# Autoantibodies Resulting from Immunization with Kidney.* (29434)

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Heymann and his associates(1,2) reported experimental production of a nephrotic syndrome in rats which had received intraperitoneal injections of rat kidney suspensions incorporated into complete Freund adjuvants. Similar immunization with rat liver suspensions also resulted in nephrosis, even though its incidence was considerably smaller than in the animals immunized with kidney suspensions. Hess *et al*(3) as well as Heymann *et al*(4) showed that experimental nephrosis may be transferred by injection of spleen cells from actively immunized animals into healthy recipient rats that had been made tolerant to the donor's tissues. These experiments made it rather plausible that experimental rat nephrosis is an autoimmune disease.

The main objective of this paper was a study of humoral antibodies resulting from immunization with kidney suspensions performed within the same species. Rabbits and guinea pigs were selected for these experients because these 2 species are better producers of humoral antibodies than rats.

*Materials and methods. Preparation of antigens.* Frozen rabbit organs were purchased from Pel-Freeze Biologicals, Inc., Rogers, Arkansas. Some individual rabbit organs and guinea pig organs were obtained in this laboratory from healthy animals after exsanguination. In a few guinea pigs the left kidney was removed by nephrectomy under Nembutal anesthesia. The organ suspensions were prepared by mincing the tissue in a Waring Blendor or Omnimixer; a convenient volume of saline was used to obtain the desired concentration of suspension. Organ extracts were obtained by centrifuging the suspensions at 20,000 rpm for 30 minutes and removing the clear supernatants. Protein concentration in organ extracts was determined by the biuret reaction, using an extinction coefficient of 2.8, as determined for a serum pool in this laboratory.

*Immunization procedures.* Flemish Giant albino rabbits weighing 3 to 4 kg and colored, mixed breed guinea pigs weighing 500 to 700 g were immunized with a 20% kidney suspension, weight/volume. Four to six inoculations were performed at one to 3 week intervals. For each inoculation a total of 1 ml of Freund adjuvant-antigen mixture was administered intradermally in several separate places. Freund adjuvants employed for the first inoculation were enriched with 8 mg/ml of a mixture of heat-killed *M. butyricum* and *M. smegmatis*. For each consecutive inoculation the amount of mycobacteria was reduced by 2 mg/ml, and accordingly for the fifth and sixth inoculations adjuvants without mycobacteria were used. All trial and final bleedings were taken 7 to 10 days after the last inoculation.

The serological tests were performed following the procedures outlined previously(5, 6). All extracts titrated in complement fixation tests had an original protein concentration of 1.5%. Readings of complement fixation tests presented in the tables were taken after 30 minutes of incubation.

*Results. Rabbit experiments.* Nine rabbits were immunized with a suspension of pooled rabbit kidney incorporated into Freund adjuvants. Seven of them formed strong antibodies combining with kidney extracts in both complement fixation and double diffusion gel precipitation tests. The antibodies could already be found after the second inoculation; the consecutive inoculations increased the strength of the reactions. When tested by the complement fixation test, the anti-kidney sera combined not only with the kidney extract but also with extracts of other rabbit organs as exemplified by the results presented in Table I. However, the kidney extract gave positive results at dilutions considerably higher than did other extracts.

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TABLE I. Complement Fixation Test with Rabbit Anti-Rabbit Kidney Serum #1705 at 1:30 Dilution and Extracts of Pooled Rabbit Organs.

| Antigen dilution 1: | Extracts of rabbit organs | | | | | | |
|---|---|---|---|---|---|---|---|
| | Kidney | Liver | Heart | Adrenal | Ovary | Testis | Brain |
| 1 | 4+ | 4+ | 4+ | 4+ | 3+ | 4+ | 4+ |
| 3 | 4+ | 3+ | 3+ | 3+ | 1+ | 4+ | 3+ |
| 9 | 4+ | 2+ | 1+ | 2+ | 1+ | 4+ | 3+ |
| 27 | 4+ | 1+ | ± | 1+ | — | 3+ | 2+ |
| 81 | 4+ | ± | — | — | — | 1+ | ± |
| 243 | 4+ | — | — | — | — | — | — |
| 729 | 3+ | — | — | — | — | — | — |

By double diffusion gel precipitation 2 lines of precipitation were obtained (Fig. 1). One of them was apparently kidney-specific and failed to appear in reactions with other rabbit organs. The other line which was located more closely to the antiserum well was non-kidney-specific since it was formed not only with kidney extract but also with extracts of other rabbit organs, represented in the figure by liver and heart.

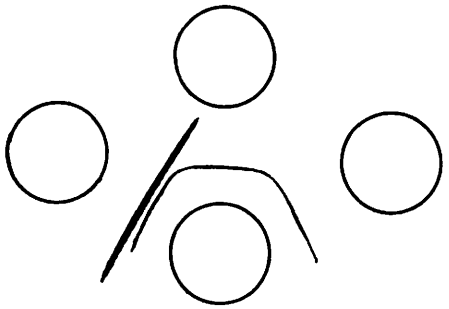

FIG. 1. Double diffusion gel precipitation. Lower well: Rabbit anti-rabbit kidney serum #1705. Upper wells: Extracts of pooled rabbit organs at a protein concentration of approximately 3.5%; left, kidney; center, heart; right, liver.

The question arose whether the antibodies under study are autoantibodies. Accordingly, an anti-kidney serum was tested simultaneously against kidney and liver extracts obtained from pooled rabbit organs and against such extracts prepared from the organs of the antibody-producing animal. As is to be seen in Fig. 2, equally strong lines of precipitation were obtained with both of these 2 kinds of antigenic preparations and the respective lines merged in reactions of identity. This proved the auto-character of both the kidney-specific and the non-kidney-specific antibodies.

A kidney antiserum was examined in complement fixation tests against kidney and liver extracts originating from three different species (Table II). Strongly positive results were obtained with a kidney extract of rabbit origin. Extracts of human and porcine kidney gave rather weak reactions. On the other hand, only rabbit and not human and porcine liver extracts gave positive results. In the double diffusion gel precipitation test, negative results were obtained with both

TABLE II. Complement Fixation Test with Rabbit Anti-Rabbit Kidney Serum #1705 at 1:30 Dilution and Extracts of Rabbit, Human and Porcine Kidney and Liver.

| | Highest extract dilution which gave 4+ or 3+ reaction | |
|---|---|---|
| | Kidney | Liver |
| Rabbit | 729 | 9 |
| Man | 9 | <1 |
| Pig | 3 | <1 |

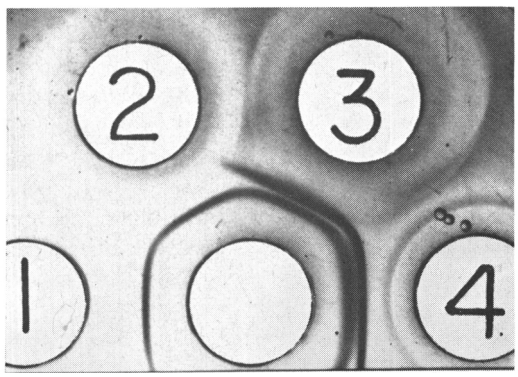


FIG. 2. Double diffusion gel precipitation. Center well: Rabbit anti-rabbit kidney serum #1786. Peripheral wells: Extracts of rabbit organs at a protein concentration of approximately 3.8%; 1, pooled liver; 2, liver of #1786; 3, kidney of #1786; 4, pooled kidney.

TABLE III. Complement Fixation Test with Guinea Pig Antiserum to Pooled Guinea Pig Kidney #141 and Extract of Pooled Guinea Pig Kidney.

| Dilution of guinea pig kidney extract 1: | Antiserum dilution 1: 5 | 10 | 20 | 40 | 80 | Saline |
|---|---|---|---|---|---|---|
| 1 | 4+ | 4+ | 4+ | 4+ | 4+ | — |
| 3 | 4+ | 4+ | 4+ | 4+ | 4+ | — |
| 9 | 4+ | 4+ | 4+ | 4+ | 2+ | — |
| 27 | 4+ | 4+ | 2+ | ± | — | — |
| 81 | 2+ | 1+ | 1+ | — | — | — |
| Saline | ± | — | — | — | — | — |

kidney and liver extracts of foreign species origin.

It was of some interest to find out whether the non-kidney-specific antibody may also be produced by immunization of a rabbit with rabbit organs other than kidney. An antiserum was investigated which was obtained by immunization with rabbit adrenal. Besides the adrenal-specific reaction, this antiserum produced a line of precipitation which was non-adrenal-specific and could be obtained with other rabbit organs. It could be shown that the non-organ-specific antigen detected by anti-adrenal serum is identical with the antigen detected by anti-kidney serum.

Histopathological studies of kidneys of the immunized rabbits were performed. Results will be presented later. It may be stated now that in some of the animals most pronounced abnormalities with up to 5-fold enlargement of kidneys were observed.

*Guinea pig experiments.* Six guinea pigs were immunized with a suspension of pooled guinea pig kidney. In another group of 6 guinea pigs, each animal was immunized with the suspension of its own kidney removed by nephrectomy. All animals formed quite potent antibodies combining with an extract of pooled guinea pig kidney as exemplified by the experiment presented in Table III. However, the reactions observed were not kidney-specific, since equally strong and sometimes even stronger reactions were obtained with the extract of guinea pig liver. On the other hand, extracts of other organs gave negative results or only rather weak reactions (Table IV).

By double diffusion gel precipitation one sharp line of precipitation was obtained. Interestingly enough, the line formed with liver extract was distinct, whereas the line with kidney extract was faint. In some experiments the only precipitation line to appear was the one with the liver extract.

The antibodies produced by guinea pigs immunized with guinea pig kidney suspensions were autoantibodies. Very similar results to those presented in Tables III and IV were obtained when the extracts of pooled guinea pig organs were replaced by extracts of organs from the antibody-producing animal. In addition, animals immunized with a suspension of their own kidneys formed antibodies similar to those produced by animals immunized with pooled guinea pig kidney (Table V).

TABLE V. Complement Fixation Test with 1:5 Diluted Guinea Pig Antisera to the Animal's Own Kidney #179 and #292 and Extracts of Pooled Guinea Pig Organs.

| Dilution of organ extract 1: | Kidney #179 | Kidney #292 | Liver #179 | Liver #292 |
|---|---|---|---|---|
| 1 | 4+ | 4+ | 4+ | 4+ |
| 3 | 4+ | 4+ | 4+ | 4+ |
| 9 | 4+ | 3+ | 4+ | 4+ |
| 27 | 3+ | 2+ | 3+ | 4+ |
| 81 | ± | — | 1+ | 2+ |

The described reactions of the guinea pig anti-kidney sera were species-restricted. Virtually negative results were obtained by both complement fixation and gel precipitation tests with kidney and liver extracts originating from ox, rabbit and rat.

TABLE IV. Complement Fixation Test with 1:5 Diluted Guinea Pig Antisera to Pooled Guinea Pig Kidney and Extracts of Pooled Guinea Pig Organs.

| | Highest extract dilution which gave 4+ or 3+ reaction: Kidney | Liver | Adrenal | Spleen | Lung | Testis | Brain |
|---|---|---|---|---|---|---|---|
| Antisera # 1 | 81 | 81 | 3 | 3 | 1 | 1 | 1 |
| #141 | 27 | 81 | n.d. | 1 | <1 | 1 | <1 |

*Discussion.* The 3 best documented experimental autoimmune diseases, allergic encephalomyelitis, experimental aspermatogenesis and experimental thyroiditis, represent organ-specific phenomena since they may be elicited only by immunization with the corresponding tissue. The experimental autoimmune diseases are frequently accompanied by formation of humoral antibodies characterized by 2 important features: a) they have an auto-character since they combine with the antigens of the antibody-producing animal, and b) they are strictly organ-specific since they act only upon the antigenic preparations from the corresponding organ and not upon antigens originating from any other organ.

Serological studies on rats with autoimmune nephrosis were performed by Hunter *et al*(7). Sera of rats immunized with kidney suspensions precipitated with kidney extracts. However, no kidney specificity of these antibodies was demonstrated; some of the sera also precipitated with rat liver extracts and the lines of precipitation formed with kidney and liver extracts merged in an identity reaction.

The pathogenic role of humoral antibodies in experimental autoimmune diseases has never been satisfactorily proven and several investigators believe that these diseases are elicited by cell mediated immunological mechanisms. Nonetheless, study of humoral autoantibodies accompanying an autoimmune disease frequently brings most valuable information by indicating which antigen or antigens may possibly be involved in the disease process.

In designing the present experiments, it was anticipated that some kidney-specific autoantibodies may be found. Even if the autoimmune nephrosis described by Heymann *et al*(1,2) is not strictly an organ-specific phenomenon, it still was observed much more frequently in rats immunized with kidney than in animals immunized with other organs. In addition, in previous studies in this department(8) some evidence for the existence of kidney-specific antigens has been achieved.

In the present study rabbits immunized with a rabbit kidney suspension formed 2 types of antibodies. One was strictly kidney-specific while the other was devoid of organ specificity. Evidence was presented that both these antibodies are autoantibodies. On the other hand, guinea pigs immunized with guinea pig kidney produced only the second type of autoantibody, *i.e.*, the one lacking organ specificity.

Formation of organ-specific autoantibodies as a result of immunization within the same species is a well established phenomenon. The present study adds kidney antibodies to the list of successfully produced autoantibodies specific for lens, brain, testis, thyroid, adrenal, salivary gland, prostate, etc. On the other hand, formation of non-organ-specific autoantibodies is a rather exceptional finding. The only other autoantibody that is devoid of organ specificity and which may be experimentally elicited is the Wassermann antibody. This antibody is directed against a ubiquitous lipid component present in various tissues of many animals as well as in several bacterial species. The antigen described here is by far less ubiquitous than is the Wassermann antigen; it has a species-restricted character since the rabbit antibody combined only with the antigen of rabbit origin and the guinea pig antibody combined only with the antigen of guinea pig origin. In rabbit, the antigen in question was present in many organs including kidney, liver, heart and adrenal. Thus far in guinea pig, it could be convincingly demonstrated only in kidney and liver; other guinea pig organs apparently do not contain this antigen at all or contain it in a very low concentration.

To obtain a definite answer as to the role of kidney-specific and non-kidney-specific autoantigens in eliciting autoimmune nephrosis, one would have to perform immunization experiments using these antigens in a purified form. It would appear, however, that possibly both of these antigens may be involved in autoimmune nephrosis. The fact that Hunter *et al*(7) failed to find kidney-specific autoantibodies in rats does not mean that the kidney-specific antigen cannot play a pathogenic role. It may well be that rats responded to the kidney-specific antigen by cell

mediated immunological mechanisms. On the other hand, the fact that in Heymann's experiments autoimmune nephrosis was also occasionally elicited by liver points to the fact that the non-kidney-specific antigen described in this study may also be involved in this disease.

*Summary.* Rabbits were immunized with a rabbit kidney suspension incorporated into complete Freund adjuvants; guinea pigs were immunized similarly with guinea pig kidney. The rabbits formed 2 types of autoantibodies, one kidney-specific and the other devoid of organ specificity. The guinea pigs formed only antibodies of the second type. The possible role of the 2 corresponding antigens in eliciting experimental autoimmune nephrosis was discussed.

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## Comparison of Enzyme Activity in Malignant and Adjacent Uninvolved Tissue.* (29435)

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The aim of the present study is identification of the site of origin of 3 serum enzymes (lactic dehydrogenase(LD), malic dehydrogenase (MD), and enolase), which are elevated in cases of adenocarcinoma of the colon(1).

There are several possible explanations for the elevated serum activities of these enzymes. First, the increase may be due to elevated production of the enzymes by the tumor mass itself. Second, the elevated activity could be caused by the stimulation of enzyme production in other tissues through systemic effects of the tumor. Third, necrosis of the tumor tissue, or of the tissue invaded by the tumor, might cause increased release of the enzyme into circulating body fluids. Finally, alterations in permeability of malignant cells, or normal cells, could account for the elevated enzyme activity in the serum. Each of these mechanisms may act alone, or in concert with the others, in producing the elevated serum enzyme levels.

This study is limited to the measurement of LD, MD, and enolase activity in tumors

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