THE ANTIBODY RESPONSE OF RABBITS TO INJECTIONS OF EMULSIONS AND EXTRACTS OF HOMOLOGOUS BRAIN

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Since the discovery of hemolysins by Bordet attempts have been made to demonstrate antibodies against practically every type of cell and tissue in the body. Most of the efforts were prompted by the idea that certain diseases, for example nephritis and diabetes, characterized by degenerative changes in the affected organs, might be due to the development of antibodies against the tissues of the particular organ involved. However, the early claims made for the specificity of organ antibodies have been discounted to such an extent by later more seasoned work that the theories evolved from them have been discarded in the majority of instances. In the period since 1926, Brandt, Guth, and Müller (1) and others (2, 3, 4) have demonstrated that brain tissue contains an alcohol-soluble lipoid which functions as a haptene and that when this lipoid is mixed with a heterologous protein a complete antigen is formed capable of inciting in animals the development of complement-fixing antibodies which are organ- rather than species-specific. This discovery again opens, for brain at least, the question of the etiological rôle of organ-specific antibodies in certain degenerative diseases and strengthens materially the hypothesis that the encephalomyelitis which follows antirabies vaccination, if not the entire group of postinfection encephalitides, is in some manner associated with the development of specific antibodies for brain.

EXPERIMENTAL

Since a number of workers (2, 4) have already shown that fresh emulsions of homologous brain possess little or no antigenicity and that only when a heterologous protein is added to the brain haptene does it become a complete antigen, we were interested to see whether certain degenerative processes such as autolysis are capable of altering

homologous brain material to such an extent that it becomes a complete antigenic complex. If this proved to be so, we planned to study the characteristics of the antigen as well as of the antibodies elicited by it. To this end rabbits were injected (1) with freshly prepared emulsions of rabbit brain, (2) with similar emulsions to which pig serum had been added, (3) with sterile emulsions of rabbit brain that had been allowed to stand at room temperature for 5 to 30 days, (4) with fresh emulsions prepared from rabbit brains experimentally infected with vaccine virus, and (5) with alcoholic extracts of rabbit brain plus pig serum. Following the injections sera of the animals were examined by means of complement fixation tests for the presence of antibodies against aqueous emulsions and alcoholic extracts of various organs.

Methods

Rabbits.—Only young mature rabbits averaging 2000 gm. in weight were used. To insure a maximum uniformity in the antibody response, only pure-strain self-blue English rabbits received injections. The rabbits which received injections of emulsions of brain infected with vaccine virus were immunized 2 weeks prior to the initiation of the experiment by means of intradermal injections of 0.25 cc. of a 1:10 dilution of vaccine virus prepared in tissue culture according to the method of Rivers (5).

Emulsions of Fresh Brain.—5 gm. of brain tissue removed aseptically from a rabbit were ground in a mortar with alundum until thoroughly macerated. 80 cc. of Locke's solution were added by increments during the process of grinding until the tissue was well emulsified. The suspension was then centrifuged at low speed for 2 minutes and the supernatant fluid removed by means of a pipette. The inoculum was prepared by the addition of 10 cc. of Locke's solution to each 40 cc. of the supernatant fluid.

Emulsions of Fresh Brain Plus Pig Serum.—Emulsions of fresh brain plus pig serum were prepared in a manner similar to that just described for the preparation of emulsions of fresh brain, except that in the final step, 10 cc. of pig serum, instead of Locke's solution, were added to each 40 cc. of the centrifuged suspension.

Emulsions of Autolyzed Brain.—Brain tissue was ground directly to a 5 per cent emulsion in a manner similar to that described for the preparation of emulsions of fresh brain. The supernatant fluid from the centrifuged suspension was transferred to a sterile flask that was then closed by a cotton plug over which were placed several layers of tin-foil. This material was allowed to stand at room temperature during the course of the experiment. As required for the injections, portions of the emulsion were removed from 5 to 30 days after preparation. Before use and from time to time during the experiment samples of the emulsion were tested for the presence of bacterial contaminants by means of aerobic and anaerobic

cultures made in meat infusion broth (pH 7.8) and on blood agar. In no case during the course of an experiment were bacteria grown from emulsions found sterile at the start.

Emulsions of Brain Infected with Vaccine Virus.—A rabbit was inoculated intracerebrally with 0.25 cc. of a 5 per cent emulsion of rabbit brain infected with the Levaditi strain of vaccine virus. On the 3rd day after injection usually at the height of the infection the rabbit was killed. A 5 per cent emulsion in Locke's solution was prepared from the brain, the technique being similar to that described for the preparation of emulsions of fresh normal brain. The absence of ordinary bacterial contaminants in the brain was shown by means of aerobic and anaerobic cultures made in meat infusion broth (pH 7.8). On each occasion an additional rabbit was inoculated intracerebrally with a portion of the emulsion in order to furnish brain material for the next immunizing injection.

Alcoholic Extracts.—Alcoholic extracts of brain were prepared in the following manner: A known amount of tissue was thoroughly macerated in a mortar to which were added 5 cc. of 95 per cent ethyl alcohol for each gram of brain. The mixture was transferred to a flask which was then closed by a well fitting cork and allowed to stand at room temperature for 5 to 10 days. Then the material was passed through filter paper. In order to make immunizing emulsions 10 cc. of the filtrate were evaporated almost to dryness on the water bath and the residue was emulsified in 20 cc. of Locke's solution to which 5 cc. of pig serum had been added. For the experiments in which alcoholic extracts of organs other than brain were used the method of preparation of the extracts was the same.

Immunization of Rabbits.—Each rabbit received intraperitoneally 10 injections of 5 cc. of the brain emulsions or brain extracts. The inoculations were made at intervals of 2 or 3 days, and 10 days after the last injection the animals were bled from ear veins to furnish sera for the complement fixation tests.

Complement Fixation Test.—The antisheep-cell system was used in the complement fixation tests. 0.2 cc. of diluted inactivated antiserum from rabbits that had received the different preparations of brain, 0.2 cc. of antigen (organ emulsions or extracts), and 0.2 cc. of guinea pig serum diluted so as to contain 2 units of complement, were mixed in a Wassermann tube and incubated for 30 minutes at 37°C. in a water bath. 0.2 cc. of 5 per cent suspension of washed sheep cells and 0.2 cc. (2 units) of antisheep-cell amboceptor were added and the tubes again incubated for 30 minutes at 37°C. The results of the tests were recorded in terms of the amount of fixation of complement, from 4+ indicating total complement fixation and no hemolysis to − designating complete laking of the sheep cells. Two types of antigen were used in the tests, aqueous emulsions and alcoholic extracts. In order to make aqueous antigens 1 gm. of the particular organ desired was thoroughly ground in a mortar with alundum, and 25 cc. of physiological saline solution were added by increments during the process of grinding until a homogeneous emulsion resulted. This suspension was filtered through a tightly folded pad of absorbent cotton to remove the alundum and large bits of tissue. When less

concentrated emulsions were used, the necessary dilutions were made from the original 1:25 suspension. The preparation of alcoholic antigens was accomplished by the evaporation of a known volume of the alcoholic organ extract almost to dryness on a water bath and the suspension of the residue in a given volume of physiological saline solution. The concentration of this antigen was designated by the ratio between the original volume of alcoholic extract used and the amount of saline solution in which the residue was resuspended.

TABLE I

Summary of Results of Complement Fixation Experiments Indicating the Presence or Absence of Antibodies in the Sera of Rabbits Injected with Various Emulsions and Extracts of Homologous Brain

Material used for immunization	Amount of complement fixed by different antisera in the presence of an aqueous emulsion of rabbit brain					
	Dilution of serum					
	1:5	1:10	1:20	1:40	1:80	1:160
Fresh rabbit brain	−	−	−	−	−	−
" " " plus pig serum	4+	4+	2+	−	−	−
Autolyzed rabbit brain	4+	4+	4+	4+	2+	−
Vaccine virus rabbit brain	4+	4+	4+	4+	1+	−
Alcoholic extract of rabbit brain plus pig serum	4+	4+	1+	−	−	−

4+ indicates complete fixation of complement.

− indicates no fixation.

The results given for alcoholic extract of rabbit brain plus pig serum were taken from another experiment and are therefore not strictly comparable to the others.

Antigenicity of Homologous Brain

In order to test the antigenic qualities of homologous brain six experiments were performed, in which 79 rabbits were used. The animals in each experiment were divided into groups of four or five, and each group of animals received injections of one of the preparations of homologous brain tissue described above. For the most part each experiment was a repetition in whole or in part of the others. The results obtained in a typical experiment have been summarized in Table I and clearly show (1) that rabbits injected with fresh emulsions of homologous brain developed few or no antibodies against brain tissue, (2) that antibrain antibodies were elicited by fresh emulsions of

homologous brain to which pig serum had been added, (3) that autolysis alone changed the nature of homologous brain tissue to such an extent that it became a complete antigen, (4) that rabbits which received injections of emulsions of fresh homologous brain infected with vaccine virus readily developed antibrain antibodies, and (5)

TABLE II

Summary of Results of Complement Fixation Tests Conducted with Sera of Rabbits Immunized with Autolyzed Homologous Brain Emulsions

Antigen	Dilution of antigen	Dilution of serum							
		1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Emulsion of rabbit brain	1:50	4+	4+	4+	4+	4+	4+	3+	–
" " " kidney	1:50	4+	4+	3+	–	–	–		
" " " liver	1:50	4+	1+	–	–	–	–		
" " " spleen	1:50	3+	–	–	–	–	–		
" " " red cells	1:25	–	–	–	–	–	–		
" " guinea pig brain	1:50	4+	4+	4+	4+	4+	4+	1+	–
" " " " kidney	1:50	4+	4+	2+	–	–	–		
" " " " liver	1:50	4+	4+	1+	–	–	–		
" " " " spleen	1:50	4+	1+	–	–	–	–		
" " " " red cells	1:25	–	–	–	–	–	–		
Alcoholic extract of rabbit brain	1:2	4+	4+	3+	–	–	–		
" " " " kidney	2:1	–	–	–	–	–	–		
" " " " liver	2:1	–	–	–	–	–	–		
" " " " spleen	1:2	–	–	–	–	–	–		

4+ indicates complete fixation of complement.

– indicates no fixation.

When aqueous emulsions were used as antigens in the tests lack of organ specificity is evident. Organ specificity is obvious in the tests in which alcoholic extracts were used.

that antibodies against brain tissue appeared in the serum of rabbits injected with emulsions of alcoholic extracts of rabbit brain to which pig serum had been added.

Specificity of the Antiserum

Having demonstrated that autolytic or disease processes acting on brain tissue can cause it to become fully antigenic for homologous animals, we proceeded to determine whether the antibodies elicited by

such altered brain material are specific for brain. For this purpose, an antiserum prepared in rabbits by means of injections of emulsions of autolyzed brain tissue was subjected to complement fixation tests in which aqueous emulsions of rabbit brain, kidney, liver, spleen, and red blood cells as well as emulsions of similar organs of the guinea pig were used as antigens. In addition, the antiserum was tested against alcoholic extracts of various rabbit organs. The results of one experiment are shown in Table II. The antiserum reacted most strongly with rabbit and guinea pig brain, yet some antibodies were also present

TABLE III

Summary of Results of Complement Fixation Tests Conducted with Sera of Rabbits Immunized with Alcoholic Extracts of Rabbit Brain Plus Pig Serum

Antigen	Dilution of antigen	Dilution of serum					
		1:5	1:10	1:20	1:40	1:80	1:160
Emulsion of rabbit brain	1:50	4+	4+	4+	3+	1+	−
" " " kidney	1:50	2+	−	−	−	−	−
" " " liver	1:50	−	−	−	−	−	−
" " " spleen	1:50	−	−	−	−	−	−
" " guinea pig brain	1:50	4+	4+	4+	2+	−	−
" " " " kidney	1:50	3+	−	−	−	−	−
" " " " liver	1:50	−	−	−	−	−	−
" " " " spleen	1:50	1+	−	−	−	−	−

4+ indicates complete fixation of complement.

− indicates no fixation.

The results of the complement fixation tests show almost complete organ specificity. Compare with results shown in Table II.

against the tissues of practically all the other organs of the rabbit and guinea pig that were tested. When alcoholic extracts were used as antigens in the complement fixation tests, however, the antiserum was organ-specific (brain-specific) within the range of antigens used. Several workers (3, 6, 7, 8) have reported similar results for antisera prepared by the immunization of animals with emulsions of heterologous brain. Further tests for organ specificity were made on antisera which had been prepared by the injection into rabbits of alcoholic extracts of homologous brain plus pig serum. The results, summarized

in Table III, show that such sera were almost completely organ-specific. The minor fixations of complement in the presence of emulsions of other organs were overshadowed by the high titer of the antisera in the presence of brain antigen. This quantitative organ specificity of antisera prepared by injections of alcoholic extracts of brain has been noted by a number of investigators (9, 10).

When one considers the complex nature of the emulsions of brain with which the rabbits were immunized, a possible cause becomes apparent for the lack of specificity evidenced by the antibodies when they were tested for ability to fix complement in the presence of aqueous emulsions of different organs. The brain undoubtedly contains certain tissues which are common to other organs. For lack of more exact knowledge we shall consider the common factor to be connective tissue. Rabbits immunized with emulsions of brain therefore develop antibodies against connective tissue as well as against the specific brain antigen. Since the connective tissue antigen apparently is not as alcohol-soluble as is the antigen of brain, complement fixation tests done with alcoholic antigens show more pronounced organ specificity. If this reasoning is correct, the serum of rabbits immunized against emulsions of brain must contain at least two, perhaps more, kinds of antibodies, one specific for brain antigen and another which is not organ-specific. A similar theory for the lack of specificity in antisera prepared in animals by the injections of emulsions of heterologous organs has already been advanced by Fleisher and his co-workers (6, 7).

Absorption of Antibodies

In order to determine whether non-specific as well as specific antibodies were present in the antibrain sera a number of absorption experiments were performed.

Method of Absorption.—A 1:25 emulsion, in physiological saline solution, of the desired organ was made in a manner similar to that used for the preparation of antigens for complement fixation tests. The technique of this procedure has already been described. The organ emulsion was then added to an equal volume of a 1:2.5 dilution, in physiological saline solution, of the antiserum, and the mixture was heated at 56°C. for 2 hours in the water bath. After standing overnight at room temperature it was centrifuged at high speed for half an hour, and the supernatant liquid was recovered with a pipette. In order to remove all of the

antigen-antibody complex, however, it was necessary to pass the mixture through a Seitz filter. As a control a second sample of the same antiserum was subjected to similar manipulations with the exception that physiological saline solution was added to it instead of an absorbing antigen. Experiments were also carried out in which an alcoholic extract of brain was used as an absorbing agent. The technique was similar to that just described except that a 1:16 dilution of the alcoholic antigens was found to yield maximum absorption.

TABLE IV

Summary of Results of Complement Fixation Tests Conducted with Antibrain Serum before and after Absorption with Aqueous Emulsions of Rabbit Kidney

Serum	Antigen	Dilution of antigen	Dilution of serum					
			1:5	1:10	1:20	1:40	1:80	1:160
Unabsorbed	Emulsion of rabbit brain	1:600	4+	4+	3+	1+	–	–
	" " " kidney	1:50	4+	4+	3+	–	–	–
	" " " liver	1:50	4+	1+	–	–	–	–
	" " " spleen	1:50	3+	–	–	–	–	–
	" " guinea pig brain	1:600	4+	4+	4+	2+	–	–
	" " " " kidney	1:50	4+	4+	2+	–	–	–
	" " " " liver	1:50	4+	4+	1+	–	–	–
	" " " " spleen	1:50	4+	1+	–	–	–	–
Absorbed with aqueous emulsion of rabbit kidney	Emulsion of rabbit brain	1:600	4+	4+	2+	–	–	–
	" " " kidney	1:50	–	–	–	–	–	–
	" " " liver	1:50	–	–	–	–	–	–
	" " " spleen	1:50	–	–	–	–	–	–
	" " guinea pig brain	1:600	4+	4+	3+	2+	–	–
	" " " " kidney	1:50	–	–	–	–	–	–
	" " " " liver	1:50	–	–	–	–	–	–
	" " " " spleen	1:50	–	–	–	–	–	–

4+ indicates complete fixation of complement.

– indicates no fixation.

The results of the absorption experiments show that an emulsion of rabbit kidney removed the non-specific antibodies from the antibrain serum.

Three kinds of absorption experiments were conducted. In the first, antibrain sera were absorbed with emulsions of rabbit kidney which, containing only non-specific antigen, should not remove specific antibodies for brain. In the second, emulsions of rabbit brain which contain both the specific and non-specific antigens were used for absorption. In this case both kinds of antibodies should be removed from

the antisera. And finally antibrain sera were absorbed with alcoholic extracts of rabbit brain which contain only or principally the specific antigen and should therefore remove from the sera only the specific antibodies for brain. The results of these absorption experiments are summarized respectively in Tables IV, V, and VI. From the results

TABLE V

Summary of Results of Complement Fixation Tests Conducted with Antibrain Serum before and after Absorption with Aqueous Emulsions of Rabbit Brain

Serum	Antigen	Dilution of antigen	Dilution of serum					
			1:5	1:10	1:20	1:40	1:80	1:160
Unabsorbed	Emulsion of rabbit brain	1:600	4+	4+	4+	3+	2+	1+
	" " " kidney	1:50	4+	4+	3+	−	−	−
	" " " liver	1:50	4+	1+	−	−	−	−
	" " " spleen	1:50	3+	−	−	−	−	−
	" " guinea pig brain	1:600	4+	4+	4+	2+	−	−
	" " " " kidney	1:50	4+	4+	2+	−	−	−
	" " " " liver	1:50	4+	4+	1+	−	−	−
	" " " " spleen	1:50	4+	1+	−	−	−	−
	Alcoholic extract of rabbit brain	1:2	4+	4+	3+	−	−	−
Absorbed with aqueous emulsion of rabbit brain	Emulsion of rabbit brain	1:600	−	−	−	−	−	−
	" " " kidney	1:50	−	−	−	−	−	−
	" " " liver	1:50	−	−	−	−	−	−
	" " " spleen	1:50	−	−	−	−	−	−
	" " guinea pig brain	1:600	−	−	−	−	−	−
	" " " " kidney	1:50	−	−	−	−	−	−
	" " " " liver	1:50	−	−	−	−	−	−
	" " " " spleen	1:50	−	−	−	−	−	−
	Alcoholic extract of rabbit brain	1:2	−	−	−	−	−	−

4+ indicates complete fixation of complement.

− indicates no fixation.

The results of the absorption experiments show that an emulsion of brain removed both the specific and non-specific antibodies from the antibrain serum.

shown in Table IV it can be seen that an emulsion of rabbit kidney absorbed the non-specific antibodies from the serum so that it failed to react to any organ emulsion except that prepared from brain. When the absorption was carried out with an emulsion of rabbit brain (Table V) which contains both the specific and non-specific antigens, both kinds of antibodies were removed and the serum failed to fix comple-

ment in the presence of any of the organ emulsions. Finally, absorption of the antiserum with an alcoholic extract of rabbit brain (Table VI) resulted principally in the removal of antibodies against brain, permitting the serum still to react with emulsions of all the organs which contained the non-specific antigen. One might wonder why the antiserum absorbed with the alcoholic extracts of brain did not react to a slight extent with emulsions of brain as well as with emulsions of other organs because brain tissue contains both specific and

TABLE VI

Summary of Results of Complement Fixation Tests Conducted with Antibrain Serum before and after Absorption with Alcoholic Extract of Rabbit Brain

Serum	Antigen	Dilution of antigen	Dilution of serum					
			1:5	1:10	1:20	1:40	1:80	1:160
Unabsorbed	Alcoholic extract of rabbit brain	1:2	4+	4+	4+	2+	1+	−
	Emulsion of rabbit brain	1:600	4+	4+	3+	2+	−	−
	" " " kidney	1:50	4+	4+	2+	−	−	−
	" " " liver	1:50	4+	3+	1+	−	−	−
	" " " spleen	1:50	4+	1+	−	−	−	−
Absorbed with alcoholic extract of rabbit brain	Alcoholic extract of rabbit brain	1:2	−	−	−	−	−	−
	Emulsion of rabbit brain	1:600	−	−	−	−	−	−
	" " " kidney	1:50	4+	4+	2+	−	−	−
	" " " liver	1:50	4+	2+	−	−	−	−
	" " " spleen	1:50	3+	−	−	−	−	−

4+ indicates complete fixation of complement.

− indicates no fixation.

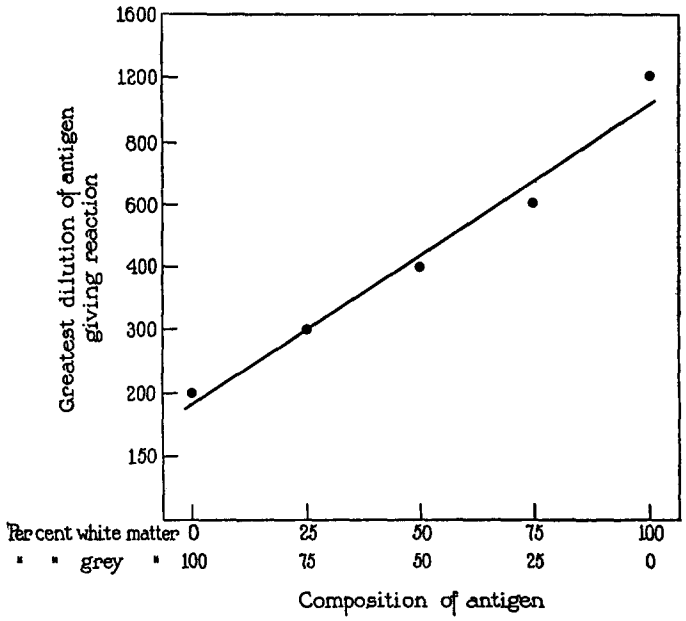
The results of the absorption experiments show that an alcoholic extract of brain removed principally the antibrain antibodies from the antibrain serum.

non-specific antigens. This is explained by the fact that in order not to mask the sensitivity of the complement fixation test by a large amount of antigen, it was necessary to use a 1:600 dilution of the emulsion of brain. At this concentration the non-specific antigen contained in brain tissue was diluted beyond its power to fix complement in the presence of the antiserum. The results of the absorption experiments just enumerated appear to indicate that two if not more antigen-antibody systems were involved, one of which was specific for brain while

the other was associated with some element or elements common to many organs.

Nature of the Antigen

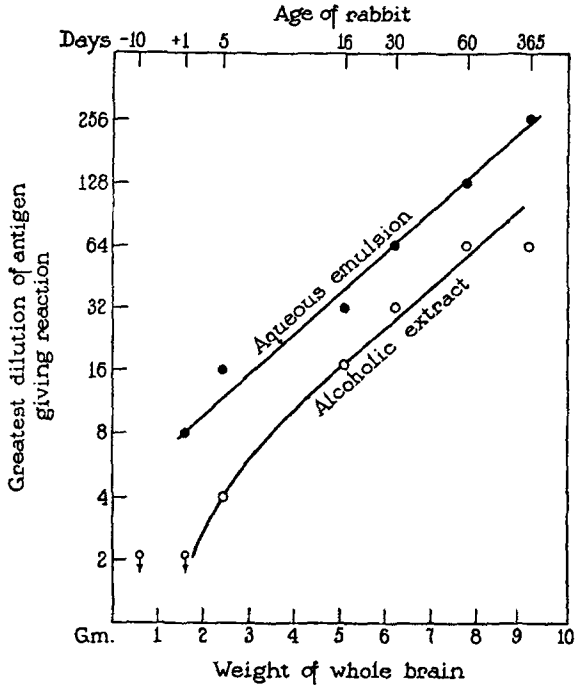
Having satisfied ourselves that emulsions of homologous brain can be made to become antigenic under certain conditions we were interested to determine what substance in the brain causes it to act as a specific antigen. It occurred to us that the specificity might be



TEXT-FIG. 1. The graph represents the relative antigenicity of white and grey matter of brain. The greatest dilution of each mixture of antigens which fixed complement in the presence of an antibrain serum has been plotted against the composition of the mixtures of white and grey matter.

associated in some manner with myelin. The most obvious way of testing such an idea was to find out whether the amount of myelin in brain antigens used for fixation of complement in the presence of antibrain sera runs parallel to the degree of fixation. A rabbit brain was therefore divided as nearly as possible into two portions, white matter which contains large numbers of myelinated nerve fibers and grey

matter which has a lower myelin content. From the two portions, aqueous emulsions were made, after which five mixtures were prepared by the combination of different quantities of them so that the ratio of grey emulsion to white emulsion varied from 100 per cent white to 100 per cent grey. The antigenicity of each of the emulsions was de-



TEXT-FIG. 2. The graph represents the relative antigenicity of brains of rabbits of different ages. The greatest dilution of antigen which fixed complement in the presence of an antibrain serum has been plotted as a function of the weights of the whole brain from which the antigen was made. The ages of the rabbits from which the brains were removed is also indicated. The ordinates for the curve for aqueous antigens are plotted as dilutions of a 1:50 brain emulsion; *i.e.*, a 1:2 dilution in the graph is actually a 1:100 emulsion of brain tissue.

termined quantitatively in complement fixation tests conducted with antibrain serum. Text-fig. 1 shows the results graphically. It is apparent from them that the antigenicity of the white matter was roughly six times stronger than that of the grey matter, a property which at least paralleled the content of myelin in the two emulsions.

Further investigation of the relation between the myelin content and the antigenicity of brain tissue was made possible by the fact that myelin is practically absent from the central nervous system of fetal or newly born animals and increases with the age of the animal. We, therefore, prepared emulsions of the brain tissue of rabbits of different ages, ranging from the fetal stage to maturity. The relative antigenic properties of these emulsions were determined by the fixation of complement in the presence of antibrain serum. Furthermore, in order to eliminate as far as possible the non-specific antigen, alcoholic extracts were made from portions of the brains of these animals and tested for antigenicity. In Text-fig. 2 the results of such an experiment are shown graphically. It is immediately apparent that in newly born rabbits the content of organ-specific antigen in the brain emulsions was negligible and that it increased steadily with the age of the animal to reach a high concentration at maturity. Alcoholic extracts of brains of fetal or newly born rabbits failed to fix complement in the greatest concentration used, while extracts of brains of adult rabbits contained sufficient antigen to fix complement in high dilutions.

Observations on the Clinical and Pathological Findings in Rabbits Injected with Emulsions and Extracts of Homologous Brain

The rabbits which received injections of the different emulsions or extracts of brain were carefully observed during and after the course of immunization for the appearance of abnormalities attributable to the procedures used. In no instance was a reaction seen immediately following primary injection. For the most part the rabbits remained in good condition throughout the experiments, although some that received injections of emulsions of autolyzed brain lost weight during the course of the experiments. In every experiment, however, a certain number of rabbits developed signs referable to the central nervous system. The course in every case was the same: the rabbits developed a slight weakness of the hind legs which progressed rapidly to a complete paralysis of the hind quarters and was accompanied by a progressive emaciation; retention of urine was noted; and death occurred after an illness of a few days.

At necropsy the central nervous system and other organs in the gross were normal in appearance. In the brains of some of the paralyzed

rabbits stained sections showed perivascular infiltration and small areas of necrosis surrounded by zones of inflammation, in others evidences of healing lesions were seen, in still others no pathological changes were noted. Demyelination was not observed in any instance in the brain and cord, and the peripheral nerves appeared to be normal. Lesions identical in appearance with those just described were also found in some of the non-paralyzed rabbits sacrificed as controls and were considered to be due to the activity of *Encephalitozoon cuniculi.* The fact that the lesions were not present in all of the paralyzed animals and that they occurred in some of the non-paralyzed rabbits led us to conclude that they should not be looked upon as the

TABLE VII

Relation between the Number of Paralyzed Rabbits and the Immunizing Materials

Material used for immunization	No. of injections	No. of rabbits injected	No. paralyzed	Per cent paralyzed
Fresh rabbit brain	5–12	19	0	0
" " " plus pig serum	5–10	14	1	7
Alcoholic extract of rabbit brain plus pig serum	13–26	8	1	12
Vaccine virus rabbit brain	5–12	10	1	10
Autolyzed rabbit brain	5–15	28	9	32

cause of the paralyses. This opinion is substantiated by the observations of Hurst (11), who, working with rabbits known to be free from *Encephalitozoon* infection, found no pathological changes in the central nervous system of animals which became paralyzed following injections of emulsions of heterologous brain.

It is interesting to note that the incidence of paralysis in the rabbits of the different groups paralleled the antigenicity of the brain emulsions and extracts that they received. In Table VII, the different immunizing emulsions and extracts are listed in the order of their antigenicity and the incidence of paralysis caused by them. None of the rabbits that received fresh emulsions of homologous brain, which has little or no antigenicity, became paralyzed, while 9 of 28 rabbits (32 per cent) injected with autolyzed homologous brain, a good antigen, showed evidences of paralysis. These observations, however,

cannot be considered evidence that the paralysis was directly related to the antigenicity of the brain emulsions, because Hurst (11) has pointed out that similar paralytic accidents follow injections of substances other than brain and also because the paralysis might have been induced by some toxic substance in the autolyzed or diseased brain tissue.

DISCUSSION

It has been shown by a number of investigators that heterologous brain (2, 3), alcoholic extracts of heterologous brain plus pig serum (1, 10), homologous brain plus pig serum (4), and alcoholic extracts of homologous brain plus pig serum (10) excite in animals receiving them the production of specific antibrain antibodies capable of demonstration either by means of complement fixation tests or by precipitin reactions. In addition to this we have been able to demonstrate that homologous brain altered by autolysis alone or by infection with vaccine virus alone becomes antigenic and is then capable of inducing the production of antibodies specific for brain tissue.[1] Furthermore, we have been able to show that the specific antigenicity of homologous brain runs parallel to the myelin content of the tissue.

In view of the facts just enumerated it is interesting to speculate about the etiology of certain diseases of the central nervous system, particularly the demyelinating maladies, such as the postinfection encephalitides, multiple sclerosis, Schilder's disease, and the encephalomyelitis occurring after antirabies vaccination. Speculation would be more interesting if it were possible to produce demyelination in animals by means of injections of brain tissue or if it were possible to demonstrate either brain antigen or antibrain antibodies in the blood of patients with demyelinating diseases. Hurst (11) from his review of the literature dealing with the effects on animals of repeated injections of brain tissue and from the results of his own experiments concluded that, although paralysis has been observed following injections of emulsions of brain, no one has definitely shown the presence of demyelination in the brains and cords of the paralyzed animals. Rivers,

[1] After our work was completed, Lewis (13) reported that antibrain sera are also specific for testicular tissue. We did not examine our antibrain sera by means of complement fixation tests in which testicular tissue was used as an antigen. Consequently we have no information regarding this matter.

Sprunt, and Berry (12), however, recorded the fact that two of eight monkeys which received repeated injections of emulsions and alcoholic extracts of rabbit brain developed paralysis associated with demyelination. In regard to the presence of brain antigen or antibrain antibodies in the blood of patients with demyelinating diseases no significant reports have been seen. Although speculation about the relation of antibrain antibodies to certain demyelinating maladies is extremely intriguing, further facts must be obtained before a reasonable hypothesis concerning the matter can be presented.

SUMMARY

Rabbits injected with fresh emulsions of homologous brain developed few or no antibodies capable of fixing complement in the presence of aqueous emulsions or alcoholic extracts of rabbit brain. Complement-fixing antibodies, however, were produced in rabbits by means of injections (1) of sterile emulsions of homologous brain which had been allowed to stand at room temperature for 5 to 30 days and (2) of emulsions of homologous brain experimentally infected with vaccine virus. The antisera that were produced following injections of emulsions of autolyzed homologous brain were shown by absorption tests to contain both specific and non-specific antibodies. The specific brain antigen was found to be approximately six times as abundant in the white matter as in the grey. It was almost absent from the brain of fetal and newly born rabbits, but increased in amount with the age of the animal to reach a maximum concentration at maturity. The specific antigen seemed to parallel the myelin content of brain tissue.

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