THE PRODUCTION OF CHRONIC GLOMERULONEPHRITIS IN RATS BY THE INJECTION OF RABBIT ANTI-RAT-PLACENTA SERUM

By BEATRICE CARRIER SEEGAL, M.D., and EMILY NICHOLS LOEB, M.D.

(From the Departments of Bacteriology and of Medicine, Columbia University, and the Research Service, First (Columbia University) Division, Goldwater Memorial Hospital, Department of Hospitals, New York)

Plates 9 and 10

(Received for publication, June 6, 1946)

Recently interest in diseases which may result from specific antibodies acting *in vivo* has been intensified by the following observations: It has been demonstrated that injection of animals with antiserum prepared from homologous kidney results in progressive renal lesions (1–5). Glomerulonephritis in rats has resulted also from the action of autoantibodies following the injection of rat kidney mixed with killed streptococci (6). Furthermore, in man it has been established by the work on erythroblastosis foetalis (7) that specific cytotoxic antibodies may arise naturally and cause serious or fatal disease.

The clinical picture of the toxemias of pregnancy has many points in common with acute glomerulonephritis; namely, the explosive development of albuminuria, cylindruria, oliguria, and hypertension. There has long been speculation about the possible rôle of the placenta in the etiology of the toxemias. This organ, derived in part from the fertilized ovum, suggests an obvious source of antigen which might prove foreign to the mother and provoke the production of injurious antibodies. With the idea in mind to test the possible renal effect of such an antigen-antibody reaction, a series of experiments was undertaken in which rabbit anti-rat-placenta serum was injected into pregnant rats. Specific anti-placenta serum has been known to interrupt pregnancy in the guinea pig and rabbit since the observations of Dobrowolski in 1903 (8). Degeneration of the placentae and fetal death likewise were found to follow the injection of rabbit anti-rat-placenta serum in rats of the Long-Evans strain (9). However, no acute renal lesions were observed at autopsy in these animals which were sacrificed at term 9 days after the antiserum was given.

Smadel and Swift (10) reported that rats of the Long-Evans strain injected with nephrotoxic serum, may require months to develop chronic glomerulonephritis. It therefore seemed of interest to allow animals injected with anti-placenta serum to remain under observation for a period of months in order to detect a possible eventual effect on the kidneys. The present paper reports these experiments controlled by studies on additional groups of rats which were injected with anti-rat-kidney serum, anti-rat-erythrocyte serum, anti-rat-serum serum, normal rabbit serum, or which were left untreated. A preliminary description of some of these results has already appeared (11, 12).

Materials and Methods

Preparation of Rabbit Antiserums.—

Anti-Rat-Placenta Serum.—Pregnant rats were sacrificed with ether 1 day before term and perfused according to the method described by Smadel (3). The placentae were then removed aseptically, cut into small pieces, washed once in sterile saline, placed in the ice box overnight in fresh saline, and washed again with saline the following day. By these procedures most of the gross blood was removed. The placentae were ground to a paste in a glazed porcelain mortar and made up to a 20 per cent suspension in physiological saline. Rabbits were immunized by the intraperitoneal injection of 0.5 to 1.0 cc. of this suspension four or five times a week for 3 weeks. After a rest period of 10 days the series of 3-week injections was repeated twice. Each rabbit received a total of approximately 10 gm. of rat placenta. The rabbits were bled out 10 days after the last injection and the serum obtained was stored in the ice box without preservative.

Anti-Rat-Kidney Serum.—This antiserum was prepared according to the method described by Smadel (3). Each rabbit received approximately 30 gm. of rat kidney.

Anti-Rat-Erythrocyte Serum.—Rabbit anti-rat-erythrocyte serum was prepared by injecting rabbits on 5 successive days with 0.5 cc. of a 50 per cent saline suspension of rat erythrocytes washed five times. The series of injections was repeated three times with a week's rest between each series. The first series was given intravenously, the remaining three intraperitoneally. The animals were bled out 10 days later as in the case of the anti-placenta and anti-kidney serums.

Anti-Rat-Serum Serum.—This serum was prepared in the same manner as was the anti-rat-erythrocyte serum except that undiluted serum was used in place of the washed red blood cells.

Antibody Titration of Antiserums.—

Antiserums were tested for precipitins against dilutions of rat placenta, kidney, liver, ovary, lung, heart, brain, and serum, and for hemolysins for rat erythrocytes.

In the precipitin tests the antiserum was used undiluted. The organs were weighed, ground, and a 10 per cent saline suspension was prepared. This was centrifuged and from the clear supernatant serial dilutions up to 1:1280 were made. Nitrogen determinations were carried out on the original 10 per cent centrifuged supernatant so that results could be interpreted in terms of the amount of protein in each antigen suspension. The immune serums and organ dilutions were taken up in glass capillary tubes 1 mm. in diameter and were examined for precipitate after 48 hours in the icebox (Swift *et al.* (13)).

Hemolysins were determined by mixing 0.3 cc. of serial dilutions of the antiserums with 0.1 cc. of 5 per cent washed rat erythrocytes. When the mixture had stood for 30 minutes at room temperature 0.1 cc. (2 units) of guinea pig complement was added and the whole incubated for ½ hour at 37°C. The titer is reported as that dilution of serum which gave complete lysis.

Animal Stock and Diet.—

Hooded rats bred from stock obtained from Dr. P. E. Smith (Long-Evans strain) were used. The animals were maintained on a diet consisting of ground whole wheat 67.5 per cent, whole milk powder 10 per cent, butter 4 per cent, cod liver oil 1 per cent, casein 15 per cent, sodium chloride 1 per cent, and calcium carbonate 1.5 per cent.

Injection of Rats.—

Injections of all antiserums, except for the anti-rat-erythrocyte serums, were given on 2 or 3 consecutive days intravenously in the foot or leg veins, without the use of an anesthetic.

The first injection in the case of the anti-rat-erythrocyte serums was only 0.12 or 0.15 cc. inasmuch as larger initial amounts resulted in fatal hemolytic anemia. The subsequent doses and intervals between injections were gauged according to the degree of anemia and the rate of recovery. Two of the serums, 1337 and 1339, required 22 to 29 days for administration, while serum 1338 required 31 to 68 days owing to the greater capacity of this serum to produce anemia. The age of the animals in all groups varied from 46 to 99 days, the pregnant animals falling into the older age group. When pregnant animals were used they were injected first on the 10th or 11th day of gestation. The date of mating was determined by examination of the vaginal smears.

Examination of Urine.—

Samples of urine were collected every 2 to 4 weeks. Albumin determinations by the heat and acetic acid method were done routinely.[1] Since normal rats, especially male animals, show small amounts of albumin in the urine, only a marked increase over the normal base

TABLE I

Allocation of Animals

Group	No. of animals		Age at time of injection		No. of pregnant animals	Serum	Amount
	♂	♀	♂	♀			
			days	*days*			*cc.*
I	16	16	46–67	72–88	12	Rabbit anti-rat-placenta Nos. A, B 515, or 103	0.9–2.0
II	5	8	68–71	75–99	7	Rabbit anti-rat-kidney No. 550	0.6–1.0
III	21	34	—	—	—	None	—
IV	10	13	52–70	52–95	7	Rabbit anti-rat-erythrocyte Nos. 1337, 1338, or 1339	1.5
V	6	8	65	68–83	8	Rabbit anti-rat-serum Nos. 538, 108, or 109	0.8–1.4
VI	—	7	—	73–85	6	Normal rabbit	1.5

line was considered significant. To establish further the existence of significant albuminuria random samples of urine at intervals of 1 to 3 months were tested quantitatively by the method of Shevky and Stafford (14).

Nature of Urinary Protein.—

In a few cases the urines of recently injected rats were examined immunologically for the presence of both rat and rabbit proteins. In the former instance, a rabbit anti-rat-serum serum was employed, whereas a horse anti-rabbit-globulin serum supplied by Dr. Michael Heidelberger was used in the latter instance. The urines were diluted 1 to 5 since more concentrated urine sometimes gave non-specific precipitation with normal rabbit or horse serum. The tests were done in duplicate in capillary tubes of 1 mm. diameter and in small precipitin tubes of 3 mm. diameter.

[1] For these determinations as well as for other technical assistance we are indebted to Miss Geraldine Haines and Miss Gertrude Herz.

Determination of Blood Non-Protein Nitrogen.—

When marked and persistent albuminuria gave evidence suggestive of kidney damage the animals were bled from the heart at intervals of 1 to 3 months and the non-protein nitrogen content of the whole blood determined.[2]

Blood Counts.—

Random red blood cell counts in approximately half the animals were made 2 to 4 days after the injection of serum to detect the possible presence of anemia. Counts were repeated on the pregnant animals 1 day prior to delivery. Where anti-erythrocyte serum was employed counts were made at least every 4 days until all the serum had been given.

Postmortem Studies.—

The animals were sacrificed with ether 3 to 14 months after injection. Unless death from renal insufficiency appeared imminent the experiment was usually terminated in 12 months. Sections of the organs removed at autopsy were fixed in Zenker's solution and stained with hematoxylin and eosin.

Allocation of Animals.—

The 144 rats used in the experiments were divided into 7 groups according to the treatment which they received. This distribution is presented in Table I.

RESULTS

Renal Pathology

Group I.—Of the 32 animals injected with anti-placenta serum 11 males and 7 females presented evidence of chronic glomerulonephritis (Table II) when sacrificed 3½ to 14 months later. The gross and microscopic renal lesions observed have been classified uniformly for all groups as Type A (moderate), Type B (severe), and Type C (very severe). The course of events in each instance as measured by the degree of albuminuria and of nitrogen retention was correspondingly moderate, severe, or very severe. Type A nephritis was characterized by a slow course marked by persistent albuminuria of 6 to 10 gm. per liter with an average terminal non-protein nitrogen of 51 mg. per cent 9 to 14 months after injection. At autopsy, except for one instance when the kidney surface was finely pitted, no gross renal lesion was observed. Microscopic examination of the kidneys showed moderate numbers of casts with flattening of the tubular epithelium. Occasional glomeruli presented thickening of Bowman's capsule, adhesions of the tuft to the capsule, and infrequent crescents. There was very little leukocytic infiltration and rarely interstitial connective tissue proliferations. This type A nephritis occurred in 3 males and 3 females of group I.

Type B nephritis, occurring in 5 males and 4 females injected with anti-placenta serum, ran a more rapid course, characterized by heavy albuminuria,

---

[2] We are indebted to Miss Emily Bidwell and Miss Ines Pingitore of the Chemical Laboratory of the Presbyterian Hospital, New York, for these analyses.

in excess of 10 gm. per liter, and an average terminal blood non-protein nitrogen value of 58 mg. per cent 7 to 11 months following injection. At autopsy the kidneys of all animals except one were pitted on the surface (Fig. 1). Moderate enlargement was noted in two instances. On section the cortex appeared narrow and the tubular markings were prominent in 5 of the 9 animals. On microscopic examination casts were numerous with varying degrees of flattening of the tubular epithelium. There was frequent thickening of the basement membrane of Bowman's capsule, and many examples of all degrees of glomerular damage from swelling of the tuft to adhesions between tuft and capsule and crescent formation. Moderate interstitial fibrosis with leukocytic infiltration was present (Figs. 2 and 3).

TABLE II

Incidence of Chronic Glomerulonephritis in Rats Injected with Cytotoxic Serums or Left Untreated

Group	No. of animals		Serum injected	Average total dose	Average lowest RBC count	Average No. of young	No. of animals with persistent abnormal albuminuria	Average age at death	No. of animals showing chronic nephritis at autopsy		
	♂	♀							Type A (moderate)	Type B (severe)	Type C (very severe)
				cc.				*mos.*			
I	16	16	Rabbit anti-rat-placenta	1.5	7.43	4.8	18	12.5	6	9	3
II	5	8	Rabbit anti-rat-kidney	0.7	7.87	7.4	11	10.8	2	6	3
III	21	34	None	—	—	—	—	12.0	0	1	1
IV	10	13	Rabbit anti-rat-erythrocyte	1.5	4.35	0.7	1	13.4	1	1	0
V	6	8	Rabbit anti-rat-serum	1.3	7.34	7.0	2	13.0	0	2	0
VI	0	7	Normal rabbit	1.5	7.72	10.2	0	14.0	0	0	0

Type C nephritis occurring in 3 males and in none of the females injected with anti-placenta serum ran a fulminating course and the animals were sacrificed in 3 to 5 months. Terminal blood non-protein nitrogen values on 2 of these animals were 112 and 190 mg. per cent, respectively. No determination was made on the third animal which was moribund when sacrificed. No quantitative determinations were made of the urinary albumin. Qualitatively the albuminuria was persistently heavy. At autopsy the kidneys of all 3 rats were greatly enlarged, pale, and finely pitted with pinpoint surface cysts (Fig. 1). In each of 2 animals where weights were determined the kidneys weighed 5.2 gm. and 11.0 gm., respectively, or approximately 2 to 5 times normal. Narrowed cortices and prominent tubular markings presented on section. Microscopic examination revealed numerous and prominent casts, flattening the tubular epithelium and distending the tubules to enormous dimensions. No normal glomeruli could be found while extensive crescent formation and

complete fibrosis of the tufts occurred. Marked interstitial fibrosis with leukocytic infiltration was present (Fig. 4).

Seven animals in group I were subjected to unilateral nephrectomy 1 to 5 months before sacrifice. The gross and microscopic findings of the surgically removed kidney only are included in those described above. The second kidney of each animal which was examined at autopsy was enlarged, in contrast to the surgically removed organ in all instances, weighing between 1.73 and 2.45 gm. The granular appearance was likewise more marked in all animals. In 5 of the animals microscopic examination of the kidney removed at laparotomy revealed a Type B nephritis, the lesions in the remaining 2 being considered Type A. The former animals at autopsy 4 to 6 weeks later still presented microscopic findings of Type B nephritis, although further advance of the lesion in two cases was suggested by an increase of 20 mg. per cent in blood non-protein nitrogen. Both of the animals presenting a Type A nephritis in the kidney removed at laparotomy, showed a definite advance to a Type B lesion at autopsy 2 and 5 months, respectively, after operation. The blood non-protein nitrogen value of 53 mg. per cent determined at operation in one animal rose to 83 mg. per cent in the terminal 8 weeks.

Fourteen animals of group I injected with anti-placenta serum failed to develop chronic glomerulonephritis. Albuminuria in excess of 5 gm. per liter was not observed and the average blood non-protein nitrogen value was 46 mg. per cent.

In 6 of the 14 animals microscopic examination of the kidneys revealed occasional small, local accumulations of leukocytes, mainly monocytes, infiltrating the cortex. These areas were at times associated with casts in adjoining tubules and rarely with fibrosis of neighboring glomeruli. These findings characterize "spontaneous" focal nephritis in the rat (15). The kidneys of the remaining 8 animals were normal.

One of the male rats in group I, included among those described as having focal nephritis, also had a moderate hydronephrosis of the right kidney. This lesion is not infrequent in this strain of rats and occurs almost always in the right kidney in male animals. It varies in degree from a slight increase in the pelvic space unaccompanied by histological evidence of parenchymal involvement to transformation of the kidney to a sac of fluid with little discernible renal tissue even on microscopic examination.

Group II.—It will be seen from the table that of the 13 animals injected with rabbit anti-rat-kidney serum, chronic glomerulonephritis developed in 11 sacrificed 3 to 12 months after injection. Type A (moderate) nephritis occurred in one male and one female, Type B (severe) lesions occurred in 2 males and in 4 of the females, whereas Type C (very severe) lesions were found in 2 males and 1 female. The rate of progression as measured by the degree of albuminuria and the blood non-protein nitrogen values in each grade of nephritis was comparable to findings observed among the rats of group I receiving anti-placenta serum. Likewise the gross (Fig. 1) and microscopic pathology corresponded to that seen in the same grades of nephritis described for group I. Two females in group II failed to develop chronic glomerulo-

nephritis. In one of these scattered small areas of focal nephritis were observed. One male with Type B nephritis presented a pronounced right-sided hydronephrosis with marked diminution of renal tissue.

Group III consisted of 55 untreated rats from breeding stock ranging from 7 to 15 months of age at death, in all but one instance. The exception was 21 months old when sacrificed. Chronic glomerulonephritis was observed in 2 of these animals.

One was the 21-month-old female which presented microscopic findings typical of Type B (severe) nephritis. The other, also a female, was sacrificed at 8 months of age because of a persistent abnormal albuminuria. At autopsy there was a maximal hydronephrosis of the left kidney, the organ being reduced to a shell of clear fluid. The right kidney was greatly enlarged, weighing 4.4 gm., and the surface was granular and presented multiple small cysts. The right ureter appeared normal, whereas no left ureter could be found grossly. The microscopic lesions revealed chronic glomerulonephritis typical of Type C. No quantitative urinary albumin or blood non-protein nitrogen determination was made on either of these 2 animals. It is interesting that the aged rat with Type B nephritis had been mated six times, had borne two litters which it failed to raise and apparently had failed to deliver the other four times. The other animal, pregnant twice, delivered one litter but did not raise it and failed to deliver the second litter.

No terminal elevation of blood non-protein nitrogen occurred in 39 of the 53 normal animals in this group in which this was measured. Abnormal albuminuria, *i.e.* in excess of 5 gm. per liter, occurred in one animal of 38 studied. This animal had 12.7 gm. per liter on one occasion and presented microscopic lesions typical of marked "spontaneous" focal nephritis. Focal nephritis occurred in 8 males and 12 females in this group. Hydronephrosis of the right kidney occurred in one female and 3 males which otherwise presented normal kidneys grossly and microscopically.

Group IV.—Of the 23 rats injected with rabbit anti-rat-erythrocyte serum, 2 presented microscopic lesions of chronic glomerulonephritis at autopsy 6½ to 13 months later. Type B (severe nephritis) occurred in one male which had shown a gradually increasing albuminuria to 10.9 gm. per liter and a terminal blood non-protein nitrogen of 68 mg. per cent. At autopsy a mild right-sided hydronephrosis was present. A pregnant female which had failed to deliver showed Type A lesions microscopically. It had a normal albuminuria, a terminal blood non-protein nitrogen of 38 mg. per cent, and normal kidneys grossly. The remaining 21 animals in the group failed to develop an abnormal degree of albuminuria, or nitrogen retention as measured by blood non-protein nitrogen. Normal kidneys were encountered in 7 instances, a marked degree of focal nephritis occurred once, and mild degrees of focal nephritis in 13 instances. Among these latter, one female and one male had also a slight right-sided hydronephrosis.

Group V consisted of 14 rats injected with rabbit anti-rat-serum serum and observed over a period ranging from 9 to 12 months. On microscopic examination 2 animals, both males, were found to have chronic glomerulonephritis

Type B. Grossly the kidneys appeared normal. The albuminuria was 12.7 and 14.4 gm. per liter. No terminal elevation of blood non-protein nitrogen was observed. In the remaining 12 animals albuminuria in excess of 5 gm. per liter did not occur. Elevation of blood non-protein nitrogen to 70 mg. per cent occurred in one animal heavily jaundiced, the cause of which was not determined. Lesions of focal nephritis slight in extent were found in 6 animals, 4 of which presented 1 to 2 coarse scars on the kidney surface.

Group VI consisted of 7 female rats, 6 of which were pregnant, injected with normal rabbit serum and observed $9\frac{1}{2}$ to $12\frac{1}{2}$ months. None developed chronic glomerulonephritis. Five presented minimal lesions of focal nephritis. There was no abnormal albuminuria nor any elevation terminally of the blood non-protein nitrogen.

Miscellaneous Pathology

There was no pathology relevant to the problem outside of that described above. A considerable degree of peribronchial leukocytic infiltration occurred in the lungs of a large number of animals. From time to time a purulent bronchopneumonia was encountered. One instance of extensive myocardial scarring was observed grossly and several instances of small focal collections of leukocytes in the heart muscle with or without evidence of scarring were observed microscopically. Two rats dying, deeply jaundiced, showed microscopic lesions characteristic of acute hepatitis. The livers of occasional animals showed a small collection of leukocytes and others had, in addition, small focal areas of necrosis. In a few instances encysted liver parasites were observed grossly. Gross evidence of *Salmonella* infection, as described by Buchbinder *et al.* (16), was found at autopsy in 21 of 144 animals. None of the above miscellaneous pathology could be correlated with the development of chronic glomerulonephritis or of spontaneous focal nephritis.

Anemia.—As can be seen from Table II, with the exception of animals in group IV receiving rabbit anti-rat-erythrocyte serum, anemia did not occur. Only one animal of all the other groups had a red blood cell count below 6 million. Anti-erythrocyte serum 1338 was extremely potent in producing anemia and resulted in the death of 8 animals (not included in group IV), whereas only one death followed the injection of each of serums 1337 and 1339. The only 2 rats in this group exhibiting renal lesions characteristic of chronic glomerulonephritis had been injected with serum 1338. Their average lowest red blood cell count was 2.75 million per c.mm., while the average count in the remaining 11 rats treated with serum 1338 was 3.91 million per c.mm. The average lowest red blood cell count among 10 rats treated with serums 1337 or 1339 was 5.06 million per c.mm.

Abortion.—It can be seen from Table II that the injection of anti-placenta serum in pregnant animals reduced the expected number of young by approximately 50 per cent. This diminution was largely due to the outstanding

capacity of serum 103 to produce abortion. Anti-kidney serum and anti-serum serum may have reduced the number of viable young in two instances each. Six of the 7 pregnant rats injected with anti-erythrocyte serum failed to deliver full term viable young.

In vitro Titration of Antiserums

Precipitin titrations of serums A, B, 103, 550, 1337, 1338, and 538 against extracts of rat placenta, kidney, liver, ovary, lung, heart, brain, and serum were carried out by the technique already described. The results failed to correlate with the activity of these serums *in vivo*.

The anti-placenta serums A, B, and 103 gave only traces of precipitate with all the organ extracts. Dilution of the organ extracts to 1–160 usually resulted in a negative test. There was no evidence of specificity for either placenta or kidney. Likewise anti-kidney serum 550 was weakly precipitating showing little more than traces of antibody for any antigen except kidney. With the latter a slightly heavier precipitate formed with the 1–10 dilution of the antigen. The anti-erythrocyte serums 1337 and 1338 and the anti-serum serum 538 produced large amounts of precipitate when tested with lung, heart, and brain extracts diluted 1–10. The tests usually became weak or negative on diluting the extracts 1–320. Antiserums 1337 and 538 also strongly precipitated placenta extract and rat serum. Other organ extracts were precipitated weakly. The nitrogen content of the 1–10 dilution of the organ extracts varied from 0.57 to 1.47 mg. nitrogen per cc. Serums 515, 1339, 108, and 109 were not tested.

The titration of hemolysins showed that the anti-placenta and anti-kidney serums contained only small amounts of this antibody. The titer varied from 1–2 to 1–8. The hemolysin titer of the anti-erythrocyte serums 1337, 1338, and 1339 was 1–160, 1–320, and 1–40, respectively.

Species Specificity of the Urinary Protein

Urines from 5 rats obtained 1, 6, 12, and 19 days after injection of rabbit anti-rat-placenta serum were tested for the presence of both rabbit and rat protein by the precipitin technique described. Both rat and rabbit proteins were present in the urines obtained 1 and 6 days after injection. Twelve days after injection rabbit protein was absent in 4 urines and the fifth urine showed only a trace. Rat protein was demonstrable as previously. Rat protein alone was found in urines obtained 19 days after injection.

DISCUSSION

Rabbit anti-rat-placenta serums when injected into young adult rats produced chronic glomerulonephritis in 18 of 32 animals. The renal lesions were indistinguishable from those obtained in 11 of 13 rats injected with rabbit anti-rat-kidney serum. Smadel (3) has reported that antiserums prepared in rabbits by the injection of rat heart, liver, serum, or erythrocytes lack nephrotoxin. Small amounts of nephrotoxin were detected in the serum of one

rabbit subjected to prolonged immunization with rat brain. It thus appears that anti-placenta serum is the only other cytotoxic serum thus far known to be capable of producing a chronic nephritis indistinguishable from that obtained with anti-kidney serum.

It will be noted from Table II that a higher incidence of nephritis occurred among animals injected with 0.7 cc. of anti-kidney serum than in those receiving 1.5 cc. of anti-placenta serum. This does not necessarily indicate that kidney is superior to placenta as an antigen for stimulating the production of nephrotoxic antibodies in rabbits, inasmuch as the amount of kidney used for immunization was roughly three times that of placenta. Moreover, the single anti-kidney serum 550 employed in this experiment was selected from others less potent, whereas the four anti-placenta serums were unselected.

The nephritis following the injection of anti-placenta and anti-kidney serums occurred in animals of both sexes and was not correlated with the presence of pregnancy or of abortion.

The lesions of chronic nephritis arising in 6 of 99 control rats were indistinguishable from those observed among animals injected with anti-placenta and anti-kidney serums. It is of interest to note that the only Type C (very severe) renal lesion occurred in an 8-month-old untreated stock female which had a marked left-sided hydronephrosis.

As might have been predicted from work previously reported on rabbit anti-rat-whole-blood serum (9), rabbit anti-rat-erythrocyte serum interrupted the course of pregnancy, only one of 7 pregnant animals delivering full term viable young. The mechanism underlying the interruption of pregnancy following the injection of this serum has not been elucidated.

The *in vitro* titration with rat organ extracts of the antibodies in the rabbit anti-rat-placenta and rabbit anti-rat-kidney serums gave no indication of their specific activity *in vivo*. This substantiates the observations reported by Smadel (3). On the other hand, rabbit anti-rat-erythrocyte serums showed relatively high hemolysin titers and were capable of producing grave anemia *in vivo*.

The experiments demonstrate that rat placenta contains an antigen capable of producing antibodies injurious to rat kidney. Whether this antigen is identical with that present in rat kidney has not been determined. It is hoped that current reciprocal absorption experiments may give information on this point.

CONCLUSIONS

1. The injection of rabbit anti-rat-placenta serums in young adult rats produced chronic glomerulonephritis in 18 of 32 animals.

2. The course of the nephritis and the renal lesions were indistinguishable from those obtained in 11 of 13 animals injected with rabbit anti-rat-kidney serum.

3. Six instances of similar renal lesions occurred among 99 control animals. In 2 the rats had been injected with rabbit anti-rat-erythrocyte serum, in 2 with rabbit anti-rat-serum serum, while in 2 others the lesions were found among normal stock.

BIBLIOGRAPHY

1. Masugi, M., *Tr. Japan. Path. Soc.*, 1931, **21,** 329.
2. Masugi, M., *Beitr. path. Anat. u. allg. Path.*, 1933–34, **92,** 429.
3. Smadel, J. E., *J. Exp. Med*,, 1936, **64,** 921.
4. Smadel, J. E., *J. Exp. Med.*, 1937, **65,** 541.
5. Smadel, J. E., and Farr, L. E., *J. Exp. Med.*, 1937, **65,** 527.
6. Cavelti, P. A., and Cavelti, E. S., *Arch. Path.*, 1945, **40,** 158, 163.
7. Wiener, A. S., Blood Groups and Transfusion, Springfield, Illinois, Charles C. Thomas, 1943, 369.
8. Dobrowolski, M. S., *Bull. Int. Sc. Cracovie*, 1903, No. 5, 256.
9. Seegal, B. C., and Loeb, E. N., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45,** 248.
10. Smadel, J. E., and Swift, H. F., *J. Exp. Med.*, 1941, **74,** 345.
11. Seegal, B. C., and Loeb, E. N., *Fed. Proc.*, 1943, **2,** 101.
12. Loeb, E. N., and Seegal, B. C., *Fed. Proc.*, 1943, **2,** 99.
13. Swift, H. F., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, **78,** 127.
14. Simmons, J. S., Laboratory Methods of the United States Army, Philadelphia, Lea & Febiger, 1935, 290.
15. Jaffé, R., Anatomie und Pathologie Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931, 268.
16. Buchbinder, L., Hall, L., Wilens, S. L., and Slanetz, C. A., *Am. J. Hyg.*, 1935, **22,** 119.

EXPLANATION OF PLATES

PLATE 9

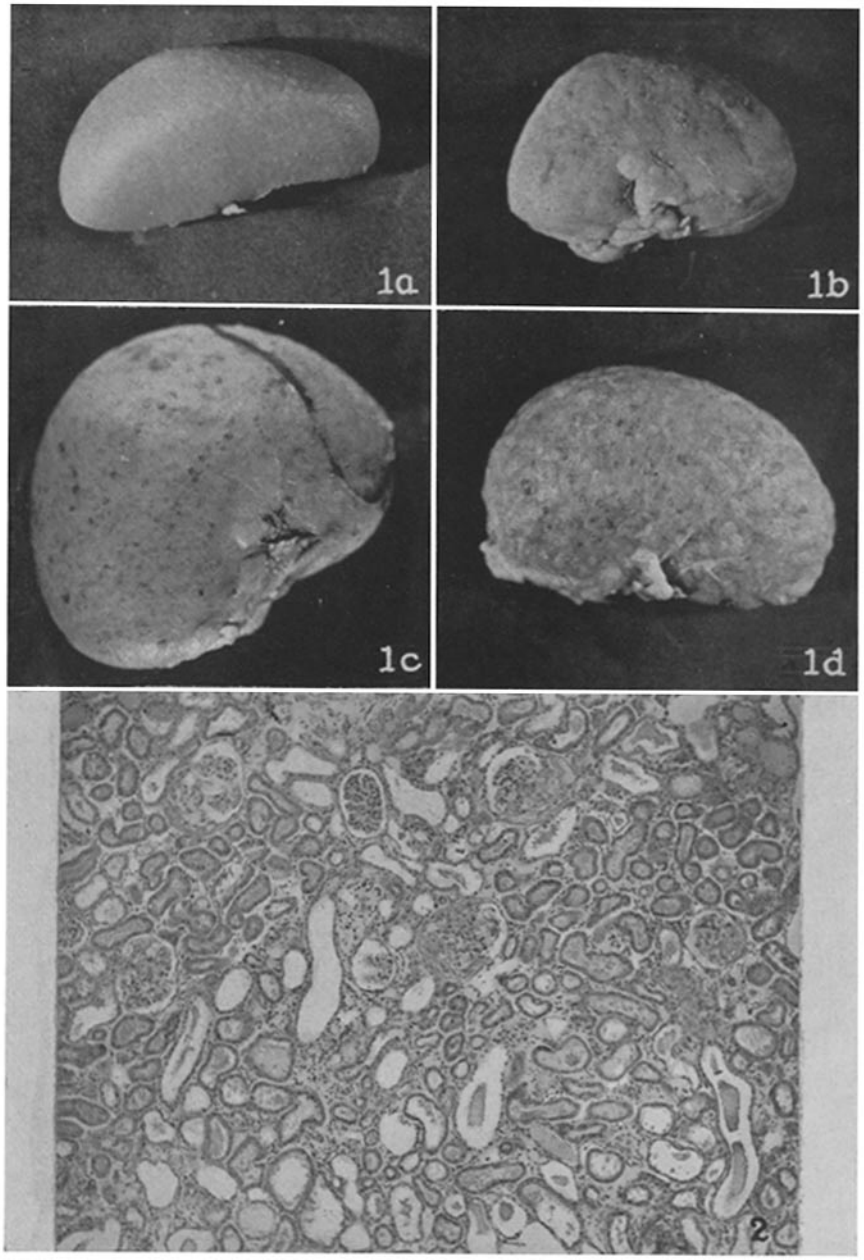
FIG. 1 (*a*) Male rat A290. Normal kidney from an animal injected 49 weeks previously with 1.5 cc. rabbit anti-rat-erythrocyte serum 1337.

FIG. 1. (*b*) Female rat A133. Type B (severe) chronic nephritis 30 weeks following injection of 2.0 cc. rabbit anti-rat-placenta serum A.

FIG. 1. (*c*) Male rat A 107. Type C (very severe) chronic nephritis 14 weeks following injection of 1.8 cc. rabbit anti-rat-placenta serum A. The mottled appearance is due to pitting and to small surface cysts.

FIG. 1. (*d*) Male rat A128. Type C (very severe) chronic nephritis 21 weeks following injection of 0.7 cc. rabbit anti-rat-kidney serum 550.

FIG. 2. Male rat 93. Type B (severe) chronic nephritis 10½ months following injection of 1.3 cc. rabbit anti-rat-placenta serum B. Note variations in the glomerular tufts from normal appearance to almost complete fibrosis. Many tubules are distended and contain casts. There is moderate interstitial leukocytic infiltration.

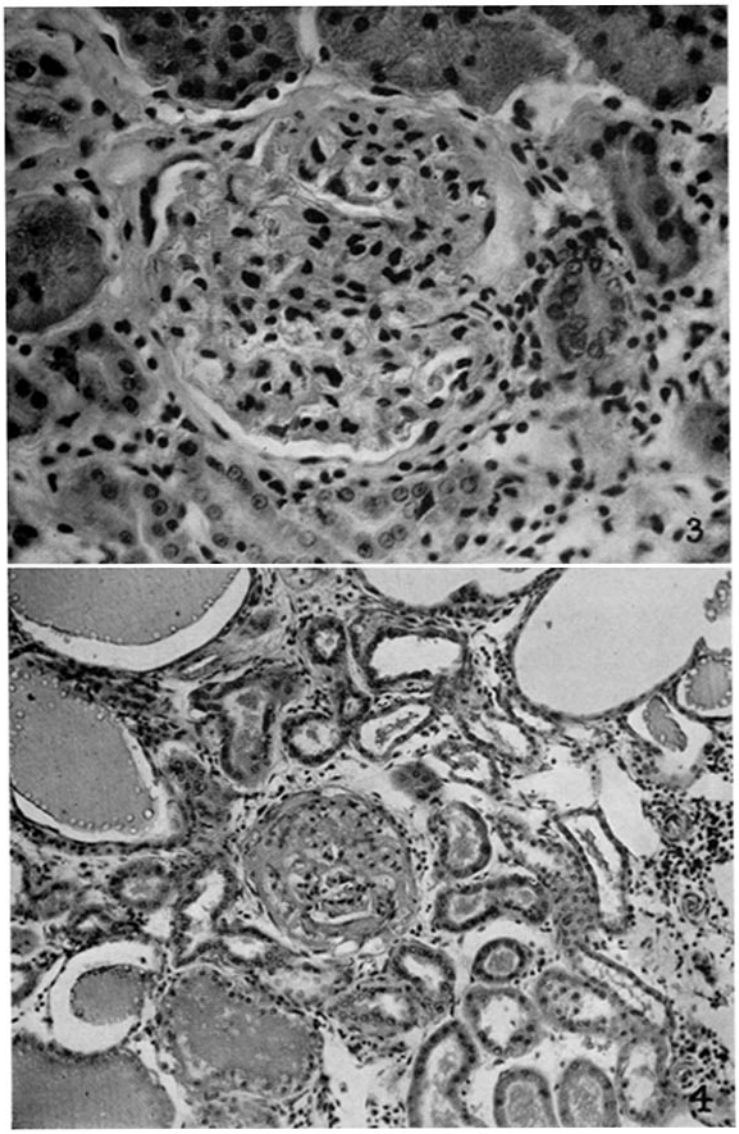


(Seegal and Loeb: Chronic glomerulonephritis)

PLATE 10

FIG. 3. Male rat 93. See above.

FIG. 4. Male rat 107. See above. Glomerular, tubular, and interstitial damage is more marked in this Type C (very severe) nephritis.



(Seegal and Loeb: Chronic glomerulonephritis)