ORGAN SPECIFICITY OF TISSUES OF THE DOG AND MAN AS SHOWN BY PASSIVE ANAPHYLAXIS IN GUINEA PIGS*

By G. HOWARD BAILEY, M.D., AND SIDNEY RAFFEL, Sc.D.

(From the Department of Immunology, Laboratories of Immunology and Filterable Viruses, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore)

(Received for publication, January 17, 1941)

In previous studies (1, 2) it was shown that prolonged immunization of rabbits with sedimented bacterial vaccines grown in infusion broths prepared from the tissues of different animals, stimulated the production of antibodies not only for the bacteria but also for the broths. It was demonstrated that fatal anaphylaxis usually followed the injection of homologous organ broths into guinea pigs passively sensitized with the antisera of rabbits immunized in this way. Thus, almost complete immunological specificity for broths made from striated muscle and brain was so obtained. It was found that some of the organ-specific substances still remained in infusions of these tissues after they had been autoclaved. The partial destruction or conjugation of the relatively small amounts of blood in the infusions by such treatment, seemed to provide a simple method which gave promise of possible application to various tissues in an effort to demonstrate organ specificity by the elimination of the numerous cross-reactions which are usually obtained in immunological tests with suspensions and extracts of unheated cells.

Reviews of the voluminous literature on recent studies of organ specificity carried out by various methods, have been made by Witebsky (3), Landsteiner (4), and others, to which the reader is referred. It should be stated, however, that most of these investigations have been made by means of either the complement-fixation or the precipitin reaction with alcoholic extracts of fresh organs. Sometimes aqueous solutions or suspensions of fresh tissues were used as antigens (5). In a few instances particular protein fractions have been tested. By serological methods, more or less strict organ specificity has been shown for brain (6, 7), testicle (7), kidney (8–10), suprarenal gland (11), placenta (12, 13), epiphysis (14), hypophysis (15), stomach and intestine (16), lens and intestine (17), leucocytes (18), carcinoma and sarcoma (19–22), and glioma (23). With some organs, the specificity of antisera has been shown *in vivo*, either directly by the action of cytotoxins, as in the case of the injection of anti-kidney sera for the production of nephritis (24, 25), or indirectly by the effects on metabolism induced by the injection of antisera for thyroid gland and the anterior body of the pituitary gland (26).

* Supported in part by a grant-in-aid from the National Research Council.

As to anaphylaxis, it has supplied a delicate technique for investigations of organ specificity (27). Anaphylaxis can be observed directly when a very small amount of a specific antigen is injected into a sensitized guinea pig, and indirectly desensitization can be noted with a much smaller amount of the specific substance. Active anaphylaxis was used by Pfeiffer (28) for the study of the organ-specific properties of the proteins of the liver, spleen, kidney, blood, etc. The results seemed to indicate the existence of such specificity, although Ranzi (29) had previously obtained entirely negative results, and Pearce, Karsner, and Eisenbrey (30) failed to confirm Pfeiffer's claims. However, Kraus, Doerr, and Sohma (31) found that guinea pigs which had been sensitized to the protein of the crystalline lens of one species would react to lens protein in general. The same thing was demonstrated for testicular protein by von Dungern and Hirschfeld (32). The results in this case, as shown by the localized reaction of Arthus, seemed to be less specific than in the case of lens.

Because of the delicacy of the reaction of anaphylaxis and the numerous species-specific cross-reactions which have been obtained with most anti-organ sera when aqueous extracts of fresh tissues were used as antigens, the method has long been neglected as a technique for the demonstration of organ specificity. Our own investigations on passive anaphylaxis with broths, already referred to, seemed to open a new approach to the subject.

EXPERIMENTAL

In view of the fact that the serological tests carried out by a number of investigators, had shown more or less specificity for different organs, not only with lipoids but also with whole cells and protein fractions, it occurred to us to determine whether such specificity could be generally demonstrated by anaphylactic tests with the water-soluble hydrolytic products of various autoclaved tissues, that is, with broths, according to the method previously used successfully with broths prepared from striated muscle (1) and brain (2). To this end, guinea pigs were passively sensitized with antisera from rabbits to determine (*a*) tissue specificity of different organs of the dog and man, (*b*) differences in the organ-specific properties of corresponding tissues in these two species, (*c*) relationship of the Forssman antigen to organ specificity, (*d*) tissue specificity of human fibromyoma and melanotic sarcoma, (*e*) relationship of the vascularity of different organs to their cross-reactions with antisera for whole blood broths. The passively sensitized guinea pigs were injected with autoclaved extracts of various organs and, if the animals survived, they were injected one hour later with the homologous extract to determine possible antigenic relationships by specific desensitization.

In the present study, two kinds of fluids have been used. As antigens for the production of tissue-specific antisera, *Pasteurella boviseptica* was

grown in certain autoclaved organ extracts to which peptone, glucose, and sodium chloride had been added. The sedimented heat-killed bacteria only, to which some of the particular broth was probably adsorbed, were injected into rabbits. Broth alone in large amounts was practically non-antigenic. We have found (1) that a number of other bacteria, as well as animal charcoal, kaolin, or collodion particles can be substituted for *Pasteurella boviseptica* when grown in ordinary beef infusion broth or added to it, and then centrifuged, heated, and used as vaccines. The antigens for anaphylactic tests in guinea pigs were autoclaved organ extracts to which sodium chloride alone was added.

Methods

Broths for Growth of Bacterial Vaccines.—The organs of normal dogs which had been sacrificed by operative ether anesthesia, were removed, dissected as free as possible from fat and connective tissue, cut into small pieces, washed under running tap water, put through a meat grinder, washed over several layers of gauze with physiological salt solution, and one part of tissue mixed with two parts by weight of distilled water. After standing in the ice box for 48 hours, the infusions were autoclaved at 122–124°C. for 20 minutes, filtered through gauze and then through paper, and 0.5 per cent by weight of sodium chloride added to them. The filtrates were adjusted to pH 7.2–7.4 with the idea of causing as much destruction of any common blood antigen as possible by the subsequent heating without complete destruction of the predominating fixed tissue antigens. The extracts were steamed in the Arnold sterilizer for 15 minutes, filtered through paper, put into 200 cc. Erlenmeyer flasks, and sterilized in the autoclave at 122–124°C. for 20 minutes. The final pH of the extracts varied from 7.0 to 7.2. The extracts of human organs were prepared in the same manner. The tissues were collected from six different individuals at autopsy and at operations. None of the materials were obtained from persons with acute infections.

Bacterial Vaccines.—After the addition of sterile Difco peptone (pH 7.6) and glucose to 1 and 0.5 per cent respectively, 100 cc. amounts of the various organ broths were inoculated with 0.01 cc. of a 24 hour beef infusion broth culture of *Pasteurella boviseptica* and incubated at 37.5°C. for 48 hours when heavy growths were obtained. The cultures were centrifuged, the supernatants decanted and discarded, each packed bacterial sediment suspended in 10 cc. of 0.85 per cent solution of sodium chloride, and heated at 56°C. for one hour.

Immunization of Rabbits.—Rabbits of mixed American Blue and Dutch stock were immunized by two or three weekly injections of increasing doses of 0.05 to 0.5 cc. of the concentrated bacterial vaccines just described, over periods of 6 to 8 weeks, until a total of 7.5 to 10 cc. had been given. Each animal was bled 50 cc. from the ear veins on the 8th day and also on the 10th day after the last injection, the serum collected, pooled, merthiolate added to 1 to 10,000, and stored in the refrigerator until needed for passive sensitization of guinea pigs to the various organ broths. Such antisera had been shown to contain antibodies not only for the bacteria but also for the broths.

Antigens for Anaphylactic Tests.—The antigens used for anaphylactic tests were

either from the same lots of autoclaved extracts of organs used for the growth of the bacterial vaccines described above or extracts of other tissues prepared in exactly the same way. Peptone and glucose were not added to these antigens. Extracts which showed turbidity were centrifuged to clear them as much as possible of any gross suspended

TABLE I

Specificity of Antisera for Broths Made from Different Organs of the Dog as Shown by Passive Anaphylactic Tests with Guinea Pigs Injected with Autoclaved Aqueous Extracts of Various Tissues of the Dog

Dog organ broth tested for production of shock	Anti-broth sensitizing sera and reactions* of guinea pigs to (1) heterologous dog organ broth and (2) homologous dog organ broth											
	Anti-skeletal muscle		Anti-heart		Anti-ileum		Anti-lung		Anti-kidney		Anti-blood	
	1	2	1	2	1	2	1	2	1	2	1	2
Skeletal muscle		4+	4+		−	4+	−	2+	±	±	−	±
Heart	4+			4+	−	4+	−	+	±	−	+	−
Diaphragm	4+		4+		−	4+	−	2+	±	−	−	+
Tongue	4+		4+		−	4+	−	2+	−	−	−	±
Esophagus	4+		4+		±	3+	+	−	±	−	−	+
Stomach	−	4+	−	4+	−	4+	−	2+	−	±	−	3+
Ileum	−	4+	+	2+		4+	3+	−	−	−	±	2+
Colon, cecum	−	4+	−	4+	±	2+	+	−	−	−	±	+
Bladder	−	4+	−	4+	+	±	4+		−	−	−	2+
Aorta	−	4+	−	4+	−	4+	±	−	−	−	±	3+
Lung	±	4+	±	4+	+	+		3+	±	−	±	−
Trachea	−	4+	−	4+	±	4+	+	−	−	−	−	2+
Liver	±	−	±	4+	−	4+	−	−	±	−	3+	−
Kidney	−	4+	±	3+	−	4+	−	±		4+	4+	
Pancreas	−	4+	−	4+	−	4+	−	2+	−	±	−	3+
Spleen	−	4+	±	4+	−	3+	−	2+	±	±	+	−
Omentum, fat	−	4+	−	4+	−	4+	−	3+	−	±	−	2+
Lymph gland	−	4+	−	4+	−	4+	±	+	−	−	±	2+
Parotid gland	−	4+	−	4+	−	4+	−	2+	−	±	−	3+
Thyroid gland	±	4+	−	4+	−	4+	−	2+	−	−	−	±
Testicle	−	4+	−	4+	−	4+	−	3+	±	−	−	3+
Brain	−	4+	±	4+	−	4+	−	3+	−	±	3+	−
Skin	−	4+	−	4+	−	3+	−	+	−	±	−	−
Blood (whole)	−	4+	±	4+	−	4+	±	2+	±	−		4+

*4+ = fatal reaction with death in 2½ to 5 minutes; 3+ = severe, almost fatal, reaction; 2+ = moderate reaction; + = slight but definite reaction; ± = very slight or doubtful reaction; − = no reaction. The signs, symptoms, and autopsy findings were all typical of anaphylactic shock.

matter. Because of the high content of epinephrin, extracts of suprarenal glands were primarily toxic by intravenous injection into guinea pigs and were not used in anaphylactic tests. None of the other organ extracts were primarily toxic for guinea pigs.

Anaphylactic Tests.—Guinea pigs of 250 to 300 gm. in weight were prepared for anaphylactic tests by intraabdominal injection of 2 cc. of the antisera for various broths previously described, and tested for passive sensitization after 48 hours by injection into

the saphenous vein with 2 cc. of autoclaved extracts of different tissues of the dog and man. In case the guinea pig lived, this injection was followed one hour later by injection into the same vein or opposite vein with 1.5 cc. of autoclaved extract of the homologous organ to test for possible antigenic relationships which might be shown by desensitization.

Organ Specificity of Antisera for Broths Made from Different Tissues of the Dog

In order to test the specificity of antisera for broths prepared from different tissues of the dog, rabbits were immunized as previously described with sedimented bacterial vaccines grown in broths made from skeletal muscle, heart, small intestine (ileum), lung, kidney, and whole blood. The results with autoclaved extracts of various tissues as antigens in anaphylactic tests in guinea pigs passively sensitized with the different antisera, are summarized in Table I. Six antisera and twenty-four extracts were used. It will be seen that the antiserum for skeletal muscle showed practically complete specificity for striated muscle as demonstrated by fatal reactions with guinea pigs injected with extracts of skeletal muscle, heart, diaphragm, tongue, and esophagus, while very slight or negative reactions were obtained with extracts of other tissues. The antiserum for heart muscle gave the same reactions as that for skeletal muscle with the exception of a definite cross-reaction with ileum. This reaction could not have been due to certain amounts of smooth muscle common to both the heart and ileum, because no reactions were obtained with heart antiserum and extracts of other tissues containing large amounts of smooth muscle. The antiserum for ileum reacted best with the homologous antigen, but definite anaphylaxis also occurred in the guinea pigs injected with extracts of bladder and lung. Smooth muscle is common to these three organs, but it is also present in other tissues, extracts of which gave completely negative reactions. It is possible that the presence of epithelial cells might account for the more or less definite cross-reactions of esophagus, colon, bladder, lung, and trachea with the antiserum for ileum. This is further indicated by the reactions of the antiserum for lung with extracts of esophagus, ileum, colon, bladder, and trachea. Furthermore, in those instances in which the reactions were not fatal, there was complete desensitization to later injections of the homologous extract of lung. It should be mentioned, however, that these tests were complicated by the fact that the antiserum for dog lung contained Forssman antibodies. The hemolytic titer for sheep red cells was about 2500 and the serum was primarily toxic for guinea pigs, causing death in 3 minutes when as small an amount as 0.2 cc. was injected intravenously. When injected intraabdominally with 2 cc. of the serum, about 50 per cent of the animals died. All survived when given 1 cc. by this route and these

guinea pigs were used in the passive anaphylactic tests with this antiserum. The antisera for other dog organs did not seem to be primarily toxic when injected intraabdominally in doses of 2 cc., although all of these antisera showed hemolytic titers somewhat higher than normal for sheep red cells. It is possible that the toxicity of the antiserum for dog lung was due not only to Forssman antibodies but also to organ-specific antibodies for lung itself.

In addition to the almost identical properties of the antisera for skeletal muscle and heart which have already been referred to, the special characteristics of antisera for kidney and whole blood deserve attention. The antiserum for kidney gave very slight reactions with a considerable number of heterologous organ extracts, but it gave a fatal reaction only with the extract of kidney itself. This antiserum was peculiar, however, in that either complete or almost complete desensitization occurred following the injections of the passively sensitized guinea pigs with any of the extracts of various heterologous tissues. Theoretically, an antiserum for such an organ would have a high degree of polyvalency, although the antibody response to any one of the complex mixture of haptens might be very slight, but sufficient to cause more or less reaction to a corresponding antigen and thus desensitization in anaphylaxis. In this connection, another antiserum which showed an even greater degree of apparent polyvalency which can be more easily explained, was that for whole blood. Guinea pigs passively sensitized with this antiserum had fatal reactions when injected with extracts of whole blood and kidney, severe reactions with those from liver and brain, and definite reactions with extracts of heart and spleen. All these organs are especially well supplied with blood and, since in the preparation of the various antigens no attempts were made to wash all of the blood out of any of the tissues, some of them probably contained a good deal of it. It is not surprising then that marked cross-reactions were obtained with the autoclaved extracts of some of the tissues mentioned above, when tested against the antiserum for blood. It is surprising, however, that a larger number of cross-reactions were not obtained with this antiserum.

Organ Specificity of Antisera for Different Human Organ Broths

Since we had been able to demonstrate almost complete tissue specificity with antisera for a number of different organs of the dog in passive anaphylaxis by the methods described above, we were interested to determine if the same technique could be applied equally well to show immunological specificity of human tissues. If such proved to be the case, it was hoped that it would then be possible to compare the anaphylactogenic properties

of normal organs and different kinds of tumors of man and animals. Accordingly, antisera for autoclaved aqueous extracts of seven representative human tissues were produced by the methods described in a previous section of this paper. The normal tissues used were skeletal muscle, lung, liver, kidney, blood, and placenta. Two tumors were available. These

TABLE II

Specificity of Antisera for Broths Made from Different Human Tissues as Shown by Passive Anaphylactic Tests with Guinea Pigs Injected with Autoclaved Aqueous Extracts of Various Human Tissues

Human organ broth tested for production of shock	Anti-broth sensitizing sera and reactions* of guinea pigs to (1) heterologous human organ broth and (2) homologous human organ broth													
	Anti-skeletal muscle		Anti-lung		Anti-liver		Anti-kidney		Anti-placenta		Anti-fibromyoma		Anti-blood	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Skeletal muscle		3+	−	3+	−	4+	−	3+	−	3+	−	+	−	4+
Diaphragm	3+	−	−	3+	−	4+	−	3+	−	4+	−	+	−	4+
Heart	4+		−	3+	−	4+	−	2+	−	3+	−	±	−	4+
Esophagus	2+	−	−	3+	−	4+	−	3+	−	3+	−	+	−	4+
Ileum	−	3+	+	−	−	4+	−	4+	−	4+	−	2+	−	4+
Uterus	−	2+	+	±	4+		±	2+	+	−	−	±	4+	
Fibromyoma	−	3+	−	4+	±	4+	−	3+	−	4+		+	±	4+
Placenta	−	2+	±	3+	±	−	−	4+		4+	−	+	4+	
Aorta	−	3+	±	2+	±	−	−	3+	−	3+	−	±	±	4+
Lung	−	2+		4+	4+		±	2+	−	3+	−	±	4+	
Trachea	−	3+	+	−	+	−	−	3+	−	3+	−	+	±	3+
Liver	−	3+	+	−		4+	±	3+	±	2+	−	2+	4+	
Kidney	−	2+	±	3+	+	−		3+	−	3+	−	±	+	+
Spleen	−	2+	−	3+	+	±	±	2+	±	2+	−	+	4+	
Lymph gland	−	3+	−	2+	−	4+	−	3+	−	3+	−	+	−	4+
Melanotic sarcoma	−	3+	−	2+	−	4+	−	3+	−	3+	−	±	−	4+
Skin	−	2+	−	4+	−	4+	−	3+	−	2+	−	+	−	4+
Fat	−	2+	−	3+	−	4+	−	2+	−	2+	−	±	−	4+
Brain (ox)	−	3+	−	4+	−	4+	−	3+	−	3+	−	+	+	2+
Blood (whole)	−	3+	−	3+	−	4+	−	2+	+	−	−	+		4+

* The signs indicating the degree of shock are to be interpreted as in Table I.

were fibromyoma of the uterus and melanotic sarcoma, the latter from two different individuals. Seven different antisera were tested with twenty autoclaved extracts. The results are given in Table II. As in the case of the tests with antisera for various organs of the dog, the only completely and definitely specific antiserum was that for skeletal muscle. This serum reacted only with extracts made from organs containing striated muscle. The antiserum for fibromyoma reacted only with the extract of this tumor but this serum was of low potency and the reactions were slight. The ex-

tracts made from fibromyoma and uterine muscle had a jelly-like consistency when first brought from the ice box and became completely liquefied only when warmed to about 25°C. They appeared to contain a considerable amount of gelatin, but they did not cause colloid shock and were not primarily toxic when injected intravenously into normal guinea pigs. Studies carried out by Bailey and Gardner (unpublished data) since the completion of these experiments, indicate that a more potent antiserum could have been obtained against the extract of fibromyoma if the tissue infusion had been adjusted on the acid side of neutrality, at pH 6.7–6.8, before each autoclaving. An alkaline reaction of about pH 7.2 was used in the present experiments.

Two other anti-human sera which appeared to be almost completely tissue-specific were those for kidney and placenta. The antiserum for the former reacted very slightly with four different antigens, but there was no desensitization. The antiserum for placenta gave a slight but definite reaction with the extract of uterus. This cannot be explained on the basis of antibodies for uterine decidua since the uterine antigen was made entirely from smooth muscle. The antisera for blood, liver, and lung also reacted with the extract of uterus. It does not seem probable that these reactions were due to the antigen of blood, because the uterine muscle used was not very vascular and could have had only a small amount of blood in it. Furthermore, antisera for the highly vascular tissues of lung and liver gave no reactions with the extract of whole blood itself. It will be noted, however, that the antiserum for lung gave a definite reaction with extract of liver, and the antiserum for liver produced a fatal reaction with extract of lung.

The antiserum for human whole blood gave fatal anaphylactic reactions with the homologous antigen as well as with extracts of uterus, placenta, lung, liver, and spleen. It even reacted slightly with an antigen made from ox brain in which the blood was heterologous. We have already mentioned that the antiserum for dog blood produced a severe reaction with an extract of dog brain. It is probable, however, that the cross-reactions of certain tissue extracts with antisera for whole blood, were due to the relatively large amounts of blood still present in such highly vascular organs as the lungs, liver, spleen, etc., from which the heated antigens were made. Extracts of tissues which normally seemed to contain only small amounts of blood, gave little or no reaction with the antiserum for whole blood.

DISCUSSION

The methods of production of immune sera and carrying out anaphylactic tests used in the present investigation proved to be very well adapted for

the demonstration of organ specificity. By the use of autoclaved extracts of aqueous infusions of various tissues, the numerous cross-reactions commonly observed in ordinary serological tests with unheated suspensions of entire organs or with alcoholic extracts of different tissues, were either entirely eliminated or greatly reduced in number. The proteins of the tissue infusions were subjected to hydrolysis by steam under pressure. Our work is somewhat similar to that of Fink (33) who studied the proteose fractions obtained by hydrolysis of egg albumin in the autoclave. This investigator found some evidence of very slight antigenic activity by means of complement-fixation, precipitin, and anaphylactic tests with certain of the hydrolytic fractions of egg white. The fractions were injected into rabbits and guinea pigs, but were not combined with a bacterial vaccine to serve as a carrier of the tissue antigen or hapten as was done in the present experiments to increase the immunizing power of the hydrolytic products.

The results of the present study are interesting because of the relatively high degree of tissue specificity which can be obtained with heated antigens. It would seem that the subjection of aqueous extracts of organs to steam under pressure might increase their immunological specificity in two ways. Zoet (34) has reported the production of artificial horse-swine "hybrid" protein by autoclaving mixtures of the sera. These synthetic or "coupled" proteins were found to possess one or more serological factors not present in horse serum or pig serum. In the case of the extracts of tissues used by us, the union of organ-specific substances with any blood still present might produce new specific immunological factors not common to blood, the organ itself, or to other organs with different combinations. It should be stated in this connection, however, that the findings of Zoet could not be confirmed by Nigg (35). The other way in which autoclaving tissues might increase their specificity would be the destruction of a more thermolabile antigen, leaving some of the greatly predominant organ-specific antigen to exert its immunizing or anaphylactic effects when injected into animals. This would be similar to boiling sheep red cells to destroy the isophile antigen common to both the sheep and the ox, leaving the thermostable heterophile antigen which, in the case of the sheep, is found almost exclusively in the red blood cells. When such boiled red cells are injected into rabbits, highly specific hemolytic sera are produced.

In this connection, it is interesting to consider some of the known thermostable antigens which might be distributed throughout the body and, because of their identity or similarity in different organs, give cross-reactions in immunological tests and thus obscure the reactions of organ-specific substances. It is known that the blood contains such thermostable antigens derived from proteins as shown by the use of boiled serum in the

production of species-specific antibodies for blood serum. Furthermore, species-specific lipoids are thermostable and antigenic under certain conditions. In addition, some lipoid-polysaccharide-protein complexes, such as the Forssman antigen, are highly thermostable. The question now arises as to whether this particular antigen might not interfere with the demonstration of organ specificity in the case of some animals in which it may be more or less uniformly distributed in all the tissues. The production of a serum containing both organ-specific antibodies and heterophile antibodies, with guinea pig brain as the antigen, has been reported by Witebsky (36). In complement-fixation tests with such an antiserum and alcoholic extracts of various organs containing the Forssman antigen, a certain amount of confusion due to cross-reactions is unavoidable. In the present study, passive anaphylactic tests were carried out with the guinea pig which belongs to the Forssman group of animals. When the results of injections of aqueous extracts of different organs of the dog, all the tissues of which contain the Forssman antigen, were compared with the results obtained with extracts of human tissues which do not contain this antigen, no definite differences in specificity were apparent. It would seem therefore that the particular method of passive anaphylaxis we have employed might be applied successfully for the demonstration of organ specificity with the tissues of various animals, irrespective of the presence or absence of the Forssman antigen or similar ubiquitous substances.

SUMMARY

The immunization of rabbits for periods of 6 to 8 weeks with sedimented, heat-killed vaccines of *Pasteurella boviseptica* grown in infusion broths made from six different tissues of the dog and seven tissues of man, caused the production of sera containing antibodies for the broths as well as for the bacteria. The broth made from human fibromyoma of the uterus was the least antigenic of all, as indicated by passive anaphylactic tests in guinea pigs. When these animals were prepared by intraabdominal injection with the rabbit antisera and tested 48 hours later by intravenous injection with autoclaved aqueous extracts of a large number of organs of the dog and man, the guinea pigs were found to be passively sensitized so that severe or fatal anaphylaxis was generally obtained with broths made from the homologous organ and in some instances with those prepared from heterologous organs of the same species. In most instances, the injection of broths from heterologous tissues did not desensitize to later injections of that from the homologous tissue. The most organ-specific antisera were

those for striated muscle, small intestine (ileum), kidney, placenta, and fibromyoma, and the least so those for whole blood, liver, and lung. The cross-reactions of the antiserum for blood were mostly with extracts of tissues which normally contain large amounts of blood. The presence of Forssman antigen in the tissues of the dog did not interfere with the demonstration of organ specificity by the methods used. In general, the results indicate that the various tissues of man and the dog contain thermostable, water-soluble, organ-specific substances which can be demonstrated by passive anaphylaxis in guinea pigs. The chemical nature of these substances has not been definitely determined, although there are some indications that they are protein split-products, probably proteoses.

BIBLIOGRAPHY

1. Bailey, G. H., and Raffel, S., *Am. J. Hyg.*, Section B, 1941, **33,** in press.
2. Bailey, G. H., and Gardner, R. E., *J. Exp. Med.*, 1940, **72,** 499; *J. Immunol.*, 1940, **39,** 543.
3. Witebsky, E., in Bethe, A., von Bergmann, G., Embden, G., and Ellinger, A., Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1929, **13,** 496.
4. Landsteiner, K., The specificity of serological reactions, Springfield, Illinois, Charles C. Thomas, 1936, 63.
5. Fleisher, M. S., and Arnstein, N., *J. Immunol.*, 1920, **5,** 437; 1921, **6,** 223.
6. Witebsky, E., and Steinfeld, J., *Z. Immunitätsforsch.*, 1928, **58,** 271, 279.
7. Lewis, J. H., *J. Immunol.*, 1933, **24,** 193; 1934, **26,** 331.
8. Hahn, F., *Z. Immunitätsforsch.*, 1935, **86,** 31.
9. Schwentker, F. F., and Comploier, F. C., *J. Exp. Med.*, 1939, **70,** 223.
10. Buchholz, A. M., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43,** 20.
11. Witebsky, E., and Klinke, J., *Z. Immunitätsforsch.*, 1933, **78,** 509.
12. de Gaetani, G. F., *Z. Immunitätsforsch.*, 1932, **77,** 43.
13. Cohen, H. R., and Freda, V. C., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43,** 22.
14. Witebsky, E., and Reichner, H., *Z. Immunitätsforsch.*, 1933, **79,** 335.
15. Witebsky, E., and Behrens, H. O., *Z. Immunitätsforsch.*, 1932, **73,** 415.
16. Witebsky, E., and Zeissig, A., *Z. Immunitätsforsch.*, 1932, **76,** 266.
17. Gotoh, Y., *Arb. med. Universität Okayama*, 1931–1933, **3,** 172, 222.
18. Witebsky, E., and Komiya, K., *Z. Immunitätsforsch.*, 1930, **67,** 480.
19. Hirszfeld, L., Halber, W., and Laskowski, J., *Z. Immunitätsforsch.*, 1929, **64,** 61, 81; 1934, **83,** 521.
20. Witebsky, E., and Morelli, E., *Z. Immunitätsforsch.*, 1933, **78,** 179.
21. Sievers, O., *Z. Krebsforsch.*, 1935, **41,** 307.
22. Mann, L. S., and Welker, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43,** 18.
23. Reichner, H., *Z. Immunitätsforsch.*, 1933, **80,** 85.
24. Smadel, J. E., *J. Immunol.*, 1936, **30,** 401; 1937, **32,** 75.
25. Ehrlich, W. E., *Proc. Soc. Exp. Biol. and Med.*, 1937, **35,** 576.
26. Kestner, O., *J. Physiol.*, 1937, **90,** 18P; 1938, **92,** 273.

27. Zinsser, H., Enders, J. F., and Fothergill, L. D., Immunity: principles and application in medicine and public health, New York, The Macmillan Co., 5th edition, 1939, 343.
28. Pfeiffer, H., *Z. Immunitätsforsch., Orig.*, 1911, **8,** 358.
29. Ranzi, E., *Z. Immunitätsforsch., Orig.*, 1909, **2,** 12.
30. Pearce, R. M., Karsner, H. T., and Eisenbrey, A. B., *J. Exp. Med.*, 1911, **14,** 44.
31. Kraus, R., Doerr, R., and Sohma, M., *Wien. klin. Woch.*, 1908, **21,** 1084.
32. von Dungern, E., and Hirschfeld, L., *Z. Immunitätsforsch., Orig.*, 1910, **4,** 257.
33. Fink, E. B., *J. Infect. Dis.*, 1919, **25,** 97.
34. Zoet, A. G., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1469.
35. Nigg, C., *J. Immunol.*, 1937, **33,** 229.
36. Witebsky, E., *Z. Immunitätsforsch.*, 1928, **58,** 297.