupled, respectively, the phosphorus content of the control diet. After 100 days on these diets, it was found that the group fed a quadruple supplement did not grow well and had a comparatively high mortality. The dental caries reduction for the groups fed the double and treble phosphorus diets was 65 and 64 per cent, respectively, and both these groups showed about the same food consumption and body weight gain. It was concluded that double phosphorus diets produced optimal caries reduction with minimal metabolic disturbance.

The next experiment was designed to determine whether the cations (ortho, pyro, tripoly, trimeta or hexameta phosphate groups) differed in their cariostatic activity when fed at the double phosphorus level. The sodium anion was common to all these compounds, and the Na/P ratio of these diets was kept the same. After 104 days on these diets supplemented with different types of sodium phosphate, it was found that all produced significant reductions of caries in the rat, that all were nutritious, and that none interfered with food consumption, body weight gain and general health. The most cariostatic phosphate cation (78% caries reduction in comparison with the control) was sodium trimeta phosphate, which is a cyclic compound.

In a subsequent experiment with inbred caries-susceptible albino hamsters it was observed that they did not tolerate sodium trimeta phosphate when fed at the double-phosphate level in a nutritionally adequate diet. The apparent difference in the sensitivity of rats and hamsters toward phosphates deserves further study.

In an attempt to determine the mechanism of phosphate action, the relative cariostatic effects of monopotassium PO_4 imbedded and not imbedded in hydrofol glyceride have been studied. The imbedded phosphate was about 3% as soluble in water as the unimbedded phosphate. After 100 days on these test diets, it was found that the group fed the imbedded phosphate had 76% less caries than the control group, whereas the group with the unimbedded phosphorus showed a 51% reduction. This difference was significant statistically. Evidently the phosphate is more effective when the phosphate is more slowly released in the mouth, or more slowly absorbed from the gut. The mechanism could be speculated as being one or a combination of the following actions: (1) through direct local retention of phosphate on and around the teeth, (2) through local action of sustained higher levels of salivary phosphate or (3) through a better mineralized, more caries resistant tooth substance.

A gastric intubation study is now under way to determine the extent to which the phosphate effect is systemic. In another experiment the relative effectiveness of potassium vs sodium ions in phosphates is being established.

It will soon be possible for us to specify the best level and type of phosphate compound for producing optimum cariostasis in rats and hamsters.

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CEREBRAL REGULATION OF SALIVARY FUNCTION: REMOTE PERIPHERAL AFFERENT
INFLUENCE UPON IT: AND ITS PHYSIOLOGICAL SIGNIFICANCE

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ASSISTED BY J. F. Kell, Jr., and M. N. Carroll, Jr.

TASK NO. NR 105-228

CONTRACT Nonr-1134(03)

OBJECTIVES

To study (a) cortical and subcortical localization of the sympathetic and parasympathetic regulation of salivary function; (b) afferent and efferent salivary pathways from periphery of the body to the cortex and subcortical centers and relays to the glands; (c) nerve endings and specific afferent fibers subserving salivary mechanisms; (d) physiological significance of peripheral salivary mechanisms and appropriate physiological stimuli; (e) effects on salivation, and gingival and dental functions of repeated brain stimulation in chronic animals.

ABSTRACT

In flaxedilized cats under ether anesthesia, submandibular salivation has been evoked in response to electrical stimulation, with controlled parameters, of the central cut-end of the sciatic nerve, other spinal nerves, and the gastric branch of the vagus as well as cortical loci on the sigmoid gyrus and gyrus proreus and through bipolar electrodes, stereotaxically placed in the medial amygdaloid nucleus, the central gray, the central tegmental superior nucleus of von Bechterew and the ventromedial hypothalamic nucleus. The salivary responses have been examined in animals with solely sympathetic or parasympathetic innervation of the gland intact. From all tested loci, an average of 0.5 to 3.0 cc of secretion for 20 seconds duration was obtained. From all subcortical loci, watery salivary response was given with the exception of loci in the ventromedial hypothalamic nucleus which responded with viscid secretion. The most copious responses from the subcortical loci were obtained from the central tegmental superior superior nucleus of von Bechterew and the ventromedial hypothalamic nucleus. Generally, cortical loci gave more copious responses than subcortical loci. Although chlordiazepoxide, ethanol, mebutamate and reserpine exerted variable, selective effects upon a variety of autonomic responses to cortical and subcortical stimulation, none of these compounds altered centrally-evoked salivation. For parasympathetic as well as sympathetic responses, the ascending pathway in the cord runs in the dorsal part of the lateral funiculus as shown both by microelectrode cord stimulation and partial cordotomy experiments. Although the afferent spinal salivary pathway overlaps the posterior spinocerebellar tract, ablation of the cerebellum does not interfere with the salivary responses. Although the sciatic salivary response (in sympathetic preparations) is still present after superior collicular decerebration, it is abolished in acute animals by complete upper cervical cordotomy. This suggests a long-circuited pathway through the brain-stem somewhere below the superior collicular level. However, a tegmental activation of sympathetic salivary responses may have been masked by autonomic spinal shock. The excitable cortical and

subcortical areas overlap those yielding vasomotor, endocrine and other autonomic responses but specific selective representation of particular autonomic responses is confirmed.

The descending pathway for sympathetic salivary responses traverses the cervical level of the spinal cord in a region restricted to the junction of the dorsal and ventral parts of the lateral funiculus. Through this region also run descending vasomotor fibers. This is the final common pathway in the cord for sympathetic salivary effects, both from the cortex, subcortical regions and from the sciatic nerves.

Whereas salivation may occur as a part of more generalized sympathetic or parasympathetic responses to cortical or subcortical stimulation, it nevertheless may occur as the sole autonomic response. This indicates that a massive cerebrogenic autonomic response is not the invariable rule but that highly selective control is possible.

THE COMPOSITION OF CALCIFIED TISSUES

William H. Horner - Georgetown University
Robert VanReen - Naval Medical Research Institute

TASK NO. NR 105-050

CONTRACT Nonr-1531(00)

OBJECTIVES

a) To study the intermediary metabolism of carbohydrates in calcified tissues; (b) to clarify the mechanism by which citric acid accumulates in bone; (c) to study the formation and metabolism of lactic acid in bone and the influence of parathormone on these relations.

ABSTRACT

One of the fascinating aspects of the normal growth and development of calcified tissues is related to the question of how such tissues form and resorb the apatite structure and reform new calcified areas. It has been proposed by some investigators that materials are formed by bone cells which cause a dissolution of apatite. A number of naturally occurring biochemical substances are known which could perform such a task. Citric acid, for example, has a high formation constant with calcium ($\log K_f$ for calcium citrate is 3.15). Calcium is also phosphatophilic with a value for the $\log K_f$ of 3.58 for calcium adenosinetriphosphate, however, the affinity of calcium for phosphorus compounds is quite nonspecific.

Evidence to support the importance of citric acid in calcium mobilization has come from studies in which a parathyroid extract was shown to stimulate the release of citric acid from calcified tissues as well as causing the release of calcium.

Other studies have demonstrated that bone preparations can form lactic acid and that its production can be stimulated by parathyroid extracts. The values for lactic acid production which appear in the literature may not actually be representative of the maximal ability of bone to form this acid for several reasons: (1) the effect of cofactors has not been studied in calcified tissues; (2) the stability of the various enzymes which mediate the conversion of glucose to lactic acid has not been studied in calcified tissues. Experiments in this laboratory indicate that there is much more rapid formation of lactic acid from fructose 1,6-diphosphate than from free glucose by rabbit femur preparations. This may be due to the lability of the early steps of the glycolytic pathway. Studies are underway to explore this subject.

Some of the enzymes of the tricarboxylic acid system have been demonstrated in calcified tissues. The enzymes which form and degrade citric acid, namely, citrogenase, aconitase, and isocitric

dehydrogenase have been shown in rabbit femur preparations, thus the accumulation of citrate is not due to the absence of these enzymes. We have recently found that injections of a parathyroid hormone extract does not influence the activity of femoral aconitase or isocitric dehydrogenase. This is contrary to the findings of other investigators and will have to be clarified. Thus, the current status is that parathyroid hormone extracts affect the level of citric acid in bone but, in our hands, this is not due to a direct reduction in the capacity to metabolize citrate in vitro. Current studies are directed toward the possible effect of the hormone on the level or availability of TPN or DPN.

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A STUDY OF THE OSSEOUS HEALING OF THE POST-EXTRACTION
ALVEOLUS UTILIZING TETRACYCLINE INDUCED FLUORESCENCE

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ASSISTED BY P. J. Boyne

TASK NO. NR 105-212

CONTRACT Nonr-2836(00)

OBJECTIVES

To develop a microscopic technic of observation of chronologically oriented tetracycline induced fluorescence in extraction wound healing in the dog, and through this technic to investigate the host acceptance of various bone implants

ABSTRACT

In order to study the chronologic nature of osseous healing of extraction wounds, and the effect of implantation of bone substitutes in the post-extraction site, tetracycline was given at intervals of from 3 to 28 days after surgery in 7 mongrel dogs. Specimens were obtained from 3 to 12 weeks post-operatively. Ground undecalcified sections were studied under ultra-violet light, and identical slides were examined by routine histologic methods, and by microradiographic technics. Each dose of tetracycline was found to have produced a lineal yellow pattern of fluorescence in the host bone. These patterns were found to have assumed a characteristic arrangement in the area of each of the following types of implants used: 1. Ethylenediamine treated, and 2. boiled-defatted heterogenous bone, 3. Freeze-dried homogenous, and 4. freeze-dried heterogenous bone and 5. autogenous bone. The fluorescent patterns correlated with initial host acceptance, and rates of resorption of the implant. Extra-alveolar bone formation in areas remote from the healing extraction sockets were also observed. These areas occurred: (a) overlying the mandibular canal, (b) subperiosteally along the alveolar ridge, and (c) in the maxillary sinus floor. The significance of the extra-alveolar repair phenomena is being investigated.

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(b) Use of Tetracycline Fluorescence Microscopy in the Study of Bone Repair. Report to NAS-NRC, 26 May 1961, Washington, D. C.

THE COMPARABLE ACCEPTANCE OF BOILED DEFATTED BONE HETEROGRAFTS
FROM COW, PIG, AND SHEEP IMPLANTED IN RATS

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ASSISTED BY J. T. Nicholson

TASK NO. NR NR 105-212

CONTRACT Nonr-2836(00)

OBJECTIVES

(a) To study the comparative usefulness of boiled, defatted bone from the cow, pig, and sheep as heterografts, and (b) to study the incidence of immunological reactions to a second boiled, defatted transplant made from the various animals.

ABSTRACT

Bovine bone has been more widely investigated in the use of heterografts than any other animal bone. The present study, using boiled defatted bone from indifferent animals, demonstrated that bone from pig and sheep also may be used for heterografts after proper processing. Animal acceptance of the heterografts was compared to a similarly prepared homograft of rat bone by implantation of bone chips into mechanical defects created in the skulls of rats. Animals with an open defect, not treated with any bone chips, served as the ultimate control.

Two operations on each animal were performed fifteen days apart with the same treatments and the same types of graft material. The animals were weighed and checked daily; differential lymphocyte counts were done at the time of each operation and at sacrifice. Antigen-antibody reactions were presumed to increase the operating risk in the second operation. This did not prove to be a serious threat. Rejections after the second operation were no more numerous than they had been after the first operation. Infection appeared to be the major operating risk in both operations.

Clinical and histologic evidence seems to demonstrate that boiled defatted bone is accepted by the host as an inert filling material regardless of the species from which it was derived. Boiling and defatting of bone is successful in destroying the species difference causing rejection of heterografts in the fresh state, but this process also destroys osteogenic potential and makes resorption of the graft difficult. Bone chips used in this investigation appear to be useful in facilitating the formation and strengthening of bone and connective tissue lattice work over an osseous defect. The bone is easy and inexpensive to prepare and seems to fulfill some of the requirements of an emergency bone material needed urgently in the time of a national disaster.

A SYSTEM FOR THE DEFINED STUDY OF MIXED CULTURES

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ASSISTED BY K. Graap, S. Berglund and B. L. Simonsen

TASK NO. NR 105-224

CONTRACT Nonr-3130(00)

OBJECTIVES

a) To develop a technique for the controlled study of mixed cultures and b) to apply this procedure in a study of the interactions between two or more bacterial species and with special reference to the metabolic products.

ABSTRACT

Most of the literature on this subject deals with results obtained in closed systems, such as test tubes, Petri dishes, etc., where the uncontrolled collection of metabolites interferes with growth or survival in tubes or vessels and growth on plates can be interpreted only as inhibited or stimulated. In practically all cases the cultural conditions are far from the natural environment.

Our approach has been to adapt continuous culture methods, especially that of the "chemostat", in which an organism can be maintained in a steady state under precisely controlled conditions, the viable numbers determined, rate of growth established and metabolites measured qualitatively or quantitatively.

To date we have developed a multiple stage system in which selected pairs of bacteria are cultured in defined media in their respective "chemostats" where they can be held at any phase of the growth curve. These are then fed into a common cell or "chemostat" in which unrestricted growth occurs. The results indicate that this technique can show interactions so subtle to be measured in no other way; that is, the effect on generation time, a term which has accepted meaning. Thus, Veillonella alcalescens and Streptococcus salivarius mutually restrict each other's growth rate when compared with the generation times of identically treated pure cultures. Because of the design of the culture system, these inhibitions do not appear to be caused by depletion of nutrients or physical factors such as pH. In contrast, there are unilateral interactions between Staphylococcus aureus growing either with V. alcalescens or Strep. salivarius: V. alcalescens dramatically prolongs the generation time of S. aureus to a point assuring its complete elimination from the system. S. salivarius also inhibits the generation time of S. aureus but not to the extent of V. alcalescens. Conversely, S. aureus does not alter the generation times of the other two species.

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(a) R. B. Parker and M. L. Snyder (1962) "Interactions of the Oral Microbiota I. A system for the defined study of mixed cultures." Proc. Exper. Biol. & Med., In Press.

(b) R. B. Parker and M. L. Snyder (1962) "Interactions of *Streptococcus salivarius*, *Staphylococcus aureus* and *Veillonella alcalescens*" Abstract for Proceedings, Soc. Am. Microbiol.

A STUDY OF DENTAL MATERIALS

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TASK NO. NR 105-360

CONTRACT N6onr-232(08)

OBJECTIVES

To cover research on the development of the best possible combination of materials and techniques for the production of (a) dental gold inlay castings and (b) partial and complete dentures.

ABSTRACT

Since this project has been in effect, a considerable amount of research has been accomplished in different phases of restorative dentistry. The different areas in which this research has been carried out are as follows: 1) dental amalgam, 2) complete dentures, 3) properties of gold wire, 4) casting gold inlays, 5) removable partial denture materials, 6) impression materials, 7) resins for dentures, 8) gypsum materials, 9) investments and hygroscopic expansion, 10) microstructure of cobalt-base alloys, 11) analysis of human teeth. A total of twenty scientific reports have been published during these years, with four others in the process of being published. From the studies on chromium-cobalt alloys, the A.D.A. Research Staff at the National Bureau of Standards was able to adapt the test procedures to the development of an A.D.A. Specification No. 14 for this type of alloy. An accurate and reproducible technic known as the "controlled water added technic of casting gold inlays" was developed from studies completed under this contract. Numerous other benefits have occurred, also through modifications and refinement of materials, as well as the training of research personnel.

Currently a study of the effects of different investments on the sulfide contamination of the embedded metals during the casting procedure was studied. Also, the bond strength of new "ceramco" porcelain to "ceramco" gold was studied. In this study it was shown that by different methods and procedures of producing porcelain fused to gold, the bond strength of the gold may increase up to 33%. Samples subjected to an oxidation of the metal for twenty minutes before fusing of the porcelain showed an increase in the bond strength of from 9.5 to 33 per cent, depending upon variables in procedure.

The casting of a three unit bridge in one piece is another study which was carried on during this period. By employing controlled water added casting technic for the investment procedure and using modified waxing procedures, it is possible to cast an accurate bridge in one piece, which eliminates the soldering operation.

During this current period, two technical papers were published. (1) Effect of casting conditions in some mechanical properties of cobalt-base alloys, and (2) Effect of microstructure on the physical properties of cobalt-base alloys.

The immediate plans are to carry on additional work on the effect of different investments on the sulfide contamination of the embedded metals during the casting procedure.

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(b) L. W. Suffert and D. B. Mahler (1955), "Reproducibility of gold castings made by present day dental casting technics". J.A.D.A., 50:1-6.

(c) F. A. Peyton, D. B. Mahler, and K. Asgar (1956), "Controlled water addition technic for hygroscopic expansion of dental casting investment". J.A.D.A., 52:155-161.

(d) K. Asgar (1956), "Chemical analysis of human teeth". J. Dent. Res., 35:742-748.

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(g) K. Asgar and F. A. Peyton (1959), "Pits on inner surfaces of cast gold crowns". J. Pros. Dent., 9:448-456.

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(i) K. Asgar and F. A. Peyton (1961), "Effect of casting conditions on some mechanical properties of cobalt-base alloys". J. Dent. Res., 40:73.

DEVELOPMENT OF AN ARTIFICIAL MOUTH FOR IN VITRO STUDIES
OF DENTAL CARIES

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ASSISTED BY H. Cueto and D. Baugh

TASK NO. NR 105-017

CONTRACT Nonr-3139(00)

OBJECTIVES

(a) The development of the Artificial Mouth and its application to the study of problems of the oral cavity, (b) the development of agents which will reduce dental caries and calculus formation, (c) the study of the nature of the difference in the resistance of teeth to decay and of protective factors at the tooth surface.

ABSTRACT

In the early work on this project, an apparatus was developed, called the Artificial Mouth, which produced decay in extracted human teeth. By a number of methods, the decay was shown to simulate closely that which occurs naturally in the human mouth. The concentration of glucose in the culture medium ('artificial saliva') was found highly important in influencing the type of attack. At concentrations of about 150 mg. per 100 ml., decalcification of enamel and attack of dentinal matrix occurred. At higher concentrations, only decalcification occurred, and at lower concentrations, only decalcified dentinal matrix was affected. Using pure strains of oral microorganisms, many types were shown to cause in vitro decay.

The procedure was then quantitated by using microhardness measurements to follow the rate of decay. The method showed that fluorides, applied topically or as dentifrice ingredients, slowed down the rate of softening. A comparison of the effect of common sugars in the nutrient medium showed that all caused similar rates of decay, with the probable exception of maltose. Soluble starch had no softening effect, but amylases were not present and hence the conditions did not correspond to oral conditions.

The use of hardness measurements provided a sensitive method of showing and measuring changes in the surface of the enamel. By applying solutions of calcium phosphates to softened surfaces, rehardening of the surfaces occurred. The presence of small amounts of fluoride (ca. one p.p.m.) accelerated the rate of rehardening which occurred in a matter of several hours. Methods of utilizing this effect for the protection of teeth are currently under study.

CURRENT REPORTS AND PUBLICATIONS

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- (b) E. Newbrun and W. Pigman (1960), "The hardness of enamel and dentine." Australian Dental Journal, 5, 210
- (c) T. Koulourides and W. Pigman (1960), "Studies on rehardening of artificially softened enamel." J. Dental Res., 39, 740
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- (g) T. Koulourides, H. Cueto, J. Reed, M.S. Kim and W. Pigman (1961), "Studies on rehardening of artificially softened enamel." J. Dental Res., 40, 698. Abstract.
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The Relationship Between Salivary Glands
and the Utilization of Foods

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ASSISTED BY

Charles Ligon and James Dumas

TASK NO. NR 105-176

CONTRACT Nonr-908(11)

OBJECTIVES

Preliminary experiments performed by us suggest that less carbohydrate is retained in the liver in animals without their salivary glands. These data will be extended in order to determine what salivary gland contributes to this effect, what carbohydrates are affective, and if protein is similarly affected. The effect of food utilization will also be studied in animals whose salivary gland physiology is modified by the administration of I^{131} and/or the addition of propylthiouracil. In addition, salivary gland extracts will be used to determine if the food utilization pattern can be brought back to normal.

ABSTRACT

As reported in our annual progress report of January 30, 1961 we planned to begin work concerning the relationship between glycogen retention in animals having various levels of salivary function.

Preliminary work on this aspect of our grant has involved the perfection of analytical methods for estimating the utilization of different carbohydrates following their ingestion under standard conditions in animals with either normal or varying degrees of salivary gland dysfunction. Carbohydrate availability is being estimated by measuring blood glucose, liver glycogen, and glucose tolerance values at various intervals following the ingestion of different carbohydrates. Blood glucose is determined by the method of Folin and Malros (J.Biol.Chem, 83: 115, 1929) and liver glycogen by the modified method of Somogyi.

A preliminary animal study was conducted to ascertain the reliability of our analytical methods. This consisted of eighty animals divided into two groups, one of which was a control and the other salivaryadenectomized. Each animal received eight grams of a standard diet for intubation (Scow) daily by means of a stomach tube for thirty days, after which the animals were sacrificed by ether inhalation. Blood glucose and liver glycogen was measured.

The results obtained were somewhat obscure because the glucose level was measured after starving the animals for twenty-four hours, instead of making a tolerance curve by giving known amounts of glucose to the animals before taking the blood samples. Also, one-half of the animals died during the study from the tubing procedure since the animals were so young at the beginning of the study. This first study, however, provided considerable information in regard to the technique required.

Preliminary studies designed to measure the effect of desalivation on glycogen retention in the livers of rats was conducted by using two

groups of animals, one of which had their major salivary ducts ligated (Group A) and the other had a sham operation performed (Group B) and served as controls. The data obtained is found in Tables 1 and 2.

Another study was conducted in which weanling male rats were used. Through surgical techniques, all the salivary ducts on 16 of the animals were ligated. This group will be referred to as Group 1. Group 2, also consisting of 16 animals, were sham or mock operated in order to induce the trauma of the operation without performing the actual ligation. Animals from Groups 1 and 2 were then paired according to weight and paired for a period of twenty days; that is, each pair consumed qualitatively and quantitatively the same food during this period.

Each of the two main groups was divided into 4 sub-groups:

- Sub-group A - fed stock corn diet
- B - fed 20 per cent sucrose diet
- C - fed 50 per cent sucrose diet
- D - fed 80 per cent sucrose diet

The purpose of the 4 sub-groups was to check the effect of sucrose intake variation upon glycogen synthesis in addition to the effect of ligation. Each day, during the experiment, the animals were weighed and the daily food consumption carefully determined.

A preliminary experiment was conducted on six adult male rats to try to ascertain whether sucrose concentration in the diet affects the extent of glycogenesis. These were divided into three groups of two with Group A receiving 30% sucrose, Group B receiving 65% sucrose, and Group C receiving 80% sucrose. The feeding was extended over a period of 30 days. Considering the small number of animals involved in the preliminary test, any resulting experimental data would, at best, be questionable. Primarily this experiment served to sharpen techniques in the glycogen determination and to check the accuracy of the method.

The cumulative data is found in Table 2. Only half of the animals in each group were analyzed since it is intended to extend the experiment for an additional ten days at which time the remaining half will be analyzed in a similar manner.

The data plainly indicates that the two groups followed the predicted course; that is, the operated group failed to gain weight as rapidly as the control.

Glycogen level appears to be of higher value among the ligated animals. This is in contrast to previous supposition; however, the data cannot be considered valid since the method of determination employed is not adaptable to such low concentrations. Extrapolation was necessary at values less than 8.2 milligrams per gram of liver.

The level of sucrose intake appears to be insignificant in glycogen synthesis. This observation is in agreement with the results from the analyses of the preliminary experiment which was mentioned.* This seems reasonable in that beyond a certain maximum glycogen level, ingested carbohydrates would preferentially enter the pathway of fat metabolism.

This does not explain the negative values within the 80 per cent-sucrose-group, although, again, the low values negate any definite conclusions. The data show the glycogen level to be highest in the corn-fed group. These animals were also in the best physical condition throughout the feeding period. Those fed on high sucrose diets suffered a downhill course and appeared grossly undernourished at the time they were sacrificed, the reason most likely being due to the very low protein intake.**

There are two main causes for the low glycogen values in the results:

1. The animals were starved for one day before they were sacrificed. It is estimated that the liver glycogen content in a fed animal is approximately 10 per cent on a wet-weight basis; for starved animals, values may run as low as .2 per cent or even zero.
2. The small aliquot of hydrolyzed glycogen that was used in the final titration. The aliquot volume was erroneously chosen as that suggested for fed animals.

The experimental results, though generally inconsequential, have uncovered some potentially interesting points. It is hoped that, with betterment of technique and elimination of errors such as those mentioned above, this study may be exploited on a larger scale with more significant results.

Another study was conducted using 40 young male rats approximately fifty days of age. These animals were divided by body weight into four groups of ten animals each. Those animals in Group 1 served as controls, those in Group 2 received a salivary gland extract (Parotin) daily, and the animals in Group 3 and 4 were salivaryadenectomized. The animals in Group 3 received a salivary gland extract daily (Parotin) while those in Group 4 received no additional supplement. All animals were housed in pairs and received distilled water ad libitum. In addition all food consumption was carefully controlled by use of tube feeding procedure with all animals receiving equal amounts at 12-hour intervals throughout the thirty day duration of the study. At this time, blood samples were collected by cardiac puncture, the animals were sacrificed by ether inhalation, and the livers removed and immediately frozen with dry ice and acetone. The results of the analyses are shown below:

Group	Mean final animal wt.(gm)	Blood Glucose (mg%)	Liver Glycogen (mg%)
1	161	910 $\pm$ 40	760 $\pm$ 40
2	159	870 $\pm$ 50	670 $\pm$ 30
3	155	950 $\pm$ 20	760 $\pm$ 30
4	157	980 $\pm$ 30	850 $\pm$ 40

It is obvious that the length of time following desalivation is an important variable to consider when attempting to demonstrate any possible relationship between the salivary glands and food utilization. This will be considered in our future studies. The amount of carbohydrates and its clinical nature will be similarly evaluated.

A follow-up study is now in progress. This is a ninety-day study using 120 rats. These are divided into three groups; desalivated, sham operated, and control. At thirty day intervals a glucose tolerance curve will be measured on all three groups. Blood phosphorus will also be measured in this study, as well as liver glycogen.

At the end of the study (120 days), all animals will be sacrificed and liver glycogen, blood glucose and phosphorus estimated. In the present study the animals are being fed a stock diet (corn) from feeding dishes instead of being tube-fed. This has decreased the mortality considerably; no animals have been lost.

Future plans involve the selective desalivation of rats and the use of the same analytical procedures in order to determine what salivary glands are most prominent in affecting "food" utilization. We also plan to test different carbohydrates, amino acids, and proteins to see if nitrogen metabolism is affected. We will also evaluate our own salivary gland extract in affecting "food" utilization, as well as to see if thyroid dysfunction affect salivary function sufficiently to modify "food" utilization.

* The specific results of this experiment have been omitted from this report. The results did show, however, no correlation between glycogen level and glucose intake. For example, the highest level and lowest level were displayed by animals on identical diets.

** Higher carbohydrate diets were prepared at the expense of protein. In all cases, vitamin, mineral, and fat concentrations were identical.

TABLE I

	Initial Weight (gms)	Final Weight (gms)	% Weight Gain	Total Food Consumed (gms)	Liver Sample Weight (gms)	Mg. Glycogen in sample	Mg. Glycogen per Gm. Liver
1A lig.	142	203	42.8	259	1.02	114.0	111.8
1B cont.	143	214	29.6	259	1.02	105.0	102.9
2A	145						
2B	145						
3A	123	170	38.2	253	1.03	69.0	67.0
3B	122	192	57.4	254	1.00	97.5	97.5
4A							
4B							
5A	134	182	35.8	256	1.01	7.5*	7.4
5B	134	193	44.0	256	1.00	64.0*	64.0
6A	147	209	42.2	268	1.04	3.0*	2.9
6B	153	192	25.5	268	1.02	38.0*	37.3
7A	137	174	26.1	232	1.02	10.0*	9.8
7B	138	186	34.7	234	1.01	2.0*	2.0
8A	137						
8B	137						
9A	132	206	56.0	246	1.02	48.0*	47.1
9B	145	205	41.4	246	1.01	21.0*	20.8
10A	134	166	23.8	254	1.00	69.0	69.0
10B	135	201	48.9	255	1.00	135.0	135.0
11A	126	140	11.1	203	1.02	neg.*	neg.
11B	128	161	25.7	203	1.01	16.0*	15.8
12A	126	185	46.8	251	1.00	neg.*	neg.
12B	131	191	45.8	250	1.00	24.0*	24.0
13A	139	186	33.8	271	1.03	70.5	68.4
13B	139	208	49.6	271	1.02	99.0	97.1
14A	140	199	42.1	261	1.03	106.6	103.4
14B	139	207	48.9	261	1.01	82.5	81.7
15A	124	148	19.3	224	1.02	90.0	88.2
15B	127	180	41.7	225	1.03	122.0	118.4
16A	141	176	24.8	256	1.01	54.0	53.5
16B	140	196	40.0	256	1.01	67.5	66.3
17A						12.0	11.7
17B						42.0	41.6

TABLE II

	Food Consumed (gm)	Weight Gain	% Gain	Mg. Glycogen Per 1 gm. Liver
Group A - (Corn Diet)				
Lig.1	76	16.0	17.8	10.62
Con.1	76	17.5	18.8	7.85
Lig.2	61.5	3.5	4.9	30.74
Con.2	60.5	7.5	10.2	22.01
Lig.3				
Con.3				
Lig.4				
Con.4				
Cumulative Average Group A				
Lig.	68.7	9.75	11.35	20.68
Con.	68.2	12.50	14.50	14.93
Group B - (20% Sucrose)				
Lig.1	76	22	26.2	12.52
Con.1	76	28.5	33.9	7.94
Lig.2	76	16	16.5	7.99
Con.2	76	29	30.2	0
Lig.3				
Con.3				
Lig.4				
Con.4				
Cumulative Average Group B				
Lig.	76	19	21.35	10.25
Con.	76	28.75	32.05	3.97
Group C - (50% Sucrose)				
Lig.1	76	14.0		8.25
Con.1	76	33.5		0
Lig.2	76	28.5	33.6	-6.77
Con.2	76	44.5	50.6	-8.20
Lig.3				
Con.3				
Lig.4				
Con.4				
Cumulative Average Group C				
Lig.	76	21.25	24.6	7.51
Con.	76	39.0	43.9	4.10
Group D - (80% Sucrose)				
Lig.1	62.5	3.5	4.9	0
Con.1	61.5	4.0	5.2	0
Lig.2	68.5	1.5	2.1	0
Con.2	68.5	9.0	11.7	0
Lig.3				
Con.3				
Lig.4				
Con.4				
Cumulative Average Group D				
Lig.	65.5	2.5	3.5	7.91
Con.	65.0	6.5	8.45	5.31

CHEMICAL STUDIES ON CONNECTIVE TISSUE RELATING
TO PERIODONTAL DISEASE

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TASK NO. NR 105-251

CONTRACT Nonr-3266(00)

OBJECTIVES

(a) To study the biochemical changes in connective tissue of normal and lathyrictic animals as an approach to the study of the degenerative diseases of connective tissue as may be involved in periodontal disease, (b) in this connection, to study the synthesis of soluble and insoluble collagen in normal and lathyrictic chick embryos, and (c) to study the metabolism of mucopolysaccharides in normal and lathyrictic chick embryos.

ABSTRACT

It has been known for some time that saline solutions of varying ionic strengths are capable of extracting collagenous fractions from connective tissues. It is now generally accepted that the metabolic precursor of the insoluble collagen fibril is present in these fractions. The demonstration that in experimental lathyrism large amounts of saline-extractable collagen are formed raises the question as to the metabolic source of this material and its relation to the normal pathways of collagen fibril biosynthesis. This is a matter of some practical significance, since it bears on the processes of connective tissue formation and degradation in normal and pathologic states.

In attempts to define the biochemical defect(s) responsible for the extensive tissue fragility of animals given lathyrogenic compounds, many compounds have been tested for such activity. The assay system employed was the 14-day chick embryo, in which a single dose of an active compound will produce extensive tissue fragility and large amounts of soluble collagen in forty-eight hours. In this system, a considerable number of hydrazides, all with a free hydrazine group, have been shown to be active. The activity of these hydrazides is not associated with pyridoxine metabolism.

To investigate the source of the excessive amounts of soluble collagen produced in lathyrism, radioactive proline was administered to normal and lathyrictic chick embryos, and the soluble and insoluble collagen fractions of skin and bone were examined for their radioactive hydroxyproline content. On the basis of specific activity and total activity of the hydroxyproline, it is concluded that the soluble collagen in lathyrism is largely newly synthesized and that the rate of its synthesis is considerably accelerated above normal. It is suspected that in lathyrism, there is interference in the conversion of soluble to insoluble collagen.

Injection of radioactive inorganic sulfate into chick embryos previously made lathyrctic by the injection of aminoacetonitrile results in significantly less uptake of the label in the long bones of these animals as compared with that in the bones of normal animals. Radioactivity determinations on the crude mucopolysaccharides resulting from digestion of the bones with papain, dialysis, and alcohol precipitation disclose that the differences in activity are located in this fraction. Paper electrophoresis results in the isolation of a metachromatic material with the mobility of chondroitin sulfate in which the radioactivity resides. The reduction in sulfate uptake appears during the first six hours after the lathyrogen is given. These results point to a relation between sulfated mucopolysaccharide synthesis and collagen deposition in the chick embryo.

CURRENT REPORTS AND PUBLICATIONS

(a) D. J. Smith (1961), "Production of lathyrism in chick embryos by hydrazides." Fed. Proc., 20, 161

(b) D. J. Smith and J. B. Tortorella (1962), "Incorporation of radioactive sulfate by bone of normal and lathyrctic chick embryos." Fed Proc., in press.

MECHANISMS OF CALCIFICATION AND RELATED PROBLEMS

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TASK NO. NR 105-025

CONTRACT Nonr 987-(01)

OBJECTIVES

(a) To gain insight of the nature of strontium deposition in bones and teeth, (b) to study inhibitors of bone crystal growth, (c) to explore the role of carbonate and carbonic anhydrase in calcification, (d) the role of organic matrix in calcification, (e) the influence of chondroitin sulfate on bone repair.

ABSTRACT

a) Our studies, using rachitic rat cartilage, indicate that in solutions of calcium and phosphate, mineralization takes place with relative ease. In solutions of strontium and phosphate, mineralization is difficult to achieve; if calcium ion is added to the solutions, the formation of strontium phosphate nuclei (mineralization) is inhibited. But if strontium phosphate nuclei are produced before calcium ion is added, then the crystal growth of strontium phosphate is not inhibited.

In another study it was shown that prior feeding of stable strontium reduces the retention of radio-active strontium by 76%.

b) A brief shaking of $\text{Ca}_3(\text{PO}_4)_2$ with little as 1 part of beryllium in 100 million parts of water inhibited subsequent crystal growth; shaking in 1 part of polyphosphates in 10 million parts of water partially inhibits crystal growth of $\text{Ca}_3(\text{PO}_4)_2$. These observations therefore provide models for cessation of crystal growth by surface inhibitors.

c) It was shown that in vitro calcification will not take place in an almost CO_2 free barbiturate buffer system. When carbonic anhydrase was added to the system, in vitro calcification occurred. Increased calcification in vitro was obtained in the presence of carbonate, also when carbonic anhydrase was added to the system. When carbonic anhydrase was inactivated with sulfanilamide or by heating, there was no increase of calcification in vitro. These findings imply that carbonic anhydrase may have a role in the calcification process.

d) Remineralization studies with bones and teeth indicate that the organic matrix of bone and dentine has the innate capacity to induce nucleation at specific sites.

e) An accelerated rate of osteogenesis was observed when chondroitin sulfate-treated demineralized bone was implanted into the trephined skull of rats. This was evidenced by complete repair of the defect in six weeks in contrast to nine weeks which were required for complete healing when untreated demineralized bone was used. In addition, acid-soluble collagen precipitated with chondroitin sulfate was more effective in the healing of the bony defect than acid-soluble collagen precipitated with NaCl. But no materials used were as effective as demineralized bone treated with chondroitin sulfate in causing the rapid repair of bone. (About 600 rats were employed in these studies.)

The possibility exists that chondroitin sulfate, or a complex of chondroitin sulfate with a protein has, in these instances, acted as an induction factor for the formation of the new bone necessary to repair the bony defect.

The studies of B.S. Kasavina (Institute of Traumatology and Orthopedy, Ministry of Health, Moscow, USSR) imply that this effect of chondroitin sulfate in accelerating healing may not be limited to bone.

CURRENT REPORTS AND PUBLICATIONS

(a) A. E. Sobel, P. A. Laurence and S. Nobel (1961), "Nuclei formation and crystal growth in mineralizing tissues". J. Indiana State Dent. Assoc., 40, 47-58

(b) A. E. Sobel, and G. R. Goldberg (1961), "Crystal dissolution in mineralizing tissues". Fed. Proc., 20, 77

(c) S. H. Cohn, S. Nobel and A. E. Sobel (1961), "Diet-induced changes in the exchange and accretion of radio-strontium by rat skeleton". Radiation Res., 15, 59-69

(d) A. E. Sobel (1961), "Remineralization of bones and teeth". Intl. Dent. J., 11, 363

(e) S. Nobel and A. E. Sobel (1961), "Dynamics of nucleation in bone". Clin. Chem., 17, 377

(f) A. E. Sobel and G. R. Goldberg (1961), "Crystal Dissolution in Mineralizing Tissues". Clin. Chem. 7, 377

(g) B. S. Sherman and A. E. Sobel (1961), "Mineralization of various collagens using bicarbonate or barbiturate buffer systems". Clin. Chem. 7, 378

THE PRODUCTION OF CLINICAL RADIOGRAPHS BY MEANS OF COMPACT,
LOW-ENERGY AND HIGH-INTENSITY RADIOACTIVE SOURCES

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TASK NO. NR 105-185

CONTRACT Nonr-495(17)

OBJECTIVES

(a) To study the gamma and x-ray spectra of radioactive nuclei which emit radiations primarily between 25 and 250 kev, (b) to study the radiographic and roentgenographic effectiveness of the radiations, (c) to search for new radioactive nuclei which possess the characteristics sought for in (a) above, and (d) to fabricate a small safe portable container for selected radioactive material which can be made into essentially a point source of very high specific activity.

ABSTRACT

Forty-seven radioactive nuclides have been experimentally examined and evaluated for the essential combination of availability, ease of production, suitability of emission spectrum, specific activity and half-life.

New or modified energy level diagrams of 19 of the 47 nuclides have been deduced from data obtained by using scintillation crystals, multi-channel pulse height analyzers, and coincidence circuits.

The existence of Tb 157, Tb 158, Tm 162, Tm 164, Yb 165, Lu 168, Ir 195 were unknown prior to this investigation.

The availability criterion is best met by the radioactive nuclides Tm 170, Ce 144, Eu 155, and W 181, since they may be purchased as stock items from The Oak Ridge National Laboratory.

Of the 47 radionuclides studied Yb 169 is the most easily produced nuclide because its precursor, Yb 168, has a very large neutron cross section, 11000 barns.

Radiations most useful for isotopic radiography are emitted by Cs 131, Gd 153 and W 181. The range of energies is between 29.8 and 136 kev.

Cs 131, Eu 155, Ce 144, Yb 169 and Hf 175 are rated best for high specific activity criterion.

The radionuclides which have the most desirable half-lives are W 181(140d), Gd 153(236d), Ce 144(290d) and Dy 159(134d). Since the intensity of the radiations from a radioactive sample is roughly inversely proportional to the half-life, nuclides having very long half-lives are therefore excluded.

Although no one nuclide possesses first position on each of the above five criteria, nevertheless there are currently ten nuclides (Cs 131, Ce 144, Sm 145, Gd 153, Eu 155, Dy 159, Yb 169, Tm 170, Hf 175 and W 181) that reasonably satisfy the criteria.

Roentgenograms produced by radiations from a conventional x-ray machine operated at various kilovoltages and filter combinations, and radiographs produced by radiations from Cs 131, Ce 144, Sm 145, Hf 175, and Yb 169 were compared for their potential diagnostic information capabilities. This comparison was carried out objectively by making densitometric measurements of aluminum penetrometer steps. The radiographs produced by radiations from the nuclides Hf 175 and Yb 169 were found to be nearly identical to the roentgenograms produced by radiations from a dental x-ray machine whose kilovoltage dial settings were between 70 and 90 and whose x-ray beam was filtered by 0.375 mm Cu and 0.50 mm of Al. Radiographs produced by the radiations of Sm 145 were found to be similar to roentgenograms produced by radiations from an x-ray machine whose kilovoltage dial setting was 70 and whose x-ray beam was filtered by 0.50 mm Al.

Radiations from the nuclides Ce 144, Hf 175 and Yb 169 were used to produce radiographs of skulls. These radiographs were characterized by good detail not only in the dense petrous portion of the temporal bone but also in the region of the thin ascending ramus of the mandible.

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(a) "Radioactive Decay of Yb 165, Tm 165, and Er 165," Bull. Am. Phys. Soc. 6, 238 (1961).

(b) "Methods of Gamma Spectroscopy: Identification of Yb 165; and Energy Levels of Er 165," Bull. Am. Phys. Soc. 6, 471 (1961).

OTHER CONTRACTS

Contracts, for which abstracts were not received in time for inclusion in this volume, are as follows:

Low Temperature Biology

Luyet, B. J., American Foundation for Biological Research
"The Recovery of White Blood Cells After Freezing"
NR 105-267 Nonr-3700(00)

Trauma and Metabolism

Darby, W. J. and Pearson, W. N., Vanderbilt University
"Problems of Malnutrition in the Middle East and Africa"
NR 105-254 Nonr-2149(04)

Dentistry

Orland, F. J., University of Chicago
"Use of Germfree Animals in the Study of Dental Caries"
NR 105-242 Nonr-2121(05)

Smith, C. E., Madin, S. H., Meyers, C. E., and Wolochow, H.
"Detection and Identification and Determination of Drug Sensitivity of Oral Pathogens"
NR 105-010 Nonr-222(76)

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