EXPERIMENTS ON THE PRODUCTION OF SPECIFIC ANTISERA FOR INFECTIONS OF UNKNOWN CAUSE.

III. Nephrotoxins: Their Specificity as Demonstrated by the Method of Selective Absorption.

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Plates 5 to 7.

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The present study was undertaken as a continuation of work begun in this laboratory on the production of antisera for infections of unknown cause. It was found[1] that the infected tissues of animals might serve in some cases as an antigen for the production of immune sera, though with such antigens the sera inevitably contained antibodies injurious to the tissues of the animal. Experiments showed, however, that the sera can be largely deprived of these latter by incubation with successive portions of red cells without impairment of their protective value. Whether there are substances injurious to special organs which cannot so be taken out has remained to be determined. The problem is closely connected with that of the specificity of cytotoxins.

For most organs truly specific cytotoxins have not as yet been produced, despite many attempts. They have been elicited, though, for certain organs more or less isolated in the body. Zinsser sums up as follows on the subject:[2]

"Recent critical studies . . . . have revealed . . . . that the specificity of a serum produced with the tissues of one organ is not strictly limited to this organ alone, and that the serum may injure other organs as well. It is true, indeed, that there are certain cells and tissues in the body such as spermatozoa, the tissues of the testicles, the ovary, the lens of the eye, and possibly

[1] Rous, P., Robertson, O. H., and Oliver, J., *J. Exp. Med.*, 1919, xxix, 283.

[2] Zinsser, H., Infection and resistance, New York, 1914, 92.

the placenta, which have chemical characteristics so well defined and individual that the cytotoxic sera induced by them have definite organ specificity. The same to a more limited extent seems true of kidney substance (Pearce). In most cases, however, in which originally a specific cytotoxin was claimed, it has been possible to show subsequently that the apparently selective injury was not due to organ specificity alone but to the fact that the injection of tissue macerates, even when sufficiently freed from blood, induced the formation of considerable amounts of hemagglutinins and hemolysins."

In the light of such facts our problem resolves itself into a comparatively simple one: to determine whether one of the so called specific cytotoxins can be removed from a serum by exhausting the latter with red cells.

In selecting an organ for the study of "specific" cytotoxic sera the kidney seemed best adapted, because injuries to it can be determined by functional as well as histological methods. Pearce[3] has brought forward evidence that a serum can be produced which is, as he says, at last "special" in its action on the kidney. He leaves in doubt whether it can be termed specific in the strict sense, for despite the use of blood-free kidneys as antigen, his sera invariably contained hemolysins and hemagglutinins which might possibly account for their damaging effect on the kidney.

We have undertaken, first, to obtain a serum such as Pearce describes and observe its effects on the kidney; second, to determine whether the principle injurious to the kidney can be removed from this serum by absorption with red blood cells or with kidney tissue; and, third, to compare the efficacy of serum exhausted with red cells and that similarly treated with kidney tissue.

Production of Antikidney Serum.

Pearce's method was followed.[3]

Large dogs were etherized and under special precautions for asepsis their kidneys were removed and placed in glass boxes. The neighboring portion of the aorta was left attached to the kidneys in order to facilitate the passage of a cannula into the renal artery; and by prolonged washing with sterile salt solution the kidneys were practically freed of blood. The capsules were then stripped,

[3] Pearce, R. M., *J. Med. Research*, 1904, xii, 1.

the kidneys weighed and put through a meat grinder. The macerated tissue was pushed through a tea strainer of fine mesh with the aid of a pestle, small quantities of salt solution being added from time to time to facilitate its passage. The entire procedure was carried on under a glass plate surrounded by a canopy of sterile muslin which effectively protected the material from contamination.

The resulting heavy suspension in salt solution passed readily through a needle of medium sized bore. The material was usually injected into animals within 48 hours of its preparation, though occasionally it was kept in a frozen condition for some days prior to use. A number of rabbits received three to five intraperitoneal injections, each of from 4 to 8 gm. of kidney tissue, at intervals of 7 days, and they were bled on the 10th day after the last one. The sera were inactivated (56°C. for ½ hour) prior to use and their hemolytic and hemagglutinating titers tested, and cultures taken.

The sera without exception possessed moderately strong hemagglutinins and weak hemolysins for dog cells. Tests were made in the usual manner with graded dilutions in salt solution. Equal portions of the serum dilutions and of a 5 per cent suspension of thrice washed dog cells were used in the agglutination tests, and for hemolysis guinea pig complement in a dilution of 1 to 10 was added.

Precipitins were tested for but when found were negligible in amount.

Effects of Antikidney Serum.

Nine dogs were given a single intravenous injection of inactivated antikidney serum. The dose was 1 to 2 cc. per kilo of body weight. In most cases extremely marked renal changes resulted, differing considerably from those described by Pearce.

Prior to injection the general condition as well as the urine of the animals was carefully followed for a period of at least 2 weeks. Many supposedly normal dogs show albumin and casts in the urine without evident cause, and much delay was often experienced in finding animals suitable for our purpose. Within 24 to 48 hours after injection the animals appeared rather lethargic and gave the general impression of being sick. This period of depression passed off completely, or persisted, according to the potency of the serum and corresponding in a general way to the damage sustained by the kidney. The changes in the urine were marked. Severe albuminuria rapidly developed, with many casts, mostly granular at first but later many of them hyaline and occasionally waxy. This always occurred within 48 hours after the injection of the serum and persisted for the few days before the dog was killed. A trace of blood was sometimes noted.

With the exception of one dog (Dog C), which was observed for 4 months, all of the dogs were chloroformed and autopsied 10 to 14 days after injection. The kidneys were removed just prior to death or immediately after it. The viscera were examined, but no lesions were ever found that could be attributed to the serum. On two occasions more than 750 cc. of clear straw-colored fluid were present in the peritoneal cavity. No general edema was ever observed.

The kidneys showed pathological changes practically always, often of marked degree. In typical cases the organs were enlarged, and on stripping the capsule the kidney surface was everywhere mottled with bright red dots, from 0.1 to 1 mm. in diameter (Fig. 1, *A*). On section these were found to be the surface indices of hemorrhages into the cortex, the most characteristic gross finding. The hemorrhages were in general wedge-shaped, with the base of the wedge at the kidney surface. The glomeruli were indistinct and apparently had no relation to the hemorrhages. The cortex was swollen and opaque, and the pale medulla occasionally showed traces of hemorrhage extending from the cortex.

Histologically glomerular lesions, not the hemorrhages, were the most marked as well as constant finding. They occurred without hemorrhages when the serum was weak. In well marked instances there was an extensive necrosis in the glomerular tufts followed by obliterative hyaline changes (Figs. 2 and 3). A large proportion of the glomeruli were thus affected, though in even the most pronounced instances a sufficient number remained for kidney function. Accompanying the early cell changes was a plugging of the glomerular capillaries with fibrin and often a fibrinous exudation into the capsular space (Fig. 4). Desquamation and proliferation of the cells of the loops with occasional mitotic figures were observed. The end-result was often a complete obliteration of the glomerulus.

According to Pearce the most striking changes occur in the tubules. In our experiments lesions there were usually not pronounced, though sometimes marked cloudy swelling and desquamation were observed.

Next to the glomerular changes already described the most frequent findings were casts and hemorrhage into the tubules (Figs. 2 and 4). When the serum was strong small groups of a dozen to twenty tubules, usually in the neighborhood of a glomerulus, had their lumina filled

with blood cells (Fig. 5). These were the punctate hemorrhages so prominent in the gross specimen (Fig. 1).

There was also observable sometimes a considerable round cell infiltration between the tubules, especially in the neighborhood of the glomeruli. Some of the destroyed glomeruli showed a very heavy infiltration with lymphocytes and polymorphonuclear leucocytes (Fig. 6).

As a control two dogs were given large doses of normal rabbit serum. The results were negative as regards changes in the kidneys.

Effect of Antikidney Serum Exhausted with Red Cells.

The sera which caused the lesions described above contained, as already stated, hemolysins and hemagglutinins, though these were never strong. Steps were now taken to determine whether the action of the serum was due to them. Pearce performed a single experiment of the sort, submitting serum to contact with washed dog red cells for 1 hour at 0°C., and he found it thereafter "only faintly hemolytic." Kidney lesions were still produced. He records no observations, however, on hemagglutinins in the serum, which are often stronger and persist longer than hemolysins. In view of their ability to cause liver necroses,[3] such antibodies might well have been responsible for the lesions occurring in the kidneys.

By repeated absorptions with washed dog red blood cells, the technique of which has been previously described in detail,[1] our sera were completely deprived of hemolysins and hemagglutinins.

The results of the injection of dogs with serum thus treated are contrasted in Table I with findings in control animals receiving injections of corresponding doses of the same serum, untreated.

As the table shows, the exhaustion of the serum with red cells does not affect its ability to cause urinary changes. The albuminuria and cast formation were slightly less marked than in the controls. On the other hand, there were to the naked eye striking gross differences in the kidneys of the two series. It has been said that the untreated serum causes punctate hemorrhages in the renal cortex, often so numerous as to mottle diffusely the kidney surface. They are almost, or completely, lacking in animals given exhausted serum

TABLE I.

Effects of Nephrotoxic Sera, Untreated and Exhausted with Red Cells.

Experiment No.	Dog.	Serum No.	Treatment.	Serum titer. Action disappears at dilution of.	Dose per kilo of animal.	Effect on urine.		Killed after.	Condition of kidneys.
						Albumin.	Casts.		
					cc.			*days*	
1	A		Untreated.	1/256 lysis. 1/128 agglutination.	1.55	+−	++	8	Slight gross changes; characteristic glomerular lesions and microscopic hemorrhages. Casts.
	Aa	1*	11 cc. of serum underwent four absorptions with 1 cc. of red blood cells in all.	No lysins or agglutinins.	1.59	−	+	8	A few casts in tubules. Marked epithelial lesions.
2	B		Untreated.	1/256 lysis. 1/128 agglutination.	1.33	−	+	8	Marked epithelial lesions. Casts.
	Ba	2*	13.5 cc. of serum underwent four absorptions with 16 cc. of red blood cells.	No lysins or agglutinins.	1.72	+	−	8	Moderate epithelial lesions.
3	C		Untreated.	1/4 lysis. 1/1,024 agglutination.	1.65	++	+++	119	Few fibrotic glomeruli. No increase of interstitial tissue.
	Ca	3	17 cc. of serum underwent five absorptions with 25 cc. of red blood cells.	No lysins or agglutinins.	1.60	+++	+++	19	Severe glomerular lesions and hemorrhages. Many casts.

4	D		Untreated.	1/10 lysis. 1/30 agglutination.	0.60	++	++	9	Moderate glomerular lesions and hemorrhages. Many casts.
	Da	5	37 cc. of serum underwent five absorptions with 17 cc. of red blood cells.	No lysins or agglutinins.	1.36	++	+	9	Changes definite, but less than in the control.
5	E		Untreated.	As in Experiment 4.	1.85	++	++	10	Marked glomerular lesions. Hemorrhages and casts.
	Ea	5	Absorbed as in Experiment 4.	As in Experiment 4.	1.86	++	+	10	As in control, but slightly less marked.
6	F		Untreated.	1/4 lysis. 1/512 agglutination.	1.66	++	++	11	Numerous hemorrhages and glomerular lesions. Many casts.
	Fa	6	22 cc. of serum underwent seven absorptions with 22 cc. of red blood cells.	No lysins or agglutinins.	1.85	++	++	11	As in control.
7	G		Untreated.	1/128 lysis. 1/128 agglutination.	1.29	+++	++	10	Moderate changes of characteristic sort.
	Ga	7	15 cc. of serum underwent eleven absorptions with 34.5 cc. of red blood cells.	No lysins or agglutinins.	1.00	+++	++	10	As in control, but slightly fewer glomerular lesions.
	H		Untreated.	1/128 lysis. 1/128 agglutination.	0.65	++	++	11	Moderate changes of characteristic sort.
8	I	4	"	1/128 lysis. 1/16 agglutination.	1.65	+++	+++	22	Characteristic but moderate lesions.

* Sera 1 and 2 were kept 3 months before their absorption and the animal tests, which may explain the mildness of their effects.

(Fig. 1, *B*). To judge from the gross appearance one would suppose that exhaustion had entirely deprived the serum of its ability to cause renal lesions. Microscopically, however, it is seen that all of the lesions previously described are present save the hemorrhages. The glomerular and tubular lesions are in some instances less marked than in the controls, but in other cases they are equally well defined.

Type Experiment 1. Action of Nephrotoxic Serum. (a) Untreated; (b) Absorbed with Red Cells.—(*a*) Dog F, male, weight 6 kilos, after 2 weeks observation, during which time the urine was frequently examined and found to be free of casts and albumin, was given, on Mar. 26, 1918, 10 cc. of Serum 6 intravenously. The dog behaved normally after the injection. (This serum was prepared by injecting Rabbits 1 and 2 with blood-free dog kidney suspension. On Oct. 1, 1917, the suspension contained 4 gm. of kidney tissue; Oct. 8, 6 gm.; Oct. 15, 8 gm.; Oct. 22, 5 gm. Oct. 30. The rabbits were bled to death and the serum preserved in the ice box. On Mar. 22 the two sera were pooled and the titer was determined. Hemolysis disappeared at a dilution of 1 to 4 and agglutination at 1 in 512. Inactivation was done at 56°C. for ½ hour.)

Mar. 27. Urine very dark amber, alkaline, cloudy. Dense ring of albumin with nitric acid test. Many epithelial cells, a few red blood cells and leucocytes. No casts. Guaiac test positive. Mar. 28. Urine shows a great deal of albumin. Guaiac test positive. Occasional granular casts. Mar. 30. Same findings. Apr. 1. Albumin present. Guaiac test negative. Very many hyaline and granular casts. Apr. 3, 5, and 6 showed the same findings.

On Apr. 6 the dog was etherized and an autopsy performed, with negative findings in all the organs except the kidneys, which were swollen and congested. On stripping the capsule innumerable fine red dots up to 1 mm. in diameter were revealed on the surface of the kidney (Fig. 1, *A*). On section the kidneys were swollen and opaque, the cortex showing a definite widening.

Histological examination showed innumerable punctate cortical hemorrhages into the tubules, filling the lumina of scattered groups. There were a few interstitial hemorrhages. The majority of the glomeruli showed marked changes. Many of the cells in the tufts were necrotic, and numerous coils were plugged with fibrin. Mitoses were not infrequent. There was some interstitial round cell infiltration here and there between the tubules, especially in the neighborhood of the glomeruli. Many casts were seen in the tubules, but only slight changes in their epithelium.

(*b*) Dog Fa; weight 5.25 kilos. Observed 2 weeks, during which the urine was free from albumin and casts. The animal was given intravenously, on Mar. 26, 1918, 9.8 cc. of Serum 6, which had been absorbed six times with washed dog red blood cells. (The total amount of serum submitted to absorption was 22 cc. 3 cc. of washed dog red cells were added to it, the tube inverted several times and incubated at 37°C. for 1 hour. Dense agglutination resulted. After centrifu-

gation the serum was pipetted into another tube, another 3 cc. of washed cells were added, and incubation was repeated. In all, seven such absorptions were carried out on this serum, after which it was tested for hemolysins and agglutinins and found to possess none. Cultures taken prior to its use for injection proved that it was sterile.)

Mar. 27. Urine showed a trace of albumin, no casts. Guaiac test positive. Mar. 28. Same findings. Occasional granular casts. Mar. 30. Large amount of albumin, many granular casts, no blood. Apr. 3, 5, and 6. Same findings. Apr. 6. The dog was etherized and all organs found to be normal except the kidneys, which were large and pale. The capsules were stripped and revealed a few pin-point hemorrhages, probably one for every fifty seen in the case of Dog F (Fig. 1, *B*).

Histological examination revealed changes almost identical with those observed in the case of Dog F, except that there were fewer hemorrhages (Fig. 2).

Effect of Ordinary Hemolytic-Hemagglutinative Serum.

In view of the fact that hemolysins have far more effect *in vivo* than *in vitro*,[4] it seemed necessary to test the effect on the kidney of an ordinary hemolytic and hemagglutinating serum produced by the injection of dog red cells. For it might be contended, as explaining the injurious effect on the kidney of our exhausted serum, that such serum still contained traces of hemolysin and hemagglutinin, which, while not demonstrable *in vitro*, were active *in vivo*. Rabbits were immunized therefore against washed dog red cells and an anti-dog serum of high agglutinin and hemolytic titer was produced. This was inactivated and injected intravenously into dogs, a part of it after absorption with dog red cells.

Type Experiment 2. Effect of Hemolytic and Hemagglutinating Serum. (a) Untreated; (b) Absorbed with Red Cells.—The serum used was prepared by injecting rabbits intraperitoneally with a 20 per cent suspension of thrice washed dog cells in salt solution. Five injections were made at 7 day intervals, and the rabbits were bled on the 8th day following the last injection. The resulting sera were pooled and found to be hemolytic in dilutions up to 1 in 256 and agglutinative in dilutions up to 1 in 1,025. A portion of the pooled serum was subjected to eleven absorptions with dog red cells and again tested, with the result that no hemolysins or agglutinins were found. In detail the method of absorption was as follows:

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[4] Muir, R., and M'Nee, J. W., *J. Path. and Bacteriol.*, 1911–12, xvi, 410.

Absorption.	Amount of serum.	Dog red cells.	Incubation.
	cc.	*cc.*	*min.*
1	20	3	30
2		8	30
3		3.5	40
4–11		4 each.	60–120

(*a*) Dog R, weight 5.5 kilos, was given, on May 6, 1918, 2 cc. of the untreated portion of the serum. May 8. The urine, previously normal, is now smoky in appearance, containing a trace of albumin and much hemoglobin but no casts. May 10. Urine dark red; faint trace of albumin; many granular casts containing brownish pigment. Tests for blood and bile positive. May 14. Urine dark amber; albumin negative; no casts. Bile pigment and blood present. May 17. Urine amber, no albumin, hemoglobin, or casts. May 18. Dog etherized and kidneys removed. Histological examination showed much brown, granular pigment in the spleen and liver. The kidneys were entirely normal except for widespread, fairly abundant, light brown pigment granules in the cells of the proximal convoluted tubules.

(*b*) Dog S, weight 5.25 kilos, was given intravenously on May 2, 1918, 12 cc. of the absorbed portion of the serum. Immediately after the injection dog vomited, was prostrated, and passed feces. Complete recovery took place in 1 hour. The urine was examined on May 3, 4, 6, 8, 10, and 14, but on none of these occasions were any abnormal constituents found. On May 14 the dog was etherized. Autopsy showed all organs normal. On histological examination the kidneys were normal.

It will be seen that the unabsorbed serum produced no important anatomical changes in the kidney despite the marked blood destruction it caused. Pearce, using such a serum, reports a fatty condition limited almost entirely to the loops of Henle. Otherwise the urinary and kidney findings which he reports in animals that survived a few days were the same as here reported. He did not try absorption, which, as we found, renders the serum innocuous to the blood as well as the kidneys. Altogether the facts warrant the conclusion that the changes produced by an antikidney serum exhausted with red cells cannot be attributed to persisting hemolysins or agglutinins. It is interesting that the most striking gross lesion caused by the antikidney serum, namely the punctate cortical hemorrhages, is not produced by an anti-red-cell-serum, although absorption with red cells deprives the antikidney serum of its ability to produce such a lesion.

Effects of the Exhaustion of the Serum with Kidney Tissue.

Can the injurious principle in the antikidney serum be removed by absorption with kidney tissue? This point was now investigated.

For the purpose of proper absorption with kidney tissue it was obviously necessary that the latter should be in as finely divided a state as possible, so as nearly to resemble in amount of absorbing surface a red cell suspension. Coarse fragments of tissue would offer relatively little surface and might be expected to yield poor results.

To obtain a fine suspension the tissues of washed kidneys of dogs were ground in the way already described, taken up in normal salt solution, and shaken with broken glass for 1 hour. The glass and larger tissue fragments were then removed by slow centrifugation, leaving a dense, milky suspension of finely divided material. On microscopic examination many free kidney cells were found in this fluid, which was now sedimented at high speed, and the sediment repeatedly washed by centrifugation until the supernatant fluid came away perfectly clear. The sediment was found to consist of parenchymal and other cells and fragments of them. For the purpose of absorption it was measured in bulk and used in suspension in the same manner as blood cells. It went into suspension readily.

Type Experiment 3 (Table II). Inactivated Antikidney Serum X Was Divided into three Portions: (a) Untreated; (b) Absorbed with Red Blood Cells; (c) Absorbed with Different Amounts of Kidney Tissue.—The untreated serum weakly agglutinated dog red cells in a dilution of 1 to 32 and hemolyzed them completely in a dilution of 1 to 4.

(*a*) *Results with Untreated Serum.*—Dog U, weight 7 kilos, with a urine negative for albumin and casts, received 1.35 cc. per kilo of the untreated serum on June 11, 1918. No symptoms following the injection. The urine on June 14, 19, 21, and 22 showed a heavy cloud of albumin and many hyaline and granular casts.

On June 22 the dog was etherized and an immediate autopsy performed. All organs were negative except the kidneys, which were swollen and congested. A few punctate hemorrhages were seen on the surface.

Histological Examination.—All organs negative except the kidneys, which showed characteristic changes. Many of the glomeruli were greatly damaged, though not a majority. All showed increase of cell nuclei, and the loops were often difficult to distinguish and appeared collapsed, apparently as a result of small occluding fibrin masses. There were some glomerular adhesions. The proximal convoluted tubules were definitely swollen, with much albuminous debris in the lumina, as well as many casts. The lumina of the descending portion of the loop of Henle were filled with red cells in places. There were small, scattered areas of edema of the interstitial tissues. The liver and spleen showed nothing abnormal.

(*b*) *Results with Serum Absorbed with Red Cells.*—Dog V, weight 8.5 kilos, received intravenously, on June 11, 1918, 1.28 cc. per kilo of Serum X, absorbed with dog red blood cells. The following was the method of absorption.

Absorption.	Amount of serum.	Dog red cells.	Incubation.
	cc.	*cc.*	*min.*
1	22	6	50
2		3.5	60
3–4		3 each.	60 and 90

After the absorptions there were no demonstrable hemolysins or agglutinins in this serum.) No symptoms. Urine negative.

June 13. Urine negative. June 14. Albumin, dense ring. No casts or blood. June 19 and 21. Urine showed much albumin, few granular and hyaline casts. On June 21 the dog was etherized. All organs were found negative with the exception of the kidneys, which were congested and swollen; no surface hemorrhages.

Histological Examination.—All organs negative with the exception of the kidneys, which showed a few interstitial hemorrhages. The usual glomerular changes were present. Few casts seen in the tubules. Findings like those of Dog U but not quite so pronounced.

Dog W, weight 4 kilos, received intravenously, on July 13, 1918, 4.4 cc. of Serum X, absorbed with red cells as above. July 15. Urine contained much albumin, occasional granular casts, no blood. July 17, 20, 22, and 24. The urine contained much albumin, many granular casts, no blood. Dog etherized and kidneys removed on July 24. Autopsy showed all organs normal with the exception of the kidneys, which were large and pale, with a very few punctate hemorrhages on their surface.

Histological Examination.—The general picture was the same as that of Dog U.

(*c*) *Results with Serum Absorbed with Kidney Cells.*—Dog X, weight 6.25 kilos, received, on June 11, 1918, 8 cc. of Serum X, absorbed three times with kidney cells in exactly the same proportion and for the same period employed with red cells as above stated. No symptoms. Urine examined June 13, 14, 19, 21, and 22. With the exception of June 14, when a very slight trace of albumin was observed, it was negative throughout. Etherization and autopsy on June 22. All organs normal by gross and histological examination.

Dog Y, weight 5.75 kilos, received, July 13, 1918, 8 cc. of Serum X, which had been absorbed three times with washed kidney cells of dogs as just described. No symptoms. Urine showed slight traces of albumin and occasional granular casts on July 15 and 17. On July 20, 22, and 24 it was negative. July 24. The dog was etherized and autopsied. Kidneys small, slightly congested, otherwise normal. All other organs normal.

Histological Examination.—Negative.

Dog Z, weight 4.50 kilos, received, on June 27, 1918, 2.2 cc. per kilo of Serum X, absorbed with kidney tissue as described. There followed a severe reaction, with vomiting, rapid respiration, and passage of feces and urine. The general picture suggested an anaphylactic crisis. After 20 minutes there was a gradual cessation of these symptoms, with complete recovery.

July 1. General condition of dog good. Urine showed albumin and a few granular casts but no blood. July 3. Urine about the same. July 5. Many casts.

TABLE II.

Relative Effects of Untreated Antikidney Serum and Serum Exhausted with Red Cells and with Kidney Cells (Type Experiment 3).

Dog.	Serum X.	Total bulk of sediment used for exhaustion per cc. of serum.	Dose of serum per kilo of animal.	Urine changes.	Kidney changes.
		cc.	*cc.*		
U	Untreated.		1.35	++	Moderate lesions in epithelium and glomeruli.
V	Four absorptions with red blood cells. Total incubation 5 hrs.	0.70	1.28	++	Slight glomerular lesions; moderate epithelial.
W	" "	0.70	1.10	++	Moderate glomerular lesions; no hemorrhages. Many casts.
Q	" "	0.70	2.17	++	Severe glomerular lesions; epithelial injury. Casts.
X	Three absorptions with kidney cells. Incubation 4 hrs.	0.50	1.26	0	Occasional cast. Otherwise negative.
Y	" "	0.68	1.39	0	Occasional cast.
Z	" "	1.00	2.2	+	Rare glomerular lesion. Few casts.

July 8. Only a trace of albumin, few casts. Dog etherized and autopsied. General examination negative. Kidneys showed no gross changes except a few hemorrhages, very few compared with control.

Histological Examination.—Occasional glomerular changes of the sort already described, but on the whole most of the glomeruli were in excellent condition. There were not infrequent characteristic hemorrhages into groups of tubules, as well as interstitial hemorrhages. Tubules generally normal, with the exception of a few casts in lumina. Liver and spleen normal.

The results of the experiment are summarized in Table II.

DISCUSSION.

Pearce's conclusion that an anti-dog rabbit serum could be produced which has a special action on the kidney has been confirmed. He, however, left the question of the true specificity of such a serum in doubt, and we must now consider whether our experiments throw any light on the subject.

An element in antikidney serum causing a very striking lesion, (abundant punctate cortical hemorrhages) is removed from the serum by the exhaustion of the latter with red cells, although an ordinary anti-red-cell-serum fails to produce such lesions. But the principle most injurious to the kidney cannot be so removed, even when the number of absorptions and the total bulk of red cells are very large. Moreover, a serum of high titer obtained by immunization with washed red cells fails to produce kidney lesions in any way resembling those of the nephrotoxic serum, which is further evidence that the injurious principle of the latter is not an hemagglutinin or hemolysin. The absorption of the antikidney serum with kidney tissue removes the injurious antibodies, even when the amount of tissue employed, and presumably the absorbing surface, are much less than were used in similar absorptions with red blood cells. Illustrations of the point are to be found in Table II.

The criticism may be made that the great absorptive power of the kidney tissue as thus demonstrated need not be the consequence of specificity but may be related to physical conditions that obtain in the emulsion, which make its absorptive power greater than that of a red cell emulsion. The experiments of certain observers on the absorption of antibodies with substances which obviously have no specific relation to them, kaolin, for instance, might here be cited. This criticism, however, is primarily directed not at the specificity of the nephrotoxin, with which we are alone concerned, but at the basic theory of the specificity of antibodies in general.

The effects of the nephrotoxic serum are exerted on the glomeruli and to a somewhat less degree on the tubules. It may be asked whether a vascular lesion will not account for all of the results. The glomerular changes are not improbably secondary to an occlusion of the coils following injury to the vascular endothelium. Our ani-

mals were autopsied too late to furnish conclusive evidence on the point. But whether or not the primary lesion is vascular, we are certain at least that the kidneys alone are affected. Were this the result of a non-specific endotheliolysin other organs should serve equally as well as the kidney for an antigen because of their content in endothelial cells. Pearce, however, has shown that a serum produced by the use of liver tissue as antigen has no effect on the kidneys.

As has been stated, our investigation was begun with a view to determining whether an antiserum produced as a result of the injection of tissues of an infected organ can be freed of injurious tissue antibodies by its exhaustion with red cells. As far as the kidney is concerned the question has been answered in the negative. A serum produced by immunization with kidney tissue should be absorbed with material from this organ. When that has been done it is no longer injurious to the kidney. Whether these principles are of general application may be doubted, for it is admitted that the kidney constitutes a special case. Notwithstanding numerous attempts by various investigators, sera specific for the spleen, pancreas, and liver have never been conclusively demonstrated. To obviate all possibility of injury from specific antibodies, however, a serum produced by the use of a tissue antigen should be exhausted with tissue of an identical sort prior to its introduction into the animal body.

SUMMARY.

As Pearce has shown, a serum highly injurious to the kidney of dogs can be produced by the immunization of rabbits with washed renal tissue of the dog. The histological findings are striking and characteristic, the most noteworthy being a glomerular lesion of special type. The renal changes differ much from those Pearce described.

The injury to the kidney is not to be explained by hemolytic and hemagglutinative elements in the serum. The complete removal of such antibodies by exhaustion of the serum with successive portions of red cells fails to lessen materially its ability to cause kidney lesions. Furthermore, an ordinary hemolytic and hemagglutinative serum produced by the use of washed red cells as antigen fails to cause similar lesions.

The distinctive, injurious principle of antikidney serum can be removed and the latter rendered innocuous by absorption with kidney tissue. To all practical intents and purposes it would seem that nephrotoxic serum of the sort here described is specific.

If infected tissue is to be utilized as an antigen for the production of therapeutic antisera the latter must in some instances be exhausted with tissue of the same sort prior to introduction into the animal body.

EXPLANATION OF PLATES.

PLATE 5.

FIG. 1. Photograph of the kidneys of Dogs F and Fa, Type Experiment 1, Table I. (*A*) The first mentioned animal received untreated antikidney rabbit serum, and its kidney shows innumerable cortical hemorrhages. (*B*) The other received an even larger amount of the same serum, from which the hemolysins and hemagglutinins had been removed by repeated absorptions with dog red cells. Though the kidney shows almost no hemorrhages, it was found microscopically to be badly damaged (Fig. 2).

FIG. 2. Section of the kidney pictured in Fig. 1 of Dog Fa, Type Experiment 1. Three glomeruli are seen, all of which show necroses of varying size. The tubules are dilated and filled with hyaline casts. There is beginning round cell infiltration in the neighborhood of the glomeruli. Bausch and Lomb, obj. $\frac{2}{3}$, oc. 1.

PLATE 6.

FIG. 3. Section from Dog Q, Type Experiment 3, which received antikidney serum absorbed with red cells. High power view of a necrotic glomerulus. Half of the glomerulus is entirely destroyed, and the other half severely compressed by the necrotic mass There is a beginning invasion of the dead material by proliferating capsular cells. Bausch and Lomb obj. $\frac{1}{6}$, oc. 1.

FIG. 4. Same animal. Weigert fibrin stain of an injured glomerulus. The necrotic area contains a large thrombus of fibrin. Three tubules are also seen filled with hyaline casts which take the fibrin stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 7.

FIG. 5. Same animal. A group of tubules, lying just beneath the capsule, their lumina filled with red blood cells. Other tubules contain hyaline casts. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 6. Same animal. An almost completely destroyed glomerulus. There is a marked infiltration of the glomerulus and the surrounding area with leucocytes, both polymorphonuclear and lymphocytic. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

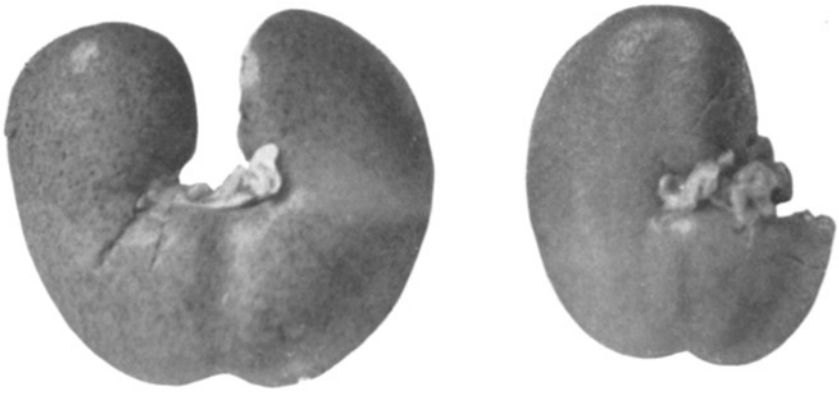

FIG. 1.

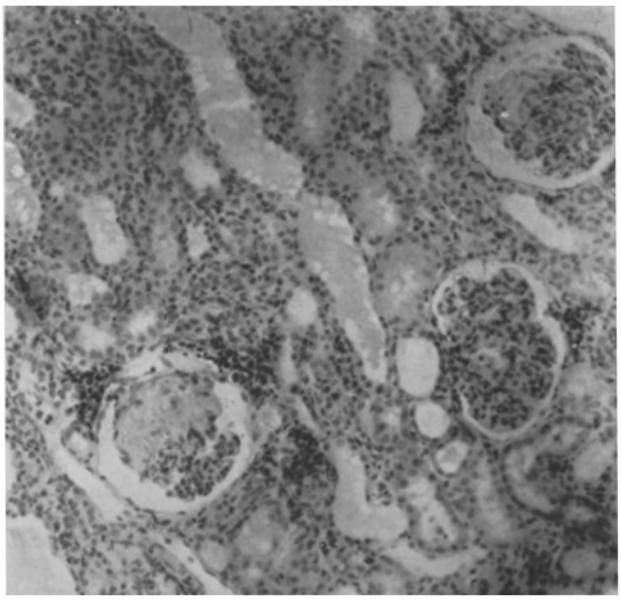

FIG. 2.

(Wilson and Oliver: Production of specific antisera. III.)

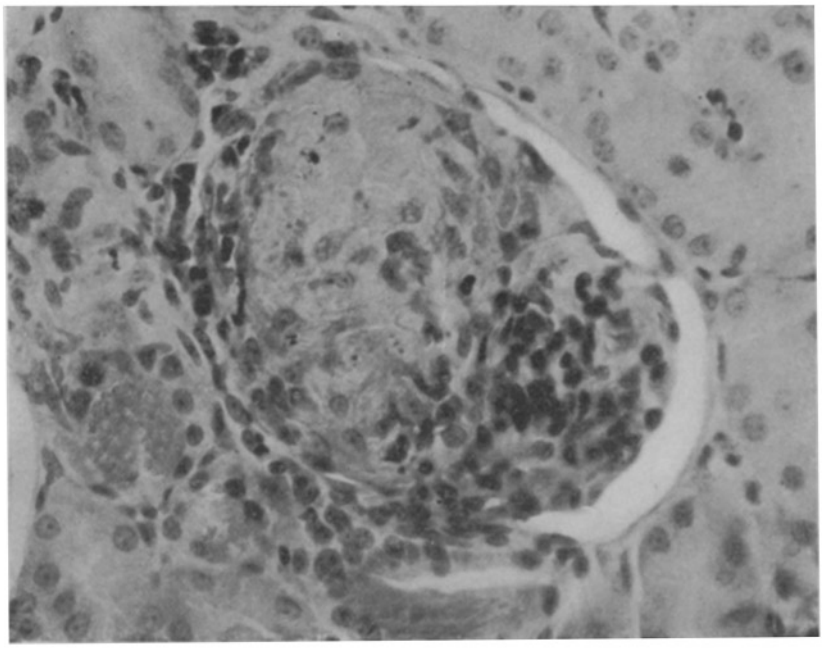

FIG. 3.

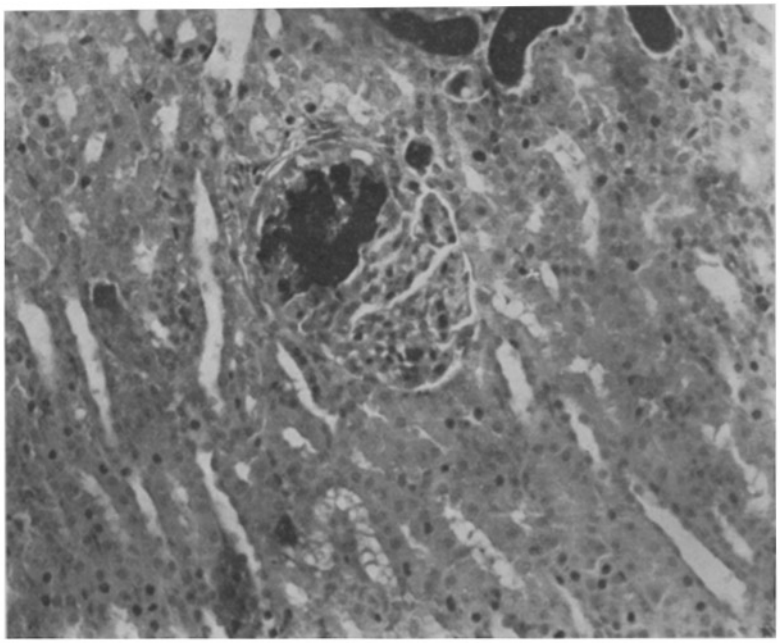

FIG. 4.

(Wilson and Oliver: Production of specific antisera. III.)

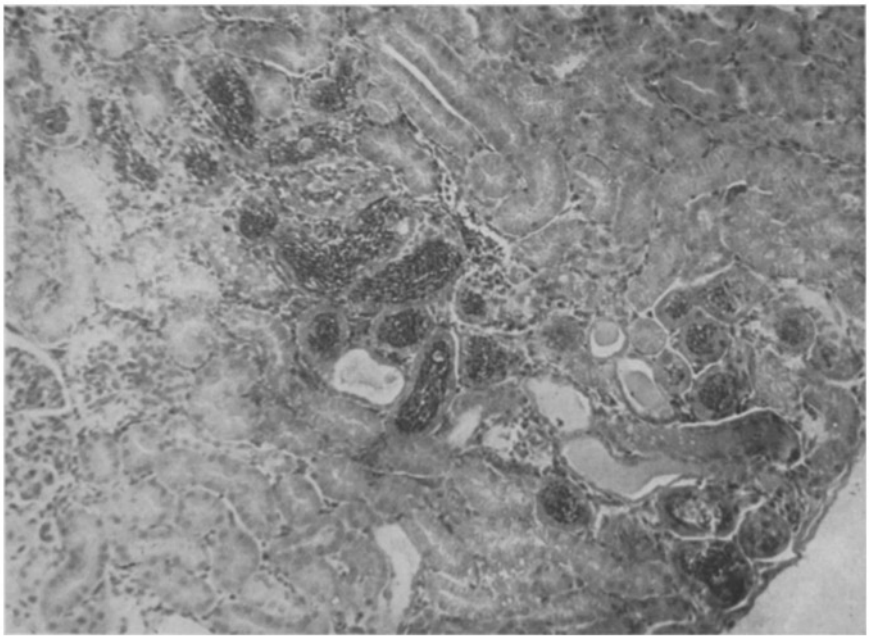

FIG. 5.

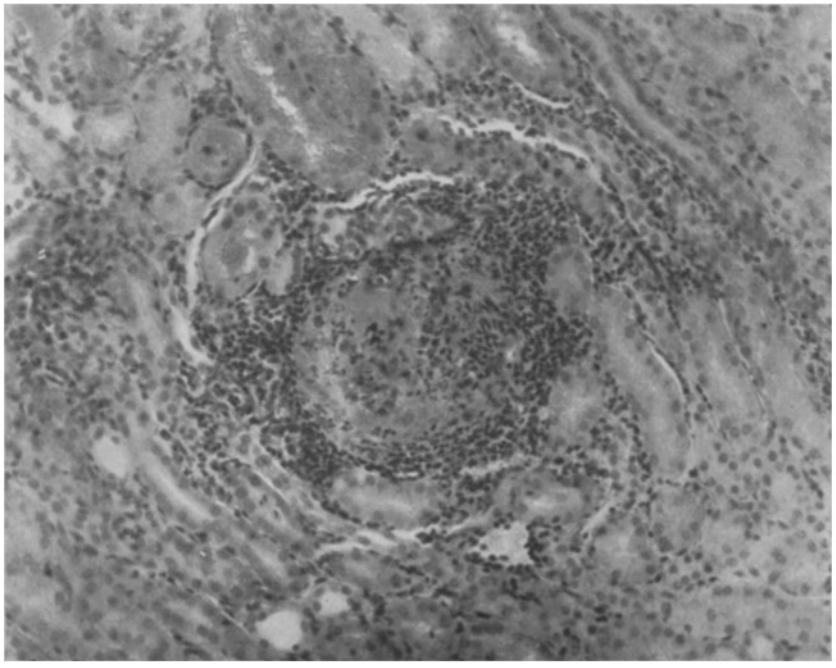

FIG. 6.

(Wilson and Oliver: Production of specific antisera. III.)