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Investigation of diagnostic allergens in brucellosis, tularemia, glanders and erysipeloid.

by Josef Parnas.

Zeitschr. f. Immunitaetsforschung, 114: 186-205 (1957).

In this paper we are reporting the results of tests designed to determine the value of the following four domestic diagnostic allergens:

1. brucellin PD
2. tularin U
3. mallein
4. rusiopestin.

Brucellin PD.

I. General data.

The basic element in the immunology of brucellosis is the condition of specific allergy. This condition does not appear instantaneously. In the initial stage of brucellosis, the serologic manifestations are predominant; in this stage the main function of the serologic reactions is represented by the uniformity of the acute form of human brucellosis. In the semi-acute and chronic forms, on the other hand, the cutaneous-allergic reaction developed by Burnet is of fundamental importance in diagnosis. It must be remembered that the human organism and the skin are conspicuous for their strong sensitivity to brucellosis allergens when infected. For this reason the development of Burnet's reaction is extremely difficult in man; we have devoted more than 10 years to this research.

Brucellin allergen has diagnostic significance when dispensed intracutaneously in small doses. At the same time, repeated and increased doses of allergen have a therapeutic effect. The curative influence of brucellin PD is based on three processes:

1. desensitization
2. the increase in specific antibody
3. the increase in phagocytosis.

Brucellin PD therefore represents the mainstay of the treatment of brucellosis, next to antibiotics. In acute forms it is secondary to antibiotics, in subcutaneous forms they are equal, in the chronic forms it dominates antibiotics.

Different preparations of allergen — diagnostic as well as curative — are produced in the various countries. Some writers claim that only corpuscular preparations, i.e. those that contain killed brucella cells, are noted for particular sensitivity of reaction and specificity. Others

claim that such preparations evoke severe and even very severe local and general reactions. Still others are of the opinion that bacterial filtrates or albumin-polysaccharide symplexes represent the best and mildest allergens. Finally there are investigators who think that these preparations are not sensitive and specific enough, and that larger doses, indispensable for obtaining the desired reaction, may cause tumultous repercussions.

After ten years of research in corpuscular and chemical allergens, we have come to the conclusion that brucellin PD is the best contemporary diagnostic allergen; it has the following advantages:

a) its production is easy and inexpensive
b) it is sensitive and specific in diagnosis
c) its effect is mild
d) it is durable and does not decompose easily.

We were also able to discover therapeutic properties in brucellin PD. In this country the different clinics, hospitals and sanitary-epidemiological stations in the "wojewods" utilise diverse curative and diagnostic allergens which are not always effective. The need arose for the delivery to interested agencies of a uniform, reliable, standardized and controlled brucellin PD preparation.

II. Selection of strains for the production of brucellin PD.

This question is of principal importance. The collection of brucella strains in this country, under the jurisdiction of the state research institute for agricultural medicine and hygiene, division of anthropozoonoses (Poland), has demonstrated allergeno-antigenic differences between the individual strains.

It was proved that the most valuable allergeno-antigenic strains are represented by the domestic strain Brucella brucei No. 24 (varietas bovis) and the foreign assay strain No. 106 (var. melitensis).

These strains are characterized by the following peculiarities:

1. their virulence, maintained constantly by animal passages;

2. biochemical and serologic properties characteristic for the varieties "bovis" and "melitensis."

These properties are constantly tested with respect to the bacteriological effect of thionin and alkaline fuchsin, the production of H_2S and by receptor analysis carried out by means of monospecific sera;

3. constant antigenic properties, tested by receptor analysis and allergometric examinations of rabbits;

4. immunogenic properties, examined by means of guinea pigs immunized

with aforesaid strains and revealing the signs of an immunizing effect (antibody, phagocytosis, histopathological lesions in the organs, allergy).

The strains are subjected to three reactions to determine the purity of phase S:

a) Burnet's thermoagglutination
b) examination of colonies by Henry's method
c) examination of colonies by Braun's method.

III. The production of the destroyed bacterial mass.

Tested standard strains are implanted in slant agar (A.G.S. medium, containing 3% agar, 1% glucose, 5% horse serum, pH 7.2). After a three-day growth and testing for cultural purity, these are rinsed off with a sterile liquid medium (peptone 1%, sodium chloride 0.6%, acid sodium carbonate 0.01%, calcium chloride 0.01%, potassium chloride 0.0075%, distilled water 100 ml). Now the culture is transferred to Roux bottles, also containing medium A.G.S. The Roux bottles are first incubated for 24 hours in order to test their sterility.

After sowing the material in Roux bottles and storage in the incubator for 3-4 days, the culture is tested for purity and rinsed off with physiological NaCl solution. The bacterial emulsion thus obtained, possessing a density of about 20 billion germs per ml, is subjected to the effect of ultrasonic frequencies of 2,375 Kc/s at 37°C for 90 minutes. The effect of the ultrasonics is examined under the electron microscope. The destroyed bacterial emulsion is then heated to 60°C within 30-40 minutes and diluted to a density of 1,300,000 brucella rods per ml according to Brown's scale. The emulsion thus diluted is conserved by the addition of 0.5% phenol. Brucellin PD for humans is produced in this manner. Brucellin PD for animals is manufactured separately with a density of 5 billion germs per ml.

IV. The internal control of brucellin PD.

The "internal control" of brucellin PD, conducted at the state research institute for agricultural medicine and hygiene, is based on the following tests:

a) control of sterility
b) control of toxicity
c) control of effectiveness in rabbits
d) control of harmlessness, particular specificity and sensitivity in connection with man.

ad a) The test of sterility is made in liquid suspension (sugar broth, Tarozzi's medium) and on solid media (Bas A.G.S. and agar with blood) under aerobic and anaerobic conditions in an atmosphere of 10% CO_2.

ad b) Toxicity is tested on ten adult white mice, of which one half are inoculated with 0.2 ml brucellin PD intraperitoneally, the other half

with 0.3 ml subcutaneously. Five adult guinea pigs serve as controls, of which three receive 0.4 ml brucellin PD intraperitoneally, and two 0.5 ml subcutaneously. No toxic manifestations were noted in the tested mice and guinea pigs, other than a depressed appearance lasting for several hours.

ad c) In order to test the allergic value of brucellin PD as a diagnostic aid, ten adult white rabbits are sensitized six to eight weeks prior to the test. This sensitization consists of the intravenous instillation of 0.5 ml emulsion of strain 24 (Brucella brucei var. Bovis) with a density of 5 billion rods per ml. After six to eight weeks the animals' fur is shorn on both sides in the hip-knee region, and carefully shaved two days before the test. The rabbits must be well tended and nourished, and completely healthy. Brucellin PD with a density of about 1 billion 300 million germs per ml is introduced intradermally, undiluted or diluted (650 million per ml, 300 million per ml, 150 million per ml). The rabbits are clinically observed for three days and Burnet's reaction is established. A dose of 0.1 ml (1 billion 300 million) evokes a positive Burnet reaction in all or in most of the rabbits after 2-3 days. The signs of this reaction are as follows: Redness of the skin with a diameter of 0.5 to 1 cm; a slight epidermal necrosis at the point where brucellin was applied. The rabbits revealed no toxic symptoms.

ad d) The test for harmlessness, sensitivity and particular specificity for humans is carried out in two groups:

1. in a group of persons free of brucellosis
2. in a group of brucellosis patients.

ad 1) The test was conducted with 60 persons unaffected by brucellosis. Burnet's reaction had a negative result in all persons. No toxic symptoms were noted.

ad 2) The test on brucellosis patients was accomplished in the clinical section of the state research institute for agricultural medicine and hygiene. The results of this test, conducted by A. Tuszkiewicz and W. Szewczykowski, are as follows:

The following properties of brucellin PD were determined in tests of 40 persons with various forms of brucellosis:

a) The harmlessness of brucellin PD in doses of 0.1 ml for the health of man and the physical condition of the patient.

b) The great allergenic value of the preparation. Brucellin PD is also used as a therapeutic by the clinical section, where no injurious effects on the health were noted. Its curative influence was established.

Human brucellin PD, tested in the aforesaid manner, was sent to the divison for testing of therapeutic sera and vaccines of the state hygienic institute at Warsaw. The results of these tests were as follows:

Rabbits infected with brucella (irulent strain) and sensitized with

brucellosis allergen revealed no weight loss, did not become depressed, and showed no toxic symptoms.

After testing the brucellin PD is placed in sterilized ampullae which are then submerged in a dye solution. The ampullae are again tested for:

a) sterility (storage in the incubator for 3 days) by sowing under aerobic and anaerobic conditions in an atmosphere of 10% CO_2.

b) air-tightness (immersion in a dye solution). After these processes the ampullaw are equipped with labels and maintained in the refrigerator at -2 to 3°C.

The brucellin PD, stored in the refrigerator, is checked every 3 months in respect to:

a) appearance of the preparation (homogeneity of the emulsion). The appearance of flakes disqualifies the preparation.

b) sensitivity and specific effect on sensitized rabbits.

c) harmlessness and sensitivity of effect on brucellosis patients.

In conformance with the regulations of the state research institute for agricultural medicine and hygiene, brucellin PD is used in the following manner on humans: 0.1 ml is injected intradermally on the inner side of the left forearm. Children receive only ½ of this dose; the same applies to older persons who have been exposed for several years. A large dose may evoke a severe reaction, which must be avoided for various reasons. After the intradermal injection of 0.1 ml brucellin PD, a small nodule with a diameter of 10 mm is formed immediately. The absence of this nodule proves that the brucellin has been instilled subcutaneously. In this case the reaction may be repeated on the right side; it must be ascertained, however, that the preparation is positively injected intradermally. The reaction site should not be treated with tincture of iodine. The skin may be cleaned with ether or alcohol.

The site of reaction is inspected after 24 hours, then after three, four or even five days.

Evaluation of the reaction:

0 = no change in the skin or a slight change of short duration.
/ = reddening of the area 2 X 1-2 cm, or insignificant infiltration (weakly positive result).
// = strong erythema and considerable infiltration in an area larger than 2 X 2 cm (positive result).
/// = very strong erythema, very strong infiltration and a central infiltration with necrosis, as well as a general reaction: Chills, headache, muscle ache, insomnia, pain and swelling of the arm, pain in the lymph glands and irritation of the lymph vessels. — Very strongly positive results appear due to excessive dosage,

which definitely is not indicated. If they occur, it is recommended to give the patient bedrest.

Tularin U.

Tularin U has an important part in the diagnosis of human tularemia and the evaluation of the immune-biological condition of the tularemia patient. This condition appears rapidly in human tularemia, usually within a few days. The tularin reaction (allergic-cutaneous) therefore is well suited to early diagnosis. The condition of sensitivity to tularin persists for a long time; it may even extend over several years. For this reason, the cutaneous-allergic tularin reaction serves a useful purpose in retrospective epidemiological research and in the case of a protracted course of the disease. The tularin reaction naturally may be considered only prior to the utilization of the vaccine. This preparation is also useful in those cases where the treatment with vaccine is used as a criterion of the patient's sensitivity. Various tularin preparations are used in different countries. Some of them have a "corpuscular character," others are filtrates or immune-chemical fractions of tularemia bacteria. Some preparations are noted for toxicity. The neutralization of such preparations usually causes the simultaneous loss of the reactive properties.

Our institute was engaged for three years in the comparison of the allergic properties of the following tularin preparations:

a) killed bacterial suspensions;
b) preparations obtained by immune-chemical means;
c) tularin "U", obtained by the destruction of tularemia bacteria with the aid of ultra sound.

Comparative tests have established the superiority of tularin "U" for diagnostic purposes.

II. The strain used in the production of tularin "U".

For reasons of safety, a viable, avirulent immunogenic strain was used in the production, obtained from the Soviet Union in the form of a vaccine against human tularemia by Elbiert and Gajski. This strain occurs in a pure phase S and is not pathogenic for test animals. It is maintained in the refrigerator at a temperature of -3 to 5°C on a medium by McCoy and Chapin, consisting of 60% egg yolk and 40% physiol. saline.

III. The production of the destroyed bacterial mass.

The controlled, standard strain is sowed on slanted McCoy media in large flasks. After a growth of 3-5 days at 37°C, the suspension is rinsed off with a sterile nutrient liquid; the suspension of tularemia rods is transferred to Roux bottles which are first maintained in the incubator for 24 hours to test the nutrient's sterility.

After sowing the material in Roux bottles and testing the culture's purity, the latter is rinsed off with physiological saline.

The suspension of tularemia rods thus obtained (density about 10 billion germs per ml) is subjected to ultra sound of about 2,800 Kc/s for 90 minutes at a temperature not in excess of 37°C.

The ultrasonic effect is controlled with the aid of the electron microscope. The destroyed bacterial mass is subsequently heated to 60°C for 30-40 minutes; it is then adjusted to 100 million bacterial cells (1 ml Brown's scale). 0.5% phenol is added. This represents allergenic tularin for humans. A similar preparation for animals — "tularin U vet" — contains 1 billion bacterial cells per ml.

IV. The internal control of tularin "U".

The "internal control" of tularin "U", conducted at the state research institute of agricultural medicine and hygiene (division of anthropozoonoses and clinical division for rural occupational diseases) and the clinic for infectious diseases of the medical academy at Poznan (Dr. Neyman, Dr. Zahradnik), consists of the following tests:

a) control of sterility
b) control of virulence
c) control of the effect on rabbits and guinea pigs
d) control of harmlessness, specificity, a d harmlessness to man.

ad a) The test of sterility is accomplished with the aid of McCoy's and Francis' media on sugar broth of Tarozzi's medium, and this under aerobic and anaerobic conditions.

ad b) Guinea pigs and white mice are used in tests of toxicity. One half of the guinea pigs receives 2 ml intraperitoneally, the other 4 ml. One half of the white mice receive 0.1 ml intraperitoneally, the other 0.2 ml. The animals, observed for 3 weeks, showed no toxic symptoms.

ad c) The testing of the allergen's effect on guinea pigs and rabbits is conducted partly with virulent, partly with the viable, fully antigenic, avirulent strain of P. tularensis. Five adult, well-nourished rabbits and five strong guinea pigs are infected subcutaneously with a virulent strain of P. tularensis (0.5 ml). The emulsion contains 100 million germs per ml. The other group of 5 rabbits and 5 guinea pigs are infected subcutaneously (guinea pigs) or intravenously (rabbits) with 0.5 and 1 ml, respectively, of the avirulent strain, the suspension having a density of 1 billion per ml. Six to eight weeks after infection the agglutination and complement fixation reactions for tularemia are conducted with all animals; later, after shearing and shaving, 0.1 ml tularin "U" is injected intradermally.

Unfortunately the tularemic reaction is not as regular and distinct in rabbits and guinea pigs as it is in animals infected with brucellosis and subsequently tested with brucellin PD. In the majority of the infected

animals, the positive tularin reaction becomes visible in the form of infiltrations of 0.5 cm or more in diameter and erythema.

ad d) The tests of specificity, sensitivity of effect and harmlessness conducted with humans naturally have the most important part in the testing of tularin "U". For this purpose a series of tests were undertaken with persons free of tularaemia. These tests have shown that tularin "U" is a completely harmless allergen that does not cause a positive reaction in the observed persons.

Tularin "U" was tested on tularemic patients in the clinic for infectious diseases at the Poznan academy of medicine. In all cases tularaemia was diagnosed on the basis of the anamnesis, the clinical examination and additional serologic reactions. In all cases tularin "U", injected in a dose of 0.1 ml intradermally, evoked a positive reaction, characterized by the appearance of a vesicle and an infiltrate (1 cm or more in diameter) as well as erythema (see Fig. 6). Similar results were later obtained in other hospitals.

V. The storage of tularin "U".

After testing, tularin "U" is placed in sterilized ampullae, which are submerged later. The immersed ampullae are checked for:

a) sterility (storage in the incubator for 3 days under aerobic and anaerobic conditions) in an atmosphere of 10% CO_2.

b) air-tightness of the ampullae (immersion in dye solution).

Now the ampullae are equipped with labels and maintained in the refrigerator at -2 to 3°C. The stored tularin U is checked every 3 months in respect to the following:

a) appearance upon shaking (homogeneous suspension). The appearance of flakes disqualifies the preparation.

b) sensitivity and specificity of effect on infected test animals and humans.

VI. Principles of utilization, reading and interpretation of the tularin U reaction in man.

The cutaneous-allergic reaction with tularin U should be used in every case where tularemia is suspected. Frequently the positive reaction occurs in the first days of the disease. The reaction with tularin U is also used in connection with retrospective occupational-epidemiological examinations. The reaction is further utilized with patients prior to vaccinal therapy for the determination of the degree of allergic sensitivity. Finally, the tularin reaction, in conjunction with serologic reactions and the opsonocytophagic index, represents an expression of immunity in persons immunized against tularemia.

After shaking, 0.1 ml tularin U is injected intradermally on the inner side of the right forearm. In order to prevent an excessive dosage, a precisely calibrated syringe and a thin, short needle must be used. The tularin must be injected intradermally, so that a small papule appears right after injection. For control purposes, 0.1 ml physiol. saline (traumatic control) and 0.1 ml peptone solution (albumin control) may be injected in the identical manner on the left forearm. A positive tularin reaction is identified by the appearance of a vesicle of about 1 cm in diameter and erythema of 1-2 cm in diameter on the second, third or fourth day. This process sometimes is accompanied by changes in the surrounding vessels and lymph glands (barely noticeable erythema and pain) and by general changes (elevated temperature up to 37.5-38°C).

A weakly positive result is distinguished by the appearance of an infiltration of about 1 cm in diameter and erythema.

The interpretation of the tularin reaction applies to its properties within the complex of the agglutination reaction, the complement fixation reaction and the opsonocytophagic index.

Mallein PS and mallein U.

For many years Hellmann's old mallein has been used in many countries, among them Poland. It is produced in this manner: The liquid culture of the glanders bacillus (glycerol broth) was killed in the autoclave, then filtered and compressed. The resulting preparation represented the mallein. It contains ballast substances emanating from the albuminous medium. The results obtained with the aid of this allergen in connection with horses and humans are not always specific, giving rise to erroneous diagnoses. Parnas and Stepkowski planned to produce a pure and very specific allergen. The result was mallein PS.

1. The strains of the glanders bacillus (pure phase S) are sowed in Roux bottles, rinsed off with sterile, distilled water, so that a density of about 10 billion bacilli per ml suspension is obtained; this is killed with a higher temperature.

2. The inactivated suspension is subjected to five times repeated freezing and melting, causing mechanical destruction.

3. The resulting allergen is mixed with 0.5% phenol and placed in ampullae as mallein PS.

4. The control of mallein PS embraces the following tests:

a) sterility
b) toxicity for guinea pigs
c) the antigenic value in Bordet and Genou's complement fixation reaction with positive glanders serum and with control sera.
d) specificity of allergenic effect on control animals infected with tuberculosis bacteria and brucella rods.

e. specificity of effect on horses free of and afflicted with glanders.

f. the negative effect on healthy persons.

The tests proved the non-toxicity of mallein PS, its great antigenic power in Bordet-Gengou's test, its specificity of effect on horses, test animals and man.

Thus a product was obtained, designated mallein PS, which was tested simultaneously with standard mallein on twelve horses. The test was conducted by a commission comprised of the chief of the veterinary section of Szczecin District, the veterinarian of the district and the county veterinary.

All positively reacting horses were sacrificed. Histopathological examination confirmed the results of malleinization. In four horses the clinical and serological tests, as well as reactions to standard mallein and mallein PS were negative. In five additional horses, standard mallein gave positive or uncertain results, while mallein PS indicated glanders in every case, proving its allergic superiority. In one horse standard mallein gave a negative result, mallein PS a positive one. Histopathological examination confirmed the result with mallein PS. In one horse, standard mallein gave a negative result, while mallein PS gave a visible indication. Histopathological examination revealed glanders. This proves that mallein PS is more sensitive than standard mallein.

We now have introduced mallein U, obtained by the destruction of the bacterial suspension with ultra sound.

Rusiopatin L and U.

The first investigations of rusiopatin L were conducted in my institute by Lorkiewicz and Mereata.

Goertler sees in erysipeloid a disease which is based on allergosis whose erysipeloid process is evoked by infectious substances in the organism due to the influence of external factors such as temperature changes, alterations in the diet, preventive vaccinations, parasites.

The allergic manifestations of erysipeloid were to be used for diagnostic purposes in man (Selby). At the same time, this reaction was tested in veterinary medicine in the case of erysipeloid infections of sheep with chronic courses (Curasson 1947) and also on piglets (Pietrow 1950).

The technique employed in the manufacture of rusiopatin L.

Method of production: 1,500 ml of a 3-day culture of a virulent strain of erysipeloid rods No. 4304 are centrifuged and washed three times with physiol. saline. 10 ccm 1/10 n NaOH are added to the sediment, shaken for about 10 minutes and maintained in the incubator at 37°C for 24 hours, accompanied by shaking every hour of the first five hours. The suspension

is then placed in the refrigerator for 48 hours at /2°C. The preparation was then tested for sterility. Amorphous masses of hydrolyzed bacterial cells were visible in preparations produced from the allergen.

Rabbit tests were conducted with allergen in fourfold dilution.

Prior to the commencement of the experiments, the 1/10 n NaOH used in the production of allergen was tested. 0.2 ml NaOH was injected intradermally into two rabbits which had not been inoculated previously. No changes were noted at the site of injection for 72 hours.

Several groups of rabbits were utilized in the testing of allergy.

The first series contained 12 rabbits. All were about 6 months old, weighed 1,500 g and were identically fed. Four rabbits were infected with 0.5 ccm of an 18-hour culture of erysipeloid rods of the strain No. 4599 (broth culture). Three injections were given, 4 days apart.

The second group of 4 rabbits was infected three times every four days with the same doses of the avirulent strain of Staub.

The third group contained 4 control rabbits, held in segregated cages without contact with the inoculated ones.

After one week, 2 rabbits of the first group (inoculated with strain 4599) received 0.100 antistin subcutaneously, three times in intervals of one day. Two rabbits each from the second and third groups also received antistin in the same manner. The allergic investigations were conducted on the day following the conclusion of antistin injection.

Eight rabbits were inoculated with the erysipeloid rods. Of these, only 4 reacted positively to the allergen, one of them with dubious results. Of 4 rabbits inoculated with Staub's strain, 2 gave a positive reaction. The positive reaction occurred once among rabbits inoculated with the virulent strain 4599. A doubtful reaction was seen once: These rabbits had received no antistin. Animals which had received antistin and strain 4599 did not react. Among 8 rabbits inoculated with virulent and avirulent strains of erysipeloid, a strong reaction developed in 1, a moderately strong reaction in 2 cases, signifying 37% positive reactions. Fluctuations in individual sensitivity clearly have exerted a decisive influence on the result of this experiment.

A second group of rabbits, part of which had previously been inoculated with strain 4599, another part with Staub's strain, were tested with rusiopatin L.

The rabbits showed no pathological manifestations during the entire period of inoculation. Two weeks after instillation, 2 rabbits received 0.1 cc antistin subcutaneously for 3 days. On the fourth day the allergen was dispensed intradermally, as were the test substances.

The results were read after 24 and 72 hours.

The positive reaction persisted for a long time in the rabbits of this group (table 2). A suppurative infiltrate was frequently observed after 2 weeks and later.

Among 12 inoculated rabbits, 8 reacted positively. Six rabbits infected with Staub's erysipelothrix showed a strong or moderately strong reaction. Rabbits inoculated with the virulent strain (4599) reacted very strongly in 2 cases. Of the 2 control rabbits, 1 reacted positively, the other indistinctly. This phenomenon is interesting in connection with control animals. We can explain it by the lack of specificity or by an infection of the rabbits with erysipelothrix.

In order to determine the specificity of the allergic reaction in erysipeloid, examinations of erysipeloid allergen were conducted with a group of rabbits, some of which were tuberculin-positive; others had been inoculated with brucella (virulent strain). Healthy, non-inoculated animals that had had no contact with infected ones, were also included.

Rabbits that had received a suspension of virulent or avirulent erysipelothrix, reacted positively to intracutaneous instillation of rusiopatin L. Non-inoculated control rabbits generally reacted negatively (in the majority of cases).

Among 14 non-vaccinated control rabbits, 1 reacted positively, 2 others doubtfully.

No interdependence between the allergic reaction and antistin was observed in these experiments. Of 8 rabbits infected with erysipeloid rods, then given antistin after two weeks, 6 reacted positively. Of 12 rabbits infected with erysipelothrix and not receiving antistin, 7 reacted positively. It appears that antistin has no influence on the cutaneous reaction in erysipeloid.

Rabbits inoculated with brucella rods and reacting positively to brucellin AS, in the majority of cases showed a negative reaction after dispensation of erysipeloid allergen.

Another phenomenon was observed in rabbits inoculated with tuberculous material. Among 7 rabbits, 4 reacted positively to rusiopatin. However, the positive reaction was never as pronounced as in the case of the positive indication in erysipeloid rabbits.

We have tested the erysipeloid allergen on persons infected with erysipeloid.

The first case concerned a veterinary student who had infected himself during dissection of a pig. Two days after section erythema appeared at the site which he had injured with the scalpel. The site was dabbed with iodine; dressings of alcohol and ichthyol salve were applied. Erythema disappeared after a few hours. After 24 hours, erythema and tumescence were noted at another point on the hand. Treatment with ichthyol salve and prontosil was unable to halt the process. Only the dispensation of 125,000 units of

penicillin and 5 ccm prontosil gradually reversed the disease process. On the fifth day after infection, 0.1 ml of the allergen was instilled intracutaneously.

Erythema and edema had appeared after 24 hours; these manifestations increased up to the fifth day, then disappeared slowly. The reaction was distinctly positive. Sensitivity was noted in the area of erythema and tumescance.

The second case concerns an assistant of the microbiological institute of M. Curie Skodowska University, who had infected herself with erysipeloid under the elbow one year ago. The symptoms were distinct. She was treated with penicillin in doses of 50,000 units (a total of 500,000 units were dispensed). The allergic reaction to rusiopatin was negative.

The third case also involved an assistant of that institute, who had infected herself with a laboratory strain of erysipelothrix. After introduction of allergen, erythema was distinct and lasted for 3 days. Following treatment with 200,000 units of penicillin, the symptoms and the cutaneous reaction disappeared.

The fourth case was diagnosed in a patient of the agricultural occupational disease ward with the clinical findings of endocarditis chronica. The anamnesis disclosed that the patient had been infected with erysipeloid one year ago. Cutaneous lesions and disturbances of the cardiac function had appeared during the first stage. These manifestations disappeared after treatment; one year later the patient was admitted to the clinical ward with suspected endocarditis chronica. The allergic reaction with rusiopatin as well as the agglutination reaction were conducted. The allergic reaction was positive (distinct erythema — 1 cm —, swelling at the site of injection). The serum showed a positive titer of 1:200. Similar results were obtained in ten cases of erysipeloid (7 ♂, 3 ♀).

Illustrations.

Fig. 1. Brucellosis allergy after Huddleson; dermatitis and petechiae.

Fig. 2a. Brucella brucei X 16,000.
Fig. 2b. The same, destroyed by ultra sound X 16,000 = brucellin PD.

Fig. 3. Burnet's test in brucellosis. Evaluation of brucellin PD. Left forearm: 2 reactions with infiltration and erythema after brucellin PD. Dose 130 million and 300 million. Right forearm: The same with a dose of 10-20 million bacteria.

Fig. 4. Burnet's test in brucellosis, with brucellin PD. Infiltration and erythema.

Fig. 5a. P. tularensis X 15,000.
Fig. 5b. The same destroyed by ultra sound X 15,000 = tularin U.

Fig. 6. Allergo-cutaneous test in tularemia with tularin U. Infiltration and erythema.

Fig. 7. Mallein PS test positive in horse with glanders. Suppurative exudate from the nose after inoculation with mallein PS. Strong provocative effect of mallein PS.

Fig. 8. Erysipeloid Rosenbach.

Table 1.

Käfig Nr.	- cage #
Kaninchen	- rabbit
mit welchem Stamm früher geimpft	- strain with which previously inoculated

// - very strong cutaneous reaction (erythema, swelling and infiltration of about 9-10 mm).

/ - distinct cutaneous reaction (erythema and swelling 5-6 mm).

\- - without reaction.

Table 2.

Käfig Nr.	- cage #
Impfstamm vorher benutzt	- strain previously used
Zahl d. Kaninchen	- number of rabbits
/ erhalten	- / dispensed
- nicht erhalten	- - not dispensed
nach 24 u. 72 Stunden	- after 24 and 72 hours.