THE PRODUCTION OF KIDNEY ANTIBODIES BY INJECTION OF HOMOLOGOUS KIDNEY PLUS BACTERIAL TOXINS*

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The discovery of Lindemann (1) that a condition closely simulating glomerular nephritis can be produced in laboratory animals by intravenous injections of antikidney serum has been confirmed by a number of investigators. A review of the literature has already been published (2). It has been shown that the animals develop albuminuria, hematuria, cylindruria, moderate oliguria, or occasionally anuria. There is an increase in blood urea, with moderate edema, and a rise in blood pressure. At autopsy the kidneys show extensive glomerular lesions which are a close approximation to those seen in human glomerular nephritis.

These experiments support the theory that acute hemorrhagic nephritis is due to the development in the patient of some nephrotoxic substance. However, in each instance, the nephrotoxic serum was produced in an animal of a heterologous species. There is therefore no explanation as to how such a nephrotoxin could be developed in human cases of hemorrhagic nephritis, where only the homologous kidney can be involved.

The following investigations were therefore undertaken to determine whether it would be possible to demonstrate in rabbits the development of antikidney antibodies following the injection of emulsions of homologous kidney to which streptococcus toxin had been added. Because the well known resistance of rabbits to streptococcus toxin might retard the formation of antibodies, a second group of rabbits was injected with emulsions of rabbit kidney to which staphylococcus toxin had been added. As control three additional groups of rabbits were included in the experiment. The animals of one of these received injections of streptococcus toxin alone, those of the second group, staphylococcus toxin, and for the third group emulsions of kidney tissue alone were used.

Following the period of injections the blood sera of these rabbits were tested by complement fixation tests against emulsions of rabbit kidney to determine the possible development of antikidney antibodies.

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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.

Emulsions of Rabbit Kidney.—5 gm. of kidney tissue removed aseptically from a rabbit were ground in a mortar with alundum until thoroughly macerated. 100 cc. of sterile normal saline 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.

Streptococcus Toxin.—Strain NY 5 of beta hemolytic streptococcus was grown for 7 days in streptococcus toxin broth (3). The culture was tested for purity, centrifuged to remove the bacteria, and filtered through a Seitz filter. After culture had proven the filtrate sterile, the toxin content was determined by comparative titration with a toxin of known value in the skin of human volunteers. The toxin contained 50,000 S.T.D. per cc.

Staphylococcus Toxin.—A strain of *Staphylococcus aureus* of known ability to produce toxin was obtained from Dr. Earl Burky of the Johns Hopkins Hospital. This was grown in broth for 10 days according to his technique (4). The culture was tested for purity, centrifuged to remove the bacteria and filtered through a Seitz filter. The filtrate was cultured for sterility and the toxin content determined by injections of 1 cc. amounts of various decimal dilutions intraperitoneally in rabbits. Two animals receiving a 1:1000 dilution showed no reaction, two injected with a 1:100 dilution were slightly ill for 2 days following injection, while a 1:10 dilution killed both of two rabbits in 18 hours.

Immunization of Rabbits.—The rabbits were divided into five groups of four rabbits each. Each animal received ten intraperitoneal injections at 2 day intervals according to the following plan:

Group 1.—Each rabbit received at each injection 5 cc. of 5 per cent emulsion of rabbit kidney mixed with undiluted streptococcus toxin. 2 cc. of toxin was added at the first injection, 2.5 cc. at the second, 3 cc. at the third, increasing by 0.5 cc. increments at each injection to 6.5 cc. at the tenth injection.

Group 2.—Each rabbit received at each injection 5 cc. of 5 per cent emulsion of rabbit kidney mixed with 1 cc. of various dilutions of staphylococcus toxin. At the first injection the dilution was 1:100; at the second 1:90; third, 1:80; etc., to 1:10 at the tenth injection.

Group 3.—Each rabbit received at each injection 5 cc. of 5 per cent emulsion of rabbit kidney.

Group 4.—Each rabbit received the same dose of streptococcus toxin given to the animals in group 1, without the addition of kidney emulsion.

Group 5.—Each rabbit received the same dose of staphylococcus toxin given to the animals in group 2 without the addition of kidney emulsion.

Complement Fixation Tests.—The anti-sheep cell system was used in the complement fixation tests. 0.2 cc. of diluted inactivated serum from the rabbits of the various groups just described, 0.2 cc. of antigen (organ emulsions) and 0.2 cc. of a 1:10 dilution of guinea pig serum for complement were mixed in a Wassermann tube and incubated for 30 minutes at 37°C. in a water bath. 0.2 cc. of a 5 per cent suspension of washed sheep

cells and 0.2 cc. of anti-sheep cell amboceptor (so diluted as to give 2 units) were added and the tubes again incubated for 30 minutes at 37°C. The results of the test 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. The antigens for the complement fixation tests were made by grinding 1 gm. of fresh rabbit kidney or brain in a mortar with alundum. 50 cc. of physiologic saline were added by increments during the process of grinding until a homogeneous emulsion resulted. This suspension was then filtered through a tightly folded pad of absorbent cotton to remove the alundum and large bits of tissue.

TABLE I

Summary of Results of Complement Fixation Experiments Indicating the Presence or Absence of Antibodies against Rabbit Kidney in the Sera of Rabbits Injected with Various Mixtures of Rabbit Kidney and Toxins

Material used for immunizing	Antigen	Dilution of serum					
		1:5	1:10	1:20	1:40	1:80	1:160
Rabbit kidney plus streptococcus toxin	1:50 emulsion rabbit kidney	4+	3+	−	−	−	−
Rabbit kidney plus staphylococcus toxin		4+	4+	4+	4+	3+	−
Rabbit kidney		−	−	−	−	−	−
Streptococcus toxin		2+	−	−	−	−	−
Staphylococcus toxin		1+	−	−	−	−	−

In all the tables:
4+ indicates complete fixation of complement.
− indicates no fixation.

Antigenicity of Homologous Kidney plus Toxin

In order to test the antigenic quality of homologous kidney when mixed with toxin three experiments were performed. Each was a repetition of the others and the results were similar in all. In Table I have been summarized the results of one experiment which show that although mixtures of homologous kidney and streptococcus toxin were slightly antigenic, the antibody response to mixtures of homologous kidney and staphylococcus toxin was far greater. This difference is probably to be explained by the fact that rabbits are resistant to the toxic effects of streptococcus toxin although susceptible to even small doses of staphylococcus toxin. Rabbit kidney alone was not antigenic; streptococcus and staphylococcus toxins alone gave only a very feeble antibody response.

Specificity of the Antiserum

Having demonstrated that mixtures of toxin with renal tissue can cause the kidney to become antigenic for the homologous animal we proceeded

to determine whether the antibodies so elicited are specific for kidney. For this purpose the antikidney sera were subjected to complement fixation tests using as antigens emulsions of rabbit brain as well as of rabbit kidney. Brain was used for this purpose because in previous experiments (7) we had learned that this organ is highly antigenic. These experiments showed that the sera contained reacting substances for brain as well as for kidney, although in lower concentration. Fixation of complement with kidney ordinarily occurred with serum dilutions up to 1:80; with brain to 1:20.

A possible reason for this apparent lack of specificity may be found in the complex nature of renal tissue. The kidney 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 kidney therefore develop antibodies against connective tissue as well as against the specific kidney antigen. If this reasoning is correct, the serum must contain at least two, perhaps more, kinds of antibodies, one specific for kidney and another which is not organ specific. A similar theory for the lack of specificity in antisera prepared by the injections of emulsions of homologous brain has previously been advanced (5–7).

Absorption of Antibodies

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

Method of Absorption.—A 1:25 emulsion, in physiologic saline, 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 the procedure has already been described. The organ emulsion was then added to an equal volume of a 1:2.5 dilution, in physiologic saline, 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 recovered. In order to remove all of the antigen-antibody complex the mixture was passed through a Seitz filter. As a control a second sample of the same antiserum was subjected to similar manipulations with the exception that physiologic saline was added to it instead of an absorbing antigen.

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

The results of a typical absorption experiment are summarized in Table II. From these results it can be seen that absorption with either brain or kidney removed all the non-specific antibodies which had previously reacted with brain. This result was to be expected since both brain and kidney tissue contain the common non-specific factor. The sera absorbed with brain showed only a slight fall in titer when tested against kidney. This drop probably represents that portion of the antibody which reacted to the non-specific antigens contained in kidney. The sera absorbed with kidney, however, showed a marked decrease in titer against the specific

TABLE II

Summary of Results of Complement Fixation Tests Done with Antikidney Serum before and after Absorption with Emulsions of Rabbit Kidney and Brain

Serum absorbed with	Antigen	Dilution of serum					
		1:5	1:10	1:20	1:40	1:80	1:160
Unabsorbed	1:50 rabbit brain	4+	4+	2+	−	−	−
Rabbit brain		−	−	−	−	−	−
Rabbit kidney		−	−	−	−	−	−
Unabsorbed	1:50 rabbit kidney	4+	4+	4+	4+	2+	−
Rabbit brain		4+	4+	4+	2+	−	−
Rabbit kidney		4+	2+	−	−	−	−

The antikidney serum was produced by injections of emulsions of rabbit kidney plus staphylococcus toxin.

kidney antigen. Theoretically the absorption should have been complete. It is well known, however, that complete absorption of antibody is often extremely difficult to obtain. A definite drop in titer is therefore usually accepted as indicating specificity of the antibody.

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 kidney while the other was associated with some element or elements common to other organs as well. This condition has already been shown to exist in the case of antibrain sera (7).

Results with Human Sera

Having satisfied ourselves that emulsions of homologous kidney become antigenic when they are mixed with staphylococcus or streptococcus toxins we were interested to determine whether antikidney antibodies are developed in the human body during the course of streptococcal infections.

The sera of 40 patients in various stages of scarlet fever from the first to the 28th day of disease were tested by complement fixation reactions against emulsions of rabbit kidney and rabbit brain as antigens. For control the sera of 29 persons showing no signs of streptococcal infection were subjected to similar tests. The technique was the same as for the animal experiments. The results are shown in Table III. 37 of the 40 scarlet fever patients, or 92 per cent, gave reactions of some degree against kidney although only 10 showed a 4 + reaction. In contrast to this only 3 of the 29 normal persons (10 per cent) exhibited reactions. For the

TABLE III

Summary of Results of Complement Fixation Tests Done against Emulsions of Rabbit Kidney and Brain with Sera from Scarlet Fever Patients and Normal Persons

Complement fixation reaction	Antigen: kidney		Antigen: brain	
	Scarlet fever	Normal	Scarlet fever	Normal
4+	10	0	0	0
3+	8 (92%)	2 (10%)	1	0
2+	9	1	0	0
1+	10	0	0	0
−	3	26	39	26
Total.....	40	29	40	26

scarlatinal sera there was no correlation between the reactions and the day of disease. Two of these patients had mild hemorrhagic nephritis. For one the reaction was 4 +, for the other 2 +. One of the sera from scarlet fever patients and none from normal persons reacted with brain.

These results indicate that most persons suffering from scarlet fever develop circulating antibodies for kidney, a reaction only occasionally seen in normal persons. With streptococci as common as they are it is possible that earlier unrecognized infections may have caused the development of antibodies in the few normal persons who showed reactions.

DISCUSSION

The period of 2 to 3 weeks which intervenes with great regularity between the onset of acute hemorrhagic nephritis in human beings and the antecedent scarlet fever suggests that the kidney condition is not directly due to the streptococcal infection but that the latter initiates some process which, in certain individuals, reaches a degree capable of damaging the kidney. The latent period suggests the possibility of the development of

some type of antibody. Many workers (2) have demonstrated that degenerative changes simulating acute nephritis can be produced in animals by the injection of antikidney sera. These sera have been made by injections of emulsions of heterologous kidney. If this theory has any basis in fact it should be possible to produce antikidney sera by injections of the homologous organ. We have demonstrated that emulsions of rabbit kidney, when mixed with streptococcus or staphylococcus toxins are capable of producing in rabbits complement fixing antibodies specific for rabbit kidney. It seems possible that a similar mechanism may be involved in scarlatinal nephritis in man. During the primary infection the circulating streptococcus toxin damages some of the kidney tissue. Ordinarily the damage is insufficient to give clinical symptoms more than the albuminuria regularly seen during the acute stage of scarlet fever. The kidney cells, which contain a specific haptene group have, however, been acted upon by streptococcus toxin, a protein substance foreign to the body and therefore capable of being a *schlepper*. The result is a complete antigen. In the process of repair these damaged cells, now complete antigens, are absorbed by the body. As a result antibodies are produced specific for kidney tissue. In certain individuals, because of circumstances not now known, these antibodies react on the kidney giving rise to the clinical and pathological picture of acute hemorrhagic nephritis. We have shown that complement fixing antibodies can be demonstrated in the blood of persons suffering with a streptococcal infection such as scarlet fever.

The exact nature of these antikidney antibodies is, of course, unknown. In our experiments we have employed the complement fixation test to demonstrate their existence. We do not, however, believe that the antibody is necessarily of this type. In fact it seems more probable that some entirely different substance or property is involved and that, because of experimental limitations, we have been measuring only a related substance. It may be that the reaction is partially or even entirely anaphylactoid in nature and that we have been concerned with only one phase of the phenomenon.

Similar experimental evidence has been advanced to explain the mechanism of some other diseases. Schwentker and Rivers (7) working on post-infection encephalomyelitis have demonstrated that under certain conditions homologous brain may become antigenic, and that the haptene involved is a lipoid which is probably myelin. These authors (8) have also produced in monkeys a clinical and pathological picture closely simulating human encephalomyelitis by repeated injections of emulsions of brain.

Burky (9) is of the opinion that, in some diseases at least, the antigen-

antibody reaction is anaphylactoid in nature. He has shown that rabbits may be made hypersensitive to either homologous lens or muscle by injections of these rabbit tissues attached to staphylococcus toxin. By this method he has been able to duplicate in rabbits the picture found in human cases of endophthalmitis phaco-anaphylactica.

We are quite aware that the entire theory discussed here is based on only a frail foundation. However, what evidence there is supports it as a possibility. Its value lies in offering a basis for further experimental work.

SUMMARY

Rabbits injected with emulsions of homologous kidney to which staphylococcus or streptococcus toxins had been added produced complement fixing antibodies which reacted with both rabbit kidney and brain. By absorption tests it was demonstrated that the sera contained at least two antibodies, one specific for kidney, the other non-specific. Similar kidney antibodies were found in the blood of a majority of patients with scarlet fever but in only a few normal persons. The possibility that a similar or related antibody may be concerned in the etiology of scarlatinal nephritis is discussed.

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