THE TISSUE SPECIFICITY OF BRAIN AND MEDULLATED NERVES AS SHOWN BY PASSIVE ANAPHYLAXIS IN GUINEA PIGS

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The organ specificity of certain kinds of antisera has been shown by ordinary serological tests, such as the precipitin and complement-fixation reactions with aqueous and alcoholic extracts of tissues as antigens, or by the action of cytotoxins on fresh suspensions of different organs and on tissue cultures. The cellular specificity of certain anti-organ sera is still not definite, however, because of confusing species-specific effects. The degree of tissue specificity obtained in various instances depends upon the organs tested and the particular method and technique employed. In the case of the brain, most investigators have used the complement-fixation test with brain immune sera and alcoholic extracts of brain. Thus, it is known from the work of Brandt, Guth, and Müller (1), Witebsky and Steinfeld (2), Heimann and Steinfeld (3), Lewis (4), and others (5–8), that the brain has immunological properties that make this tissue almost as organ-specific as the lens of the eye.

It has been demonstrated that an alcohol-soluble lipoid of brain functions as a hapten and that when it is mixed with heterologous protein, such as pig serum, a complete antigen is formed capable of inciting in animals the development of complement-fixing antibodies. By immunizing with foreign brain suspensions, Witebsky and Steinfeld obtained two varieties of immune sera. Some were organ-specific as well as species-specific, while others exhibited organ specificity by reacting only with substances contained in alcoholic extracts of the brains of various animals. It was found that most brain immune sera react with boiled as well as native suspensions of brain, showing that the specific hapten has considerable thermostability. It was further shown that fresh suspensions of homologous brain possess little or no antigenicity. However, Schwentker and Rivers (9) obtained complement-fixing antibodies by immunization of rabbits with sterile emulsions of autolyzed rabbit brains and those prepared from the brains of rabbits experimentally infected with vaccine virus. The specific antigen seemed to run parallel with the myelin content of the brains of rabbits of different ages. The exact chemical

nature of the specific hapten has not been determined. The work of Rudy (10) and Sachs and Schwab (11) indicates that the active substances are lipoids and that there are two or more of them of different chemical constitution and physical properties, as shown by differences in their solubilities in hot and cold alcohol. They were found to be more or less insoluble in water except in the case of a purified and saponified fraction of brain prepared by Rudy.

In previous studies, Bailey and Raffel (12) and Bailey and Gardner (13) investigated the specificity of various organ immune sera by means of the anaphylaxis reaction with water-soluble specific substances in broths prepared from a large number of tissues. It was found that prolonged immunization of rabbits with sedimented bacterial vaccines grown in broths made from different organs of animals and man, caused the formation of specific antibodies for the various broths as well as for the bacteria. It was demonstrated that fatal anaphylaxis usually followed the injection of the homologous broth, as *e.g.* that prepared from striated muscle, into guinea pigs passively sensitized with the antisera of rabbits immunized in this way. These methods have proved to be especially well adapted for the demonstration of organ specificity with autoclaved extracts of various tissues, particularly with brain.

EXPERIMENTAL

Since a number of workers (2, 3) have already shown the organ specificity of brain by the method of complement-fixation with brain immune sera and alcoholic extracts of fresh brain, we were interested to see whether such specificity could be demonstrated with water-soluble products of heated brain, that is, with brain broth. If this proved to be so, we planned to study some of the chemical characteristics of the antigen and the neural distribution of the substance from which it is derived. To this end, anaphylactic tests with guinea pigs passively sensitized with rat brain immune rabbit serum were carried out to determine (*a*) organ specificity of brain as shown by injections of autoclaved extracts of partially autolyzed aqueous suspensions of brain and other tissues of the rat, (*b*) antigenic differences between such extracts made from the white and gray matter of the nervous system, (*c*) presence or absence of a thermostable specific anaphylactogen in broths made from the brains of different species of animals, fetuses, and embryos of different ages, (*d*) antigenic relationships of autoclaved extracts of carcinoma 256, sarcoma 10, and brain of the rat, (*e*) relation of the hydrogen ion concentrations of autoclaved extracts of brain to the activity of the specific anaphylactogen, (*f*) activity of autoclaved residues of alcoholic extracts of brain, and (*g*) effect of dialysis on the anaphylactic activity of

brain broth. The passively sensitized guinea pigs were injected with the various extracts and, if an animal survived, it was injected one hour later with the homologous rat brain extract to determine possible antigenic relationships as shown by specific desensitization.

Methods

Brain Broth for Growth of Bacterial Antigen.—The brains of normal Philadelphia albino rats, sacrificed by opening the thorax and clipping the heart after slight etherization, were removed, weighed, and ground in a mortar with two parts by weight of distilled water. The pH of the suspension at this stage was 6.9. After infusion in the ice box for 48 hours, the pH had changed to 7.1. The somewhat autolyzed infusion was adjusted to pH 6.9, autoclaved at 122°C. for 15 minutes, centrifuged, the supernatant saved, 0.5 per cent by weight of sodium chloride added to it, put in 200 cc. Erlenmeyer flasks and sterilized in the autoclave at 122°C. for 20 minutes. The pH of the extract was then tested and found to be unchanged. No further adjustment was made.

Bacterial Antigen.—After the addition of sterile Difco peptone and glucose to 1 and 0.5 per cent respectively, 100 cc. of the brain broth described above was inoculated with 0.01 cc. of a 24 hour beef infusion broth culture of *Pasteurella boviseptica* and incubated at 37.5°C. for 36 hours when a very heavy growth was obtained. The culture was centrifuged, all of the supernatant decanted and discarded, the 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 were immunized by two or three weekly injections of increasing doses of 0.05 to 0.5 cc. of the concentrated bacterial vaccine 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 ice box until needed for passive sensitization of guinea pigs to brain broth.

Antigens for Anaphylactic Tests.—Normal rats (Philadelphia albino), guinea pigs, and rabbits were sacrificed by almost complete exsanguination from the heart after slight etherization. The various organs of the rats were removed, adherent connective tissue and fat dissected away, washed with distilled water, weighed, and thoroughly ground in mortars. The tissues were suspended in two times their weights of a 0.5 per cent solution of sodium chloride, infused in the refrigerator at 8°C. for 48 hours, autoclaved at 122°C. for 15 minutes, centrifuged at high speed while still hot, and the supernatants saved and stored in the ice box for 72 hours to allow most of the fats to rise to the surface and solidify. While cold, the layers of fat were punctured with capillary pipettes and the nearly clear fluids drawn off, made up to the original volumes with 0.5 per cent saline, tubed, and sterilized in the autoclave at 122°C. for 20 minutes. After cooling the pH of the different extracts was then determined by the colorimetric method. With the exception of the antigens prepared from the various brains and other parts of the nervous system, the pH of the different tissue extracts was not adjusted at any time. Infusions of brain were divided into two or more lots before heating and the pH adjusted to neutrality or on the acid or alkaline side of this point. The infusions were then autoclaved at 122°C. for 15 minutes and treated in the same manner as the other extracts except that the pH of each lot was readjusted to that desired before the second and final autoclaving.

Autoclaved Residue of Alcoholic Extract of Ox Brain.—Crude protogon or "white substance" was prepared from the white matter of ox brain. The tissue (100 gm.) was cut into small pieces and dehydrated and hardened by two extractions for 24 hours each time in the ice box with three volumes of cold 90 per cent alcohol. By this treatment the proteins were coagulated and most of the salts and extractives removed. The hardened brain substance was then put through a meat grinder and further reduced to a fine purée in a mortar. The material was then extracted five times successively by shaking in a flask with 85 per cent alcohol at 70°C., and filtered through paper on a hot funnel after each extraction. The combined filtrates were put in the ice box and after 2 days a large quantity of a white precipitate which appeared crystalline under the microscope, but which was not homogeneous, had settled out. It was collected on a filter in the cold, spread out in a thin layer, and freed from alcohol with an electric fan. The material (5 gm.) was ground up in a mortar with 25 cc. of a 0.5 per cent solution of sodium chloride, infused in the ice box for 48 hours as for the preparation of broth, and the pH tested and found to be about 6.5. After autoclaving at 122°C. for 15 minutes, the emulsion was centrifuged at high speed, and the turbid supernatant saved and sterilized in the autoclave at 122°C. for 20 minutes. It was used in anaphylactic tests.

Dialysis of Autoclaved Aqueous Extract of Ox Brain.—Sterile peptone-free ox brain broth (10 cc.) was pipetted into a No. 600 cellophane (E. I. du Pont de Nemours) bag attached at the top with rubber bands to a broken test tube stoppered with cotton. The bag and attached tube had been previously sterilized in the autoclave after wetting with water and wrapping in paper. The bag containing the brain broth was suspended for 6 days in a large beaker into which tap water at 20°C. and with a pH of 8.5, was kept running through a glass tube. After this period of dialysis, the bag was suspended in a jar containing 2000 cc. of distilled water at pH 5.8, in the ice box for 24 hours. The level of the liquids was adjusted so that the dialysate would concentrate to about one-half the volume of the original brain broth. This dialysate was tested for anaphylactogenic activity.

Separation of Ox Brain Broth into Two Fractions with Trichloroacetic Acid.—Cold peptone-free ox brain broth (10 cc.) was put in a chilled 50 cc. centrifuge tube and treated with 10 cc. of cold 32 per cent trichloroacetic acid added slowly with shaking. A heavy white turbidity developed and after standing in the ice box for 18 hours a flocculent precipitate had settled out. This was centrifuged off in the cold, the supernatant decanted and saved, the precipitate suspended in 25 cc. of cold 95 per cent alcohol, stirred well, and the white flocculi centrifuged down while cold. This washing was repeated twice with 10 cc. of cold alcohol, the final precipitate dried with an electric fan, and the brown amorphous material almost completely dissolved in 2.5 cc. of 0.85 per cent solution of sodium chloride, centrifuged, and the somewhat opalescent supernatant saved for an anaphylactic test.

The first trichloroacetic acid supernatant (20 cc.), in a cold 250 cc. centrifuge bottle, was treated with 225 cc. of cold absolute alcohol and 2 cc. of 25 per cent sodium acetate, put in the ice box for 18 hours, centrifuged, and the supernatant discarded. The precipitate was suspended in absolute alcohol, removed to a Petri plate, dried with a fan, and desiccated over calcium chloride. The small amount of white amorphous powder which completely dissolved in 2 cc. of 0.85 per cent solution of sodium chloride, was used for an anaphylactic test.

Anaphylactic Tests.—Guinea pigs of 225 to 275 gm. in weight were prepared for anaphylactic tests by intraabdominal injection of 2 cc. of the previously described pooled

antiserum for broth made from rat brain, and tested for passive sensitization after 24 hours by injection into the saphenous vein with 2 cc. of autoclaved extracts of different organs of the rat and the brains and other parts of the nervous system of several species of animals. In case the guinea pig lived, the primary injection was followed one hour later by injection into the same vein or opposite vein with 1 cc. of autoclaved extract of rat brain to test for possible antigenic relation which might be shown by desensitization.

Organ Specificity of an Antiserum for Rat Brain Broth

In order to test the specificity of an antiserum for rat brain broth, autoclaved infusion extracts previously described were used. These had been prepared from twenty-six normal tissues of the rat, including brain, and also mixed white and gray matter of normal ox brain. In addition, extracts made in the same manner from rat carcinoma 256 and sarcoma 10, were tested for comparison with extracts from normal tissues. The results of the tests for organ specificity, as shown by passive anaphylaxis in guinea pigs, are summarized in Table I. It will be observed that with the extracts of normal tissues there was almost complete specificity for nervous tissue, as shown by rapidly fatal anaphylaxis with the antigens obtained from rat and ox brains and that from the sciatic nerve of the rat. A definite reaction occurred with the extract of abdominal muscle. It is possible that this was due to the presence of medullated nerve fibres in the muscle from which the extract was prepared, since no attempt was made to free any of the tissues from their normal neural elements. Excluding brain and sciatic nerve, the most marked reaction in a guinea pig passively sensitized to brain broth, was obtained with carcinoma 256. This transplantable tumor of the rat originated in the mammary gland. It will be seen that an autoclaved extract of the tumor caused a severe, almost fatal, reaction. There was a slight reaction to sarcoma 10 which came originally from the rat liver.

All of the extracts used in this series of tests had hydrogen ion concentrations between pH 6.5 and 7.0. Within this range, the unadjusted variations of pH did not appear to affect the reactivity of the antigens or the uniformity of the results of the anaphylactic tests. None of the broths used were primarily toxic for guinea pigs, not even those with a pH as low as 6.5. In the next section of this paper, some further attention will be given to the effects of acid, alkaline, and neutral reactions on the specific antigen of broths made from tissues of the nervous system of different species.

Conditions Affecting the Amount of the Specific Anaphylactogen in Brain Broths

Experiments were carried out to determine how the amount of the specific anaphylactogen in broths prepared from tissues of the nervous system, is

TABLE I

Organ Specificity of an Antiserum for Rat Brain Broth as Shown by Anaphylactic Tests with Guinea Pigs Injected with Autoclaved Aqueous Extracts of Different Tissues

Guinea pigs passively sensitized to rat brain broth	Rat organ broth tested for production of shock		Reaction* after injection of		Result—lived (L) or time of death in min.
	Prepared from	pH	Heterologous rat organ broth	Homologous rat brain broth	
1	Brain	7.0		4+	$3\frac{1}{2}$
2	" (ox)	7.0		4+	$3\frac{1}{2}$
3	Gluteal muscles	6.5	±	3+	L
4	Abdominal "	6.5	+	±	L
5	Diaphragm	6.6	±	2+	L
6	Heart	6.8	−	4+	$2\frac{3}{4}$
7	Stomach	6.5	±	2+	L
8	Small intestine	6.5	−	4+	$3\frac{3}{4}$
9	Colon and cecum	6.5	−	4+	4
10	Bladder	7.0	±	4+	45
11	Aorta and vena cava	7.0	−	4+	$2\frac{3}{4}$
12	Lung, bronchi	7.0	±	+	L
13	Liver	6.6	−	4+	$3\frac{1}{2}$
14	Kidney	6.8	±	±	L
15	Pancreas	6.5	−	2+	L
16	Omentum	6.5	−	2+	L
17	Spleen	6.9	±	−	L
18	Thymus	6.9	−	4+	$5\frac{1}{2}$
19	Uterus	6.8	±	4+	$3\frac{1}{4}$
20	Sciatic nerve	6.8	4+		$2\frac{1}{2}$
21	Testicle	6.9	−	4+	30
22	"	6.8	−	3+	L
23	Seminal vesicle, sperm	6.8	−	4+	$5\frac{1}{4}$
24	Prostate	6.8	−	4+	$5\frac{1}{2}$
25	Lens	6.9	−	3+	L
26	Skin	6.7	±	±	L
27	Cartilage (sternal)	6.8	−	4+	$4\frac{1}{2}$
28	Fat (perirenal)	7.0	±	2+	L
29	Blood (whole)	7.0	−	4+	$3\frac{3}{4}$
30	Carcinoma 256	6.9	3+	−	L
31	Sarcoma 10	6.9	+	2+	L
32†	Brain	7.0		−	L
33†	" (ox)	7.0		−	L
34†	Sciatic nerve	6.8		−	L
35†	Carcinoma 256	6.9	−		L

* 4+ = fatal reaction; 3+ = severe, almost fatal, reaction; 2+ = moderate reaction; + = slight but definite reaction; ± = very slight or questionable reaction; − = no reaction. The signs, symptoms, and autopsy findings were all typical of anaphylactic shock.

† Normal controls used to test for primary toxicity of the broths which gave definitely positive reactions in passively sensitized animals.

related to the species, age, and stage of development of the animal and is affected by pH, dialysis, and certain other conditions. The results of these tests are shown in Table II. It is evident that the highly thermostable, water-soluble antigen of autoclaved brain is not species-specific, because severe or fatal anaphylaxis was obtained in guinea pigs passively sensitized with an antiserum for rat brain when they were injected with extracts of the brains of either the rat, guinea pig, rabbit, ox, or man. Furthermore, the positive results with extracts of the white matter of ox brain and the sciatic nerves of the rat and rabbit, demonstrate that the antigen is closely associated with myelin and is probably derived from it. This is also indicated by the fact that an extract of the gray matter of ox brain gave only a very slight or doubtful reaction. In addition, the stage of development of the particular animal and the degree of myelinization are definitely related to the amount of the specific antigen present in the nervous system. A study of these relationships was made possible by the fact that myelin is practically absent from the central nervous system of fetal and newly born animals of certain species and increases with the age of the animal. In order to test this fact in relation to the presence or absence of the specific anaphylactogen of brain, broths were prepared from the brains of rats, guinea pigs, and rabbits of different ages ranging from the fetal stage to maturity. The results of tests with these broths are shown in Table II. It is immediately apparent that in fetal and newly born rats and rabbits the brain-specific antigen is so small as to be almost negligible, since the sensitive anaphylactic tests for its presence were very slight or entirely negative. In the case of the rat, the antigen probably does not appear in the brain in definitely detectable amount until some time between the 9th and 17th days after birth. However, there seemed to be at least a sufficient amount of the antigen to cause more or less complete desensitization of the guinea pigs injected with the extracts of the brains of rats in various stages of their prenatal and postnatal development, except in the case of a 9th day embryo. In this instance, there was only slight desensitization. An extract of the brain of a fetal rabbit, the age of which was not known, also did not desensitize very much, while that from a rabbit one hour after birth produced no reaction, but it did completely desensitize. This was also true for the brain of a guinea pig embryo. However, the antigen was present in the brain of a guinea pig 2 days after birth and apparently in about the same amount as in adult guinea pigs. The pH of these particular extracts was adjusted purposely to 7.2 in order to be definitely alkaline and somewhat destructive of the anaphylactogen, with the aim of showing any differences in the amounts of the specific substance in the brains of guinea pigs at different

TABLE II

Amount of Specific Anaphylactogen in Autoclaved Aqueous Extracts of Brain as Influenced by Species and Stage of Development of Animal, pH, and Dialysis of Heated Infusion

Guinea pigs passively sensitized to rat brain broth	Brain broth tested for production of shock: Species source and age of animal	pH	Reaction* after injection of: Primary test broth	Known active brain broth	Result—lived (L) or time of death in min.
1	Rat (10 mos.)	7.3	+	−	L
2	" (10 ")	6.8	4+		3¾
3	" (10 ")	6.5	4+		3
4	" (9 ")	7.4	−	−	L
5	" (9 ")	7.0	4+		2¾
6	" (9 ")	6.8	4+		2½
7	" (8 ")	7.4	±	−	L
8	" (8 ")	7.1	4+		2¾
9	" (8 ")	6.8	4+		2½
10	" (6 ")	6.8	4+		2½
11	" (5 ")	6.8	4+		3
12	" (2½ ")	7.1	4+		2¾
13	" (2½ ")	6.7	4+		2½
14	" (17 days)	7.1	3+	−	L
15	" (17 ")	6.8	4+		2¾
16	" (9 ")	6.8	±	−	L
17	" (12 hrs.)	6.8	±	±	L
18	" (1 hr.)	6.8	−	±	L
19	" fetus (17th day)	6.8	±	±	L
20	" embryo (9th ")	6.8	−	3+	L
21	" sciatic nerve (8 mos.)	6.8	4+		2¼
22	Guinea pig (6 mos.)	6.8	4+		3½
23	" " (6 ")	7.2	2+		L
24	" " (2½ ")	7.2	3+	−	L
25	" " (25 days)	7.2	2+	−	L
26	" " (2 ")	7.2	2+	−	L
27	" " (embryo)	7.2	±	−	L
28	Rabbit (5 yrs.)	7.0	4+		3½
29	" (2 ")	7.0	4+		3¾
30	" (1 yr.)	7.0	4+		2¾
31	" (1 hr.)	7.0	−	−	L
32	" (fetus)	7.0	−	3+	L
33	" spinal cord	7.1	4+		3½
34	" sciatic nerve	6.8	4+		3¼
35	Hog	7.1	4+		2¾
36	Ox cerebral white matter	7.0	4+		3¼
37	" " gray "	7.0	±	±	L
38	" white matter, dialyzed		4+		8
39	" protogon, soluble in 85% alcohol	6.5	−	4+	2¾
40	" brain broth, 16% CCl_3COOH precipitate	7.0	−	−	L
41	" " " " " " supernatant	7.0	−	−	L
42	Human white, gray matter	7.1	3+	−	L

* The signs indicating the degree of shock are to be interpreted as in Table I. The broths were prepared only from brains except in the cases of the spinal cord and sciatic nerve. In the case of 9th day rat embryos, whole heads were used.

stages of development. No definite differences could be detected in the brains of these animals after birth.

The results of tests for the thermostable brain-specific antigen shows that there are variations in amount, not only according to age but also with the species. Furthermore, the results with the brains of rats, guinea pigs, and rabbits are in agreement with what might be expected when the periods of gestation of these different species are considered. At the time of birth the guinea pig is well advanced in its development, almost with the same physical activity and habits as the mature animal, while the rat and the rabbit at this period are still essentially fetal in these respects. The development and myelinization of the central nervous systems of the last two animals are known to be considerably delayed after birth. These facts are in agreement with the present findings in that the thermostable specific anaphylactogen of brain seems to run parallel with the myelin content of this organ, although no anaphylaxis or desensitization whatever was obtained with the lipoids of myelin in the form of autoclaved protogon.

At this point we should consider further the effects of hydrogen ion concentration on the specific substance of brain broth. These effects are well shown in the tests with guinea pigs 1 to 15, and 22, 23, and 24 (Table II). It is evident that when aqueous suspensions of brain at pH 7.3 to 7.4 are autoclaved at 122°C. for 15 minutes the specific antigen is more or less completely destroyed, while reactions between pH 6.5 and 7.0 are not appreciably injurious. There appears to be slight destruction even at pH 7.1 as shown by a comparison of the anaphylactic tests in guinea pigs 14 and 15. These results are in agreement with what is known about the action of alkalis on antigens in general. In a previous study (12) it was found that the specific anaphylactogen of broth made from ox striated muscle was completely destroyed by boiling for 2 hours in N/5 NaOH but was entirely resistant to such treatment in N/5 HCl. This antigen was also resistant to autoclaving at pH 7.6. It would seem therefore that there is considerable difference in the thermostability of the organ-specific anaphylactogens of brain and striated muscle in alkaline solution.

As to certain other properties of the antigen of brain broth, we have determined that the specific substance does not dialyze through a cellophane membrane, is highly soluble in water but insoluble in 75 to 90 per cent ethyl alcohol, and does not appear to be a lipoid, at least it does not possess the lipoidal properties of the brain-specific antigens described by other investigators. After treatment in the cold with strong trichloroacetic acid and alcohol, neither the precipitate so obtained nor any substance remaining in the supernatant fluid still possessed the power to produce anaphylaxis, but

both had desensitizing properties for untreated brain broth. A further study of the chemical properties of the active substance of this broth is now being carried on.

DISCUSSION

The present investigation is of special interest not as a further proof of the organ specificity of brain, but because such specificity has been demonstrated by the reaction of anaphylaxis and with the demonstration of a highly specific substance in broth prepared from a definite part of brain. It was shown that autoclaved extracts of the white matter of the central nervous system and medullated peripheral nerves contain water-soluble substances which are immunologically indistinguishable in anaphylactic tests. Such antigenic identity was to be expected from anatomical considerations and in fact had been previously shown by others (7, 14) by means of complement-fixation tests with alcoholic extracts of the tissues. The results with such extracts, however, had led most investigators to conclude that the brain-specific antigens or haptens have the properties of lipoids only. Two lipoid haptens, one specific for white matter and the other for gray matter, have been reported (14). These have been further differentiated, on the basis of differences in their solubilities in alcohol and other lipoid solvents, as the ordinary brain hapten and as protogon (11). A purified serologically active preparation of the latter made by Rudy (10) was found to be free of phosphorus and to contain nitrogen and bound sugar. It was not definitely determined, however, whether nitrogen and sugar are characteristic of this brain hapten. The specific activity was not associated with stearin, cerebroside, or creatin. Progressive purification of the substance increased its dialysis through parchment membrane and its solubility in water, especially in alkaline solution in which it appeared to be stable and to retain its serological activity even after more or less complete saponification. It was believed that the substance had a fat-like structure and that the primary high solubility in alcohol and low solubility in water were due to its adsorption on lipoids.

The anaphylactogen we have studied in the present investigation was found to be characteristic only of the white matter of the central nervous system and of medullated peripheral nerves, and to be very thermostable, the antigens of gray matter being relatively thermolabile. These results show that the antigenic substance of brain broth is derived from myelin. This is further demonstrated by the results with autoclaved extracts of the brains of animals in various stages of development. The active substance is insoluble in strong alcohol and is unstable in hot alkaline solution.

Although it is very soluble in water, it does not dialyze through an ordinary cellophane membrane. That the substance is not a derivative of a lipoid, such as protogon, is shown by the fact that an autoclaved residue of an alcoholic extract of brain was completely inactive as an anaphylactogen. All of these properties indicate that the antigen of brain broth has a high molecular weight and that it may be either a polysaccharide complex or a protein derivative produced by the subjection of brain to steam under pressure. The latter possibility would seem to be the more probable in view of the work of Fink (15) on the hydrolytic products of egg albumin. Furthermore, our findings of an antigenic relationship between rat brain and rat carcinoma 256, derived from a mammary gland, would seem to point in the same direction and to suggest that the substance common to both may be keratin or a precursor of this substance. The chemical similarity of the keratins of the brain and skin, both of ectodermal origin, has been shown by Block (16). It has long been known that the myelin sheaths of medullated nerves contain so called neurokeratin which can be demonstrated by histological and chemical methods (17). It is known too that tumors from squamous epithelium may develop keratin. In addition, it has been found that brain and cancer tissues are much alike in certain of their biochemical activities, such as rapid glycolysis. Further studies are now being carried on by us in an attempt at the isolation and identification of the substance which rat brain and carcinoma 256 appear to have in common.

SUMMARY

Prolonged immunization of rabbits with a sedimented, heat-killed vaccine of *Pasteurella boviseptica* grown in an infusion broth prepared from rat brain resulted in the production of antisera containing antibodies for the broth as well as for the bacteria. When guinea pigs were prepared by intra-abdominal injection with such antisera and tested 24 hours later by intravenous injection with autoclaved extracts of different organs and tumors of rats, they were found to be passively sensitized, so that severe or fatal anaphylaxis was obtained with extracts of brain and carcinoma 256,—a transplantable tumor of the rat which originated in the mammary gland,—and very slight or negative reactions with extracts of other tissues. The brain antigen was found to be organ-specific but not species-specific. It was present in the white matter of the central nervous system and in sciatic nerve, but was almost completely absent from the brains of fetal and newly born rats and rabbits. It was absent from the brain of the fetal guinea pig but was present very soon after birth. The amount of the specific brain

antigen seemed to be dependent upon the length of the period of gestation, the stage of development of the animal at birth, and the degree of myelinization of the central nervous system. The anaphylactogen of brain broth was soluble in water and insoluble in strong ethyl alcohol. It was thermostable in neutral and slightly acid solutions but more or less thermolabile on the alkaline side of neutrality.

BIBLIOGRAPHY

1. Brandt, R., Guth, H., and Müller, R., *Klin. Woch.*, 1926, **5**, 655.
2. Witebsky, E., and Steinfeld, J., *Centr. Bakt., 1. Abt., Orig.*, 1927, **104**, 144; *Z. Immunitätsforsch.*, 1928, **58**, 271, 279.
3. Heimann, F., and Steinfeld, J., *Z. Immunitätsforsch.*, 1928, **58**, 181.
4. Lewis, J. H., *J. Immunol.*, 1933, **24**, 193; 1934, **26**, 331.
5. Plaut, F., and Kassowitz, H., *Z. Immunitätsforsch.*, 1929, **63**, 428.
6. Moran, F., *Z. Immunitätsforsch.*, 1930, **67**, 115.
7. Plaut, F., *Z. Immunitätsforsch.*, 1934, **82**, 65.
8. Krüpe, M., *Z. Immunitätsforsch.*, 1935, **85**, 487.
9. Schwentker, F. F., and Rivers, T. M., *J. Exp. Med.*, 1934, **60**, 559.
10. Rudy, H., *Biochem. Z.*, 1933, **267**, 77; *Klin. Woch.*, 1934, **13**, 4.
11. Sachs, H., and Schwab, E., *Schweiz. med. Woch.*, 1935, **16**, 547.
12. Bailey, G. H., and Raffel, S., *Am. J. Hyg.*, 1941, in press.
13. Bailey, G. H., and Gardner, R. E., *J. Immunol.*, 1940, in press.
14. Reichner, H., and Witebsky, E., *Z. Immunitätsforsch.*, 1933–34, **81**, 410.
15. Fink, E. B., *J. Infect. Dis.*, 1919, **25**, 97.
16. Block, R. J., *J. Biol. Chem.*, 1932, **94**, 647; *Yale J. Biol. and Med.*, 1936–37, **9**, 445.
17. Hatai, S., *J. Comp. Neurol.*, 1903, **13**, 149.