THE PRODUCTION OF EXPERIMENTAL NEPHRITIS BY REPEATED PROTEID INTOXICATION.*

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PLATES 56 TO 65.

During the last two years there have been under observation at the Presbyterian Hospital occasional cases of nephritis, characterized by exacerbations of general edema, fever, albuminuria, and, in a few instances, urticaria and eosinophilia, which in these particular attacks, have a close resemblance to serum disease. It was through the suggestion made by other workers, and derived from a study of these cases, namely, that nephritis might in certain instances be dependent for its origin upon an anaphylactic state, that the present investigation was undertaken.

Comparatively little attention has been paid to the anatomical alterations that may take place during anaphylactic shock, and almost no observations have been made upon the effects of repeated non-fatal anaphylactic shock upon the animal tissues. It is known that man may develop a hypersensitiveness, or allergy, as von Pirquet calls it, to some food stuffs, among which may be mentioned egg-white, fruits, and shell-fish, and under these circumstances it is possible that he may be subjected from time to time to many non-fatal attacks of intoxication by the proteins of these substances. It is important, therefore, to determine if in animals artificially sensitized to foreign protein, repeated intoxications of these foreign proteins may cause injurious effects upon the body.

Recently the question that has absorbed the interest of most investigators is the manner in which the hypothetical intoxicating substance, anaphylatoxin, is

* Received for publication, July 23, 1913.

formed during the anaphylactic shock. The views formulated by Vaughan,[1] Biedl and Kraus,[2] Friedberger,[3] and Schittenhelm and Weichard,[4] and well summarized by Friedemann,[5] tend to explain the phenomenon by the formation, during the period of sensitization, of a ferment, or of an antibody towards the foreign substance, which, at the time of the second injection of protein, two or three weeks after sensitization, acts upon this protein in such a manner that it is split into substances that are simpler chemically, but, like peptones, highly toxic. Vaughan considers that this process takes place within the body cells by the action of an intracellular ferment, but the more common conception is that of antibody formation such as takes place in the production of agglutinins or precipitins.

Attempts have been made to discover whether these antibodies reside in the cells of various organs; and discussion has arisen as to whether the liberation of the toxic substance takes place in the cells of organs or in the circulation. So far, with one or two questionable exceptions, investigators have failed to demonstrate antibodies for foreign protein in any of the cells of the sensitized animals. Recently, however, Felländer and Kling[6] have shown that antibodies may be found in the bone marrow and leucocytes of sensitized animals. On the other hand, it has been shown repeatedly by various methods that the serum of sensitized animals contains antibodies that will act upon the specific protein to which the animal is sensitive to form highly toxic substances (Friedberger). There are, however, certain observations which go to show that a reaction may take place during anaphylaxis between the foreign protein and the body cells; for the experiments of Schultz,[7] and particularly their amplification by Dale,[8] demonstrate definitely that the smooth muscle of the uterus, intestine, and bronchi, washed free of blood, from the guinea pig sensitized to horse serum will contract as it does *in vivo* during anaphylaxis, when brought into contact *in vitro* with dilutions of horse serum which have no effect upon the musculature of the organs from normal guinea pigs.

The actual anatomical changes in guinea pigs dying acutely in anaphylaxis have been studied extensively by Gay and Southard.[9] They describe hemor-

[1] Vaughan, V. C., Cumming, J. G., and McGlumphy, C. B., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 16. McFarland, W. L., *ibid.*, 458. Vaughan, V. C., Vaughan, V. C., Jr., and Wright, J. H., *ibid.*, xi, 673.

[2] Biedl, A., and Kraus, R., *Wien. klin. Wchnschr.*, 1909, xxii, 363.

[3] Friedberger, E., and Goldschmidt, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 369. Friedberger, E., and Hartoch, O., *idem*, 1909, iii, 581. Friedberger, E., and Gröber, A., 1911, ix, 216. Friedberger, E., and Nathan, E., *ibid.*, 567.

[4] Schittenhelm, A., and Weichardt, W., *Ztschr. f. exper. Path. u. Therap.*, 1912, x, 412, 448; xi, 69.

[5] Friedemann, U., *Jahresber. ü. d. Ergebn. d. Immunitätsforsch.*, 1910, vi, 31.

[6] Felländer, J., and Kling, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1912, xv, 409.

[7] Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1909–10, i, 549; 1910–11, ii, 221.

[8] Dale, H. H., *Jour. Pharmacol. and Exper. Therap.*, 1913, iv, 167.

[9] Gay, F. P., and Southard, E. E., *Jour. Med. Research*, 1907, xvi, 143; 1908, xviii, 407.

rhages in the heart muscle, pleura, stomach wall, and cecum, and have called attention particularly to a fatty degeneration of the capillary endothelium which they believe is the direct cause of the hemorrhages. Fatty degeneration was also observed in the nerves, epithelium of the stomach, heart, and voluntary muscles. Anderson and Rosenau[10] in repeating this work lay but little stress upon the hemorrhages and believe that the occasional fatty changes they found may occur in other conditions and are not characteristic of the anaphylactic intoxication. These changes described by Gay and Southard may or may not be characteristic of anaphylaxis in the guinea pig, but they occur in my experience quite frequently, though of course the acute emphysema to which Auer and Lewis[11] first called attention is constant. By repeating the shock two or three times in guinea pigs by intraperitoneal injections Gay and Southard[12] failed to produce chronic changes.

Under certain circumstances, nevertheless, the body cells may react with resulting anatomical changes towards repeated doses of foreign protein. Thus the local edema, inflammation, and necrosis that is seen in the skin of a rabbit after subcutaneous inoculations of horse serum, repeated at intervals of six to seven days, and studied by Arthus,[13] is familiar. Lucas and Gay[14] have noted the same thing in children receiving repeated injections of diphtheria antitoxin. Another evidence of local reaction is the cellular pneumonia described by Friedberger,[15] which he was able to produce in the lungs of sensitized guinea pigs by spraying horse serum into the trachea. Ishioka[16] has repeated and confirmed these experiments, and Schlecht and Schwenker[17] have made a careful histological study of the lungs in this condition. They describe the proliferation of cells lining the alveolar walls, and the exudation of eosinophilic leucocytes into the alveoli with the formation of fibrin which gives rise to a type of bronchopneumonia. Moreover, they have studied the effects of repeated intraperitoneal inoculations of horse serum in guinea pigs as well as the Arthus phenomenon and find under these circumstances infiltrations of the omentum and peritoneal cavity in the one instance and infiltration of the subcutaneous tissues in the other by eosinophilic and small mononuclear cells. Intravenous injections of serum in dogs resulted in an intestinal catarrh with infiltration of the mucosa and submucosa of the intestines by many cells among which were eosinophils.

There is, therefore, a certain amount of evidence to show that during anaphylaxis some of the body cells may be injured, and a

[10] Rosenau, M. J., and Anderson, J. F., *Jour. Med. Research,* 1908, xix, 37.

[11] Auer, J., and Lewis, P. A., *Jour. Exper. Med.,* 1910, xii, 151.

[12] Gay, F. P., and Southard, E. E., *Jour. Med. Research,* 1908, xix, 17.

[13] Arthus, M., *Compt. rend. Soc. de biol.,* 1903, lv, 817. Arthus, M., and Breton, M., *ibid.,* 1478. Arthus, M., *Arch. internat. de physiol.,* 1908–09, vii, 471.

[14] Lucas, W. P., and Gay, F. P., *Jour. Med. Research,* 1909, xx, 251.

[15] Friedberger, E., *Deutsch. med. Wchnschr.,* 1911, xxxvii, 481.

[16] Ishioka, S., *Deutsch. Arch. f. klin. Med.,* 1912, cvii, 500.

[17] Schlecht, H., and Schwenker, M. G., *Deutsch. Arch. f. klin. Med.,* 1912, cviii, 405.

few observations tend to support the idea that this injury may be followed by a reaction on the part of the fixed and migratory body cells. A series of experiments, therefore, was planned to determine if, during repeated anaphylactic shocks in animals, any permanent injury might be done to the body cells, particularly the kidney, and whether there might be any reaction visible microscopically on the part of the tissues to such repeated intoxications.

METHODS.

For the experimental investigation of the problem several species of animals were used as one species might react differently from another; therefore guinea pigs, rabbits, cats, and dogs were selected. Horse serum and egg-white were chosen as foreign proteins. Guinea pigs were sensitized by the intraperitoneal injection of 0.01 to 0.1 of a cubic centimeter of horse serum and 0.5 of a cubic centimeter of undiluted egg-white. In order to render rabbits sensitive much larger and repeated doses were required and consequently two to three doses of the protein were injected at two to three day intervals. As a rule, one to three cubic centimeters of the protein were given intravenously, intraperitoneally, subcutaneously, or by a combination of these routes. The cats were injected once with one to four cubic centimeters of horse serum or egg-white either intravenously or subcutaneously. The dogs, according to their weight, were usually given three to five cubic centimeters of the protein either subcutaneously or intravenously. In some experiments the animals were sensitized both to horse serum and egg-white in order that a single animal might receive the greatest possible number of intoxicating doses.

Twenty to twenty-five days after the preliminary inoculation, the secondary series of injections was started and thereafter injections were made at two to three day intervals in some experiments, at five to seven day intervals in others, and in still a third group at two to three week intervals. In the guinea pigs the intoxicating doses were given intraperitoneally, and the same method as well as an occasional subcutaneous inoculation was used with a few rabbits and cats, but by far the greatest number of rabbits, cats, and

dogs received intravenous inoculations. In cats the injections were made into the ear vein, in dogs into the external jugular or saphenous veins.

It was at first difficult to determine the dose of horse serum or egg-white which would cause definite symptoms without fatal shock and consequently several animals were sacrificed before the proper dosage could be arrived at. Another difficulty that was encountered was the development of an anti-anaphylactic state. This was most rapidly acquired in guinea pigs. They ceased to show symptoms usually after the second or third intraperitoneal inoculation. Rabbits and cats, however, could be given doses of horse serum increasing from 0.1 to 2 to 4 cubic centimeters on six or eight separate occasions before they ceased to react, and in the same manner dogs received doses of horse serum up to ten cubic centimeters before they became insensitive. To large amounts of egg-white the rabbits and cats were never refractory. Dogs and guinea pigs reacted to egg-white as they did to horse serum.

The symptoms following the intoxicating doses in guinea pigs consisted of scratching at the nose, ruffling of the hair, defecation and urination, collapse with partial or complete paralysis of the hind legs, with cramp-like convulsions and complete paralysis in the severest cases. The rabbits showed preliminary excitement, followed by weakness and collapse. The animals flattened their bellies against the floor, lay with their heads back, passed urine and feces, and showed rapid respirations. In the severest shock the rabbits lay upon their sides, occasionally showed spasmodic running movements, or during their muscular contractions flopped about the table. The majority of the animals that reached this stage died. In the acutely fatal cases the animals would run across the floor, then fall on their sides, show the running motions, perhaps scream, and with bulging eyes and dilated pupils die in extreme spasm with marked opisthotonos. The cats regularly became excited, ran a short distance, then swayed for a moment and fell upon their sides. Immediately there were violent muscular contractions and the animals stiffened out in extreme opisthotonos. After half a minute to a minute they would relax and lie sprawled out in collapse. Urina-

tion, repeated defecation with bloody movements, and occasionally vomiting would follow. During this time there were contractions of the abdominal muscles and rapid respirations. After a period of ten to twenty minutes the cats would recover, and crawl to a corner where they sat quietly huddled up for some time.

The dogs after a preliminary short excitement vomited, urinated, swayed, stood with their heads hung down, and then lay upon their sides, completely relaxed and collapsed, but evidently quite conscious. A diarrhea often severe with blood and mucus followed. This condition lasted for ten to thirty minutes depending upon the severity of the attack. There were spasmodic contractions of the abdomen. Three dogs developed on several occasions severe generalized urticaria with extreme itching. Though the temperature of these animals was not regularly observed, it was recorded in a few instances and in all cases when definite shock was produced the temperature fell 1° to 1.5° C.

It may therefore be seen that these symptoms accord well with the clinical picture of anaphylactic shock as described for guinea pigs by Rosenau and Anderson,[18] for rabbits by Arthus,[19] Friedberger,[20] and Auer,[21] for cats by Schultz,[22] and for dogs by Biedl and Kraus,[23] and Pearce and Eisenbrey.[24]

The experiments have shown that repeated injections of egg-white and horse serum bring about serious damage to the cells of various organs and tissues in all these animals. A single intraperitoneal inoculation in guinea pigs, rabbits, and cats produced no local reaction on the part of the omentum or peritoneum, but after the repeated intraperitoneal injections in sensitized animals, the omentum and peritoneal tissues at autopsy appeared rather thick, opaque, and white, and microscopical examination showed that this appearance was due to the presence of chronic inflammatory proc-

[18] Rosenau, M. J., and Anderson, J. F., *Hygienic Laboratory Bulletins*, 1906, No. 29; 1907, No. 36; 1908, No. 45.

[19] Arthus, M., *Arch. internat. de physiol.*, 1908–09, vii, 471; 1910, ix, 156.

[20] Friedberger, E., and Gröber, A., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 216.

[21] Auer, J., *Jour. Exper. Med.*, 1911, xiv, 476.

[22] Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1912, iii, 299.

[23] Biedl, A., and Kraus, R., *Wien. klin. Wchnschr.*, 1909, xxii, 363.

[24] Pearce, R. M., and Eisenbrey, A. B., *Jour. Infect. Dis.*, 1910, vii, 565.

esses in these tissues. The fat of the omentum in guinea pigs was infiltrated with great numbers of eosinophilic cells, together with small round cells and fibroblasts. In the rabbits and cats eosinophilic cells were rare, but the peritoneal tissue showed a chronic inflammation consisting of the presence of very cellular connective tissue which thickened the peritoneal surfaces and often converted the folds of omentum into cellular fibrous knob-like processes (figure 1).

In the lungs of guinea pigs which had received only repeated intraperitoneal injections, changes similar to those described by Friedberger, Ishioka, and Schlecht and Schwenker were found, though they were less extensive and presented the appearance of small bronchopneumonic patches and peribronchial infiltrations rich in eosinophils.

In the livers of rabbits and cats there were seen areas of necrosis often periportal in situation which could be traced in many animals to undergo organization by growth of connective tissue proceeding from the portal spaces. This led to a mild grade of portal cirrhosis which has been described in detail elsewhere[25] (figure 2).

Similar necroses were likewise observed in the myocardium in each group of animals, but most frequently in rabbits (figure 3). These necroses, too, healed with the formation of scars which in the animals that had received the greatest number of injections led to a focalized and occasionally diffuse chronic myocarditis.

In a large proportion of all animals changes were also found in the kidneys. They occurred in 79.3 per cent. of the twenty-nine inoculated rabbits, in seven of the twelve cats, in all of the thirteen guinea pigs, and in ten of the twelve dogs. Since the changes were practically the same in all groups of animals, the following description will apply to all the experiments.

When the animals died acutely the cells of the convoluted tubules showed extensive cloudy swelling and there was extreme congestion, especially marked in the glomeruli. In some of the rabbits and guinea pigs the tissues about the papilla were edematous.

Besides the diffuse degenerative changes, other microscopic lesions which appeared to be the earliest evidence of any destructive change

[25] Longcope, W. T., *Tr. Assn. Am. Phys.*, 1913, xxviii (in press).

in the kidney were seen in many rabbits and occasionally in cats, guinea pigs, and dogs that had received several intoxicating injections. These consisted of small areas in the papilla, the midzone, and the cortex in which the cells lining tubules showed necrosis and desquamation. In the papilla the cells lining the collecting tubules were affected (figure 4), in the midzone the epithelium of the loops of Henle, and in the cortex of the convoluted tubules the epithelium was involved (figure 5). The nuclei of these cells appeared pyknotic or fragmented, the protoplasm swollen and frayed, and the entire cell was loosened from the basement membrane and had a distorted appearance. Occasionally the lumen of the tubule was filled with desquamated cells. In the smallest areas the surrounding capillaries were filled with small mononuclear cells. In larger areas a single collecting tubule or groups of convoluted tubules formed the center of a dense area of round cell infiltration where the round cells were collected in great numbers between these degenerating tubules (figure 7). At the same time collections of round cells were observed about the vessels in the intermediate zone and sometimes surrounding the vessels as they ran into the cortex. These foci varied considerably in number. Occasionally there were ten to fifteen in a section, and in other instances only one or two were found.

The majority of the animals, especially the rabbits, showed what was taken to be a more advanced stage of the same process. The kidneys in some instances appeared normal on macroscopic examination. In other instances minute pale areas could be made out in the cortex or pale striæ radiated from the midzone towards the capsule. In three rabbits the kidney appeared swollen and pale, but the surface was smooth. On section there was marked swelling of the papilla. The boundary between the papilla and cortex was ill defined, while the cortex looked pale and the normal markings were practically obscured. In the dogs grayish streaks were sometimes observed in the cortex and the arcuate vessels, or the tissues about them appeared abnormally dense and pale.

On microscopical examination the kidneys presented extensive changes. Masses of round cells in enormous numbers infiltrated the intertubular tissue extending in rays from the intermediate zone

towards the capsule, occurring in great patches in the papilla or running from the intermediate zone down into the papilla. More or less focalized areas of round cell infiltration could also be seen in the cortex. In the largest areas the arrangement of the tubules could scarcely be made out and frequently it was only possible to see here and there a few epithelial cells, sometimes shrunken into small clumps which showed the remnants of tubules (figures 6 and 8). Occasionally groups of tubules in the cortex showed marked dilatation and in the lumen were hyaline casts. In these areas it could be seen that the round cell infiltration was accompanied by an increase in connective tissue which appeared as a rather delicate meshwork with Mallory's and Van Gieson's stains. Though the cellular infiltration and tubular degeneration were always patchy, in three rabbits the patches were so large and close together that there was little normal tissue remaining. In the guinea pigs, too, though the lesions never compared in intensity with those in the rabbits, they often assumed a rather diffuse character. In the dogs the focal nature of the lesion was perfectly evident and in the cat where they were least marked there were rarely more than one or two patches in a section.

As a rule, the glomeruli showed little change from normal in the kidneys. There was congestion of the capillary tufts with occasionally a small coagulum in the glomerular space, but in five rabbits, one of which showed no other changes, distinct abnormalities of acute type were seen in the glomeruli. These consisted in swelling and proliferation of the capillary endothelium with occasionally a karyorrhexis of the nuclei of the cells. The capillaries were almost free of red blood cells, but scattered through the loops small numbers of small round cells were observed (figure 9). Not all glomeruli were affected but usually one or two in every microscopic field showed some degree of change, while the most marked alterations occurred in those glomeruli that were situated in the cellular areas.

In what was assumed to be the third stage of this process, the connective tissue increased and glomerular lesions formed the most conspicuous part. It was less frequently encountered than the more acute cellular infiltrations, possibly because most of the ani-

mals were killed before there was time for their development. But these changes were present in six rabbits, one dog, two cats, and three guinea pigs. They consisted in the presence of dense fibrous rays extending from the midzone into the cortex, and, as a rule, following the course of a blood vessel, sometimes to the capsule. Often there was more or less round cell infiltration of the connective tissue together with increase in connective tissue about the arcuate vessels (figures 10 and 11). The strands of connective tissue were not very wide and not numerous, averaging about the thickness of two or three convoluted tubules in cross section, and rarely more than two or three patches were seen to a section. In these areas the tubules were atrophied and contained hyaline casts. Occasionally a more diffuse increase in connective tissue was seen between well preserved tubules.

The glomeruli both in the fibrous rays and sometimes in isolated instances through the cortex showed the most marked departure from normal. In the fibrous patches scarcely any normal glomeruli were found (figure 12). There was marked fibrous thickening of the capsule, which in a few instances appeared as a hyaline ring, and the capillary endothelium showed advanced proliferation, so that the capillary tuft appeared as a mass of cells totally devoid of red blood corpuscles (figure 13). Some glomeruli showed complete hyaline degeneration. In three guinea pigs a few isolated glomeruli were seen which showed a proliferation of the capsular epithelium leading to a characteristic crescent formation, and in a fourth about two thirds of all the glomeruli showed swelling and proliferation of the capsular epithelium with thickening of the capillary tufts.

Though I have described the lesions in the kidneys of these animals as a sequence of events in the development of a nephritis sometimes of a severe grade, it would only be possible to do so from the study of a considerable series of cases, for in an individual instance two of these types of lesions might occur in the same kidney and it was not unusual to see the focal necrosis of tubular epithelium in combination with the more extensive cellular infiltration or even with the advanced connective tissue formation. The appearance of these lesions, too, was not absolutely uniform in sequence, though the production of connective tissue was not observed except in ani-

mals that had been inoculated over a period of several weeks. Two rabbits, for instance, one of which had been subjected to three intravenous intoxicating doses of horse serum and five of egg-albumen, the other to three intravenous intoxicating doses of horse serum and six of egg-albumen, over a period of sixteen days, presented extensive cellular infiltration of the kidney, while the third animal in the series sensitized in the same manner at the same time and given intravenously three intoxicating doses of horse serum and six of egg-white over a period of sixteen days, showed only a few foci of small round cell infiltration in the cortex. As a rule, however, the most extensive and advanced lesions were seen in the animals that had received the greatest number of intoxicating doses over the longest period of time.

Of the six rabbits that showed normal kidneys at autopsy one had received six, another three intoxicating doses of horse serum, one had received three intoxicating doses of horse serum and four of egg-white, while the other three had received egg-white alone, one two, one four, and the third eleven intoxicating doses intravenously.

Of the five cats that showed normal kidneys, one had received three, one six intoxicating doses of horse serum, one three intravenous injections of horse serum and five of egg-white, one four of horse serum and four of egg-white, and one twelve injections of egg-white alone. Of the four dogs that showed normal kidneys at autopsy, one very large dog, weighing 15,000 grams, had received seven doses of egg-white intravenously and one seven inoculations of egg-white.

The following protocols illustrate the characteristic lesions and the phases of the experiments.

PROTOCOLS.

Rabbit 9.—Weight 1,800 gm.
Oct. 21, 1912. Sensitization with 0.1 c.c. horse serum intraperitoneally.
Oct. 23, 1912. Urine clear; no albumin.
Nov. 22, 1912. 5 c.c. horse serum subcutaneously. No symptoms.
Nov. 25, 1912. 4 c.c. horse serum intravenously. No symptoms.
Dec. 4, 1912. 3 c.c. horse serum subcutaneously. No symptoms.
Dec. 28, 1912. 3 c.c. horse serum intravenously. Slight symptoms.
Dec. 30, 1912. Urine dark; heavy trace of albumin.
Jan. 5, 1913. 1 c.c. horse serum intravenously. Slight symptoms.
Jan. 8, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 9, 1913. 12 hour specimen of urine; heavy trace of albumin; no casts.

Jan. 10, 1913. 1 c.c. horse serum intravenously. Slight symptoms.

Jan. 10, 1913. Urine shows trace of albumin; a few hyaline and granular casts.

Jan. 11, 1913. Urine shows heavy trace of albumin; many hyaline and granular casts.

Jan. 13, 1913. 1.5 c.c. horse serum intravenously. Moderate symptoms.

Jan. 15, 1913. 1.5 c.c. horse serum intravenously. Moderate symptoms.

Jan. 17, 1913. 2 c.c. horse serum intravenously. Marked symptoms.

Jan. 20, 1913. 3 c.c. horse serum intravenously. No definite symptoms.

Jan. 22, 1913. 4 c.c. horse serum intravenously. No definite symptoms.

Jan. 24, 1913. Urine pale, cloudy; heavy trace of albumin; many hyaline and granular casts.

Jan. 24, 1913. 5 c.c. horse serum intravenously. No definite symptoms.

Jan. 27, 1913. 10 c.c. horse serum intravenously. Moderate symptoms. Killed at 4 P. M. Weight 1,580 gm.

Autopsy.—Lungs collapsed. Heart's blood fluid. Liver smooth, appearance normal. Spleen small and red. Stomach and intestines normal. Kidneys small, capsule strips readily, surface smooth. On section cortex and medulla not well defined; pale streaks through the papilla.

Microscopical Examination.—Kidneys show degeneration of groups of tubules near the midzone with infiltration of the round cells about them. These areas occur in patches most often about the arcuate vessels, extending thence into the cortex and medulla. Many tubules contain casts. Glomeruli often contain blood but they appear cellular. Endothelium of capillary tufts swollen, and the nuclei often show karyorrhexis. Many small round cells in loops.

Rabbit 15.—Weight 1,415 gm.

Sensitized with the following injections: Dec. 31, 1912, and Jan. 2, 1913. 2 c.c. horse serum intravenously. Jan. 3, 1913. 5.5 c.c. horse serum intravenously. Jan. 6, 1913. 2 c.c. egg-white intravenously. Jan. 7, 1913. 2.5 c.c. egg-white intravenously.

Jan. 15, 1913. Urine very cloudy. No albumin.

Results of injections intended to produce anaphylactic shock.

Jan. 27, Feb. 6, and Feb. 11, 1913. 1 c.c. horse serum intravenously. After each dose very severe symptoms.

Jan. 28, 1913. 0.1 c.c. egg-white intravenously. Very slight symptoms.

Jan. 30, 1913. 0.4 c.c. egg-white intravenously. Very severe symptoms.

Feb. 4, 1913. 0.5 c.c. egg-white intravenously. Moderate symptoms.

Feb. 7, 1913. 0.5 c.c. egg-white intravenously. Very severe symptoms. Urine clear; moderate amount of albumin.

Feb. 11, 1913. Urine clear; large amount of albumin, detritus, many hyaline casts.

Feb. 12, 1913. 0.5 c.c. egg-white intravenously. Very severe symptoms. Death in 27 minutes.

Autopsy.—Blood fluid. Heart and lungs normal. Spleen soft. Liver large, dark brown, showing gray lines on surface. Kidney rather large and pale, capsule strips easily. Surface smooth, mottled gray and red. On section the papilla is greatly swollen, the cortex pale, differentiation between the two is poor, and the whole surface is streaked with gray lines.

Microscopical Examination.—Kidneys show most extensive areas of small round cell infiltration obscuring portions of the cortex and midzone. Necrosis of cells of collecting tubules with extensive surrounding small round cell infiltration. In places delicate strands of connective tissue are seen running into the cortex. Many glomeruli show increase of tuft cells with infiltration of small round cells.

Rabbit 17.—Weight 1,610 gm.

Sensitized with the following injections: Dec. 31, 1912. 3 c.c. horse serum intravenously. Jan. 2, 1913. 2 c.c. horse serum intravenously. Jan. 3, 1913. 5 c.c. horse serum intravenously. Jan. 6, 7, and 9, 1913. 2.5 c.c. egg-white intravenously.

Jan. 15, 1913. Urine slightly turbid; no albumin.

Results of injections intended to produce anaphylactic shock.

Jan. 27, and Feb. 6 and 11, 1913. 1 c.c., 1.5 c.c., and 2 c.c. horse serum intravenously. Slight symptoms following each dose.

Jan. 27, 1913. 0.1 c.c. egg-white intravenously. No definite symptoms.

Jan. 28, 1913. 0.25 c.c. egg-white intravenously. Slight symptoms.

Jan. 30, 1913. 0.5 c.c. egg-white intravenously. Slight symptoms.

Jan. 24, 1913. 0.6 c.c. egg-white intravenously. No definite symptoms.

Feb. 7, 1913. 0.8 c.c. egg-white intravenously. Moderate symptoms.

Feb. 12, 1913. 1 c.c. egg-white intravenously. Death within 2 minutes.

Autopsy.—Heart contracted. Lungs collapsed, blood fluid. Heart and lungs normal. Liver brown, white streaks on surface. Kidney appears normal except for a few depressed points on surface. Section shows an occasional gray streak in the cortex.

Microscopical Examination.—There are a few patches of round cell infiltration between the tubules, with occasional increase in connective tissue.

Rabbit 28.—Weight 1,495 gm. Control for rabbits 25 and 30.

Feb. 25, 27, and 28, 1913. Sensitizing doses of 1, 1.5, and 1.5 c.c. of egg-white intravenously.

Feb. 28, 1913. Killed. All organs appear normal.

Microscopical Examination.—Kidneys normal.

Rabbit 30.—Weight 1,420 gm.

Feb. 25, 27, and 28, 1913. Sensitizing doses. 1.2 and 1.5 c.c. egg-white intravenously, and 2 c.c. egg-white intraperitoneally.

Mar. 7, 1913. Urine (12 hour specimen) cloudy; faint trace albumin; no casts.

Mar. 24, 25, 26, 27, and Apr. 4, 1913. 0.1, 0.2, 0.2, 0.4, and 0.5 c.c. egg-white intravenously, respectively. After 1st and 2d doses severe symptoms; after 3d, 4th, and 5th mild symptoms.

Apr. 8, 1913. Urine (12 hour specimen) shows marked trace of albumin; scattered hyaline and granular casts.

Apr. 8, 12, 14, and 19, 1913. 0.5, 1, 2, and 2 c.c. egg-white intravenously, respectively. After each dose severe symptoms.

Apr. 26, 1913. 1.5 c.c. egg-white intravenously. Very severe symptoms. Death in 48 hours.

Autopsy.—Heart dilated, lungs collapsed and showed reddish patches. Liver showed numerous pin-point sized opaque gray areas. Kidneys pale, medium size, occasional gray streaks from medulla and cortex.

Microscopical Examination.—The kidneys show very few changes in the cortex. Almost all the glomeruli appear abnormal. The capillary tufts contain no blood, and the endothelial cells are swollen, show many karyorrhectic figures, and many small mononuclear cells.

Rabbit 25.—Weight 1,440 gm.

Sensitized with the following injections: Feb. 25 and 27, 1913. 1 and 1.5 c.c. egg-white intravenously, respectively. Feb. 28, 1913. 2.5 c.c. egg-white intraperitoneally. Mar. 3, 4, and 5, 1913. 2.5 c.c. horse serum intravenously.

Mar. 5, 1913. Urine clear; no albumin.

Results of injections intended to produce anaphylactic shock.

Mar. 24 and 25, 1913. 0.1 c.c. egg-white intravenously, marked symptoms.

Mar. 27, 1913. 0.2 c.c. egg-white intravenously; slight symptoms.

Apr. 4 and 8, 1913. 0.5 c.c. egg-white intravenously. Marked and moderate symptoms.

Apr. 14, 1913. 1 c.c. egg-white intravenously, slight symptoms.

Apr. 21 and 28, and May 13, 1913. 2 c.c. egg-white intravenously. Severe to moderate symptoms.

May 19 and 26, 1913. 2.5 c.c. egg-white intravenously. Moderate symptoms.

Mar. 25, 1913. 0.2 c.c. horse serum intravenously. Very severe symptoms.

Apr. 7, 1913. 0.4 c.c. horse serum intravenously. Mild symptoms.

Apr. 12, 1913. 1 c.c. horse serum intravenously. Moderate symptoms.

Apr. 19, 1913. 1.5 c.c. horse serum intravenously. Slight symptoms.

Apr. 26, 1913. 2.5 c.c. horse serum intravenously. Violent symptoms.

May 10, 1913. 2 c.c. horse serum intravenously. Very severe symptoms.

May 15, 1913. 2.5 c.c. horse serum intravenously. Mild symptoms.

May 21, 1913. 4 c.c. horse serum intravenously. Mild symptoms.

May 27, 1913. 8 c.c. horse serum intravenously. Mild symptoms.

June 2, 1913. 3 c.c. egg-white intravenously. Very severe symptoms. Death in 6 minutes.

Autopsy.—Heart contracted, blood fluid, lungs collapsed, normal. Spleen normal. Liver shows slight lobulation. Kidneys soft, swollen, pale, few red dots on surface. On section the papilla is swollen and gray, and shows streaks extending into the cortex which is pale with poorly marked striæ.

Microscopical Examination.—Kidneys show very extensive cellular fibrous streaks extending from midzone to cortex. Here and there are areas of degenerated tubules with surrounding round cell infiltration. Glomeruli show swelling of capillary endothelium.

Rabbit 101.—Weight 1,250 gm.

Sensitized with the following injections: Mar. 18 and 19, 1913. 1.5 c.c. egg-white intravenously. Mar. 20, 1913. 2.5 c.c. egg-white intraperitoneally.

Results of injections intended to produce anaphylactic shock.

Apr. 10, 1913. 1 c.c. egg-white intravenously. Severe symptoms.

Apr. 17, 1913. 1.5 c.c. egg-white intravenously. Very severe symptoms Death in 5 minutes.

Autopsy.—Heart contracted, lungs collapsed, blood fluid. All organs appear normal, except for extreme congestion.

Microscopical Examination.—Kidneys show marked congestion and cloudy swelling of tubular epithelium.

Guinea Pig 3.—Weight about 400 gm.

Oct. 21, 1912. Sensitizing dose. 0.01 c.c. horse serum intraperitoneally.

Nov. 12, 1912. 2 c.c. horse serum intraperitoneally. No definite symptoms.

Dec. 27, 1912. 1.5 c.c. horse serum intraperitoneally. Mild symptoms.

Jan. 20, 1913. 2 c.c. horse serum intraperitoneally. No symptoms.

Feb. 7, 1913. 3 c.c. horse serum intraperitoneally. Slight symptoms.

Mar. 3, 1913. 2 c.c. horse serum intraperitoneally. No symptoms.

Mar. 3, 1913. 5 c.c. horse serum intraperitoneally. No symptoms.

Mar. 19, 1913. 3 c.c. horse serum intraperitoneally. No symptoms.

Apr. 15, 1913. 4 c.c. horse serum intracardially. Violent symptoms. Died in 7 minutes.

Autopsy.—Lungs insufflated, stand alone. No hemorrhage in any organ. Extreme congestion.

Microscopical Examination.—Kidneys show well marked cellular infiltration of midzone and cortex, extending down into the papilla. Most of the glomeruli show extreme congestion. A few show proliferation of the capsular epithelium, forming typical crescents.

Guinea Pig 8.—Weight about 400 gm.

Oct. 21, 1912. Sensitizing dose. 0.01 c.c. horse serum intraperitoneally.

Nov. 22, 1912. 2 c.c. horse serum intraperitoneally. Moderate symptoms.

Dec. 27, 1912. 2.5 c.c. horse serum intraperitoneally. Marked symptoms.

Jan. 20, 1913. 3 c.c. horse serum intraperitoneally. No symptoms.

Feb. 11, 1913. 4 c.c. horse serum intraperitoneally. Slight symptoms; scratching at nose.

Mar. 3, 1913. 3 c.c. horse serum intraperitoneally. Slight symptoms. 5 c.c. horse serum intraperitoneally. No symptoms.

Apr. 7, 1913. 4 c.c. horse serum intraperitoneally. No symptoms. 5 c.c. horse serum intraperitoneally. No symptoms.

Apr. 21, 1913. 1.5 c.c. horse serum intracardially. Slight symptoms.

May 21, 1913. 2 c.c. horse serum intracardially. Very severe symptoms. Death May 22.

Autopsy.—May 22, 1913. Heart and lungs normal. Stomach enormously distended showing many hemorrhages. Hemorrhage over colon. Spleen large and soft. Liver large, pale, mottled with red. Kidneys intensely congested.

Microscopical Examination.—Kidneys show increase in connective tissue about arcuate vessels with round cell infiltration and occasional thickening of glomerular capsules.

Guinea Pig 1.—Weight 295 gm.

Oct. 18, 1912. Sensitized with 0.5 c.c. egg-white intraperitoneally.

Nov. 11. 2 c.c. egg-white intraperitoneally. Very severe symptoms. Death in 30 minutes.

Autopsy.—Typical emphysema of lungs. Congestion of all organs.

Microscopical Examination.—Kidneys show congestion with acute degeneration of tubular epithelium.

Cat 2.—Weight 3,250 gm.

Nov. 19, 1912. Sensitizing dose. 3 c.c. horse serum subcutaneously.

Dec. 9, 1912. 2 c.c. horse serum intravenously. Very severe symptoms.

Jan. 2, 1913. 2 c.c. horse serum intravenously. Very severe symptoms. 22

minutes later 4 c.c. horse serum intravenously. Very slight symptoms. 17 minutes later 8 c.c. horse serum intravenously. No symptoms.

Jan. 3, 1913. Urine (12 hour specimen) clear; albumen ¼ of 0.1 per cent.; no casts.

Jan. 16, 1913. 2.75 c.c. horse serum intravenously. Very severe symptoms. Death in 7 minutes.

Autopsy.—Blood fluid. Lungs collapsed. Heart contracted. Liver and spleen congested. Kidney pale, fatty.

Microscopical Examination.—Kidneys show congestion, much fat in tubular epithelium. No change in glomeruli nor interstitial tissues.

Cat 4.—Weight 3,510 gm.

Nov. 19, 1912. Sensitized with 3 c.c. horse serum subcutaneously.

Dec. 9, 1912. 1 c.c. horse serum intravenously. Severe symptoms.

Jan. 2, 1913. 8 c.c. horse serum intraperitoneally. No definite symptoms. 25 minutes later 11.5 c.c. horse serum intraperitoneally. No symptoms.

Jan. 16, 1913. 10 c.c. horse serum intraperitoneally. No immediate symptoms. Diarrhea.

Jan. 27, 1913. 6 c.c. horse serum intraperitoneally. Moderate symptoms.

Feb. 11, 1913. 8 c.c. horse serum intraperitoneally. Mild symptoms.

Feb. 24, 1913. 6 c.c. horse serum intraperitoneally. Mild symptoms. 30 minutes later 10 c.c. horse serum intraperitoneally. No symptoms.

Mar. 12, 1913. 5 c.c. horse serum intraperitoneally. No definite symptoms. 12 minutes later 3.5 c.c. horse serum intraperitoneally. Severe symptoms. 8 minutes later 3 c.c. horse serum intraperitoneally, Slight symptoms.

Mar. 27, 1913. 5.5 c.c. horse serum intraperitoneally. Moderate symptoms.

Apr. 12, 1913. 6.5 c.c. horse serum intraperitoneally. No symptoms.

Apr. 29, 1913. 7 c.c. horse serum intraperitoneally. No symptoms.

May 28, 1913. 8.5 c.c. horse serum intraperitoneally. No symptoms.

June 11, 1913. Killed. Has lost weight, looks badly. Weight 2,400 gm. Omentum and retroperitoneal tissues thickened and opaque. Mesenteric and retroperitoneal lymph nodes large and soft. Heart and lungs normal. Kidneys large, red, and show on section a few whitish streaks.

Microscopical Examination.—Kidneys show much thickening of connective tissue about arcuate vessels, with considerable round cell infiltration. There are connective tissue striæ that extend into the cortex. In these areas tubules are atrophied. There is thickening of glomerular capsules with hyaline degeneration of capillary tufts. There are also a few isolated areas of round cell infiltration beneath the capsules.

Cat 8.—Weight 3,095 gm.

Mar. 12, 1913. Sensitizing dose. 2 c.c. egg-white intravenously.

Apr. 8, 1913. 0.4 c.c. egg-white intravenously. Mild symptoms. 25 minutes later 0.8 c.c. egg-white intravenously. No symptoms.

Apr. 19, 1913. 1 c.c. egg-white intravenously. Severe symptoms. After last dose cat continued ill.

Apr. 23, 1913. Killed.

Autopsy.—Heart shows a few hemorrhages over the left auricle. Lungs normal. Liver large, soft, mottled gray and red. Kidneys appear normal.

Microscopical Examination.—Kidneys show swelling of tubular epithelium

and occasionally cellular streaks extending along blood vessels from the midzone out towards the capsule. Some of the glomeruli in these areas show thickening of the capsule.

Dog 4.—Weight 6,890 gm.

Nov. 7, 1912. Urine by catheter clear; faintest trace of albumin; no casts.

Nov. 7, 1912. Sensitizing dose of 5 c.c. horse serum subcutaneously.

Nov. 27, 1912. 12.5 c.c. horse serum subcutaneously. Moderate symptoms.

Dec. 30, 1912. 2.25 c.c. horse serum intravenously. Very slight symptoms. 15 minutes later 3 c.c. horse serum intravenously. Very slight symptoms. 25 minutes later 3.5 c.c. horse serum intravenously. No symptoms. 10 minutes later 5 c.c. horse serum intravenously. No symptoms. 40 minutes later 8 c.c. horse serum intravenously. Vomiting and diarrhea. 25 minutes later 10 c.c. horse serum intravenously. Continued vomiting.

Dec. 31, 1912. Urine (24 hour specimen) 650 c.c., clear; faint trace of albumin; no casts.

Jan. 15, 1913. 2.5 c.c. horse serum intravenously. Mild symptoms.

Jan. 22, 1913. 4 c.c. horse serum intravenously. No definite symptoms.

Jan. 29, 1913. 10 c.c. horse serum intravenously. Mild symptoms.

Jan. 30, 1913. Killed.

Autopsy.—Liver and kidneys rather pale, but all organs appear normal.

Microscopical Examination.—Throughout the midzone of the kidneys there is a streaky cellular infiltration, extending into the cortex and occasionally into the papilla. It is not very marked. No changes in the glomeruli.

Dog 1.—Brindle. Weight 6,060 gm.

Sensitized with the following injections: Oct. 25, 1912. 5 c.c. horse serum subcutaneously. Jan. 6, 1913. 5 c.c. egg-white intravenously.

Oct. 29, 1912. Urine clear, amber; faintest trace of albumin; no casts.

Nov. 22, 1912. 10 c.c. horse serum subcutaneously. Moderately severe symptoms.

Nov. 23, 1912. Urine heavy trace albumin.

Dec. 9, 1912. 10 c.c. horse serum subcutaneously. Mild symptoms.

Dec. 10, 1912. Urine faint trace albumin.

Dec. 11, 1912. Urine heavy trace albumin.

Jan. 6, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 7, 1913. 1.1 c.c. horse serum intravenously. No symptoms.

Jan. 8, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 9, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 10, 1913. 1 c.c. horse serum intravenously. Much scratching.

Jan. 11, 1913. 2 c.c. horse serum intravenously. Moderately severe symptoms.

Jan. 13, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 15, 1913. 1.5 c.c. horse serum intravenously. No symptoms.

Jan. 16, 1913. 2 c.c. horse serum intravenously. No symptoms.

Jan. 17, 1913. 4 c.c. horse serum intravenously. No symptoms.

Jan. 18, 1913. 5 c.c. horse serum intravenously. No symptoms.

Jan. 20, 1913. 7 c.c. horse serum intravenously. No symptoms.

Jan. 24, 1913. 8.5 c.c. horse serum intravenously. No symptoms.

Jan. 27, 1913. 0.5 c.c. egg-white intravenously. No symptoms.

Jan. 28, 1913. 2.5 c.c. egg-white intravenously. No symptoms.

Jan. 31, 1913. Urine (over night) clear; large amount albumin.

Jan. 31, 1913. 2.5 c.c. egg-white intravenously. No symptoms.

Feb. 4, 1913. 5 c.c. egg-white intravenously. Severe symptoms. Lost considerable weight; looks badly. Weight 5,310 gm.

Feb. 6, 1913. Killed.

Autopsy.—Heart, lungs, spleen, and liver normal. Kidneys dark, capsule adherent, surface slightly irregular. On section the medulla is large and pale, the arcuate vessels are well marked with white streaks extending from the medulla into the cortex.

Microscopical Examination.—Marked cellular infiltration of the papilla and midzones of the kidney, with increase in connective tissue about the vessels and rays of cellular connective tissue extending into the cortex. Glomeruli do not show any marked changes.

Dog 9.—Fox terrier type. Control. Weight 3,200 gm.

Mar. 3, 1913. 3 c.c. horse serum subcutaneously.

Mar. 3, 1913. 2 c.c. egg-white subcutaneously.

Six days later developed pneumonia and died.

Autopsy.—Mar. 10, 1913. Lungs show extensive consolidation. Other organs show cloudy swelling and congestion.

Microscopical Examination.—Kidneys show cloudy swelling of tubular epithelium. No other changes.

Dog 10.—Fox terrier type. Weight 4,360 gm.

Mar. 3, 1913. Sensitizing dose. 3 c.c. horse serum intravenously.

Mar. 3, 1913. Sensitizing dose. 2 c.c. egg-white intravenously.

Mar. 24, 1913. 1 c.c. egg-white intravenously. Slight symptoms.

Apr. 4, 1913. 3 c.c. egg-white intravenously. Very severe symptoms. Death in 24 minutes.

Autopsy.—Blood fluid. Lungs collapsed, heart normal, liver large, dark, bloody. Kidneys regular, pale and gray. On section the medulla and cortex show a few gray lines and streaks.

Microscopical Examination.—Infiltration of small round cells in the midzone, papilla, and cortex. Congestion of glomeruli, some of which appear very cellular.

The nephritis that has appeared in these animals seems to be very much like that which Dickson[26] describes as resulting from the injection of uranium nitrate, though most of the animals in my experiments did not show as extensive lesions as Dickson pictures. The changes in the epithelial structures of the kidney that I have observed point to the fact that there is some direct toxic action from the repeated injections of horse serum and egg-white upon the epithelium, particularly of the cells lining the loops of Henle and the collecting tubules. This is followed or perhaps accompanied by an infiltration of small round cells producing the histological appearance of a chronic inflammation, and leading later to new connective

[26] Dickson, E. C., *Arch. Int. Med.*, 1909, iii, 375; 1912, ix, 557.

tissue formation. It is probable that these round cells come from the circulating blood and not from the fixed connective tissue cells of the kidney, for in a number of sections the capillaries surrounding small foci of necrosis were seen to be filled with small mononuclear cells.

Besides the necrosis of the epithelium there is a direct injury to the capillary tufts of the glomeruli, for the acute changes described were often seen in glomeruli far distant from any areas of tubular degeneration and atrophy and occasionally in kidneys that presented no other marked abnormality. Some of the more advanced glomerular changes in the areas where tubules were atrophied and where there was much increase in interstitial tissue might be considered as secondary to the destruction of the tubule unit, but in many instances, when such factors were lacking, there must have been primary injury to the glomerulus.

It is known that injection of uranium nitrate may produce chronic nephritis in animals, and recently Baehr[27] and Christian[28] have shown that with the same drug true glomerular lesions may be obtained in rabbits. Ophüls[29] has described a chronic nephritis as developing in rabbits intoxicated over long periods with lead, and consequently it is not so much the possibility of producing in animals a chronic or subacute nephritis that I should like to emphasize as the method of bringing about this lesion through repeated injections of foreign protein.

In all these experiments it is necessary to keep in mind the fact that laboratory animals and especially rabbits and dogs are occasionally subject to a spontaneous nephritis which might lead to a grave error in interpreting the results. With a view to determining the frequency of spontaneous nephritis in animals, Ophüls[30] has examined the kidneys of fifty supposedly normal rabbits and one hundred guinea pigs. Many of these were old animals. Of the fifty rabbits, twenty-eight had normal kidneys, nine showed slight parenchymatous lesions, three a few small areas of cellular infiltration, and ten well marked increase in the interstitial tissue. Changes

[27] Baehr, G., *Beitr. z. path. Anat. u. z. allg. Path.*, 1913, lv, 545.
[28] Christian, H. A., and O'Hare, J. P., *Jour. Med. Research*, 1913, xxviii, 227.
[29] Ophüls, W., *Jour. Med. Research*, 1908, xviii, 497.
[30] Ophüls, W., *Proc. Soc. Exper. Biol. and Med.*, 1910–11, viii, 75.

in the glomeruli were never seen. Of the guinea pigs, sixty-three had absolutely normal kidneys, and thirty-five showed foci of small round cell infiltration through the cortex. In a few instances the glomerular capsules were thickened. I have examined as a control the kidneys of twenty-four supposedly normal rabbits. The kidneys in sixteen were absolutely normal, in four they showed well marked old fibrous scars in the cortex, which did not resemble the lesions in the experiments that I have described, and in four there were one or two patches of small round cells between the tubules of the cortex which were not unlike the isolated areas of round cell infiltration seen in some of my experiments. In no instance were glomerular lesions encountered. The kidneys of fifteen cats, some taken directly from the street, others kept for several weeks in the laboratory, have also been examined, and with one exception, proved normal. The kidneys of this cat appeared coarsely granular and microscopically showed old connective tissue scars running through the cortex. There were no glomerular lesions. In my experiments I have made every attempt to exclude the possibility of using animals that might have spontaneous nephritis. Young animals were always employed. The rabbits usually weighed between 1,000 and 1,500 grams, and were selected from different lots. The guinea pigs weighed from 200 to 400 grams. An attempt was also made to use young dogs, but the ages of the cats were not known.

Before the inoculations an examination of the urine for albumin and casts was made and often repeated two or three times. If the urine contained more than the faintest trace of albumin, or cells or casts on centrifugalization, the animal was discarded. During the inoculations, as may be seen from some of the protocols, the urine of many animals showed considerable amounts of albumin with great numbers of casts, both hyaline and granular. This was most marked during the few days following an inoculation.[31] Occasionally great numbers of epithelial cells and mononuclear cells were seen, especially in dogs.

Finally, to exclude even more definitely the possibility that I might be dealing with spontaneous lesions, three dogs sensitized to

[31] When egg-white was employed, the albumin was always discountenanced, since egg-albumin after parenteral inoculations is eliminated unchanged in the urine.

egg-white and horse serum were subjected to operation with the assistance of Dr. Burnap, and a portion of one kidney of each dog was excised for control examination before the secondary series of inoculations was begun. The microscopical examination of this resected portion showed that at operation the kidneys of all three dogs were normal. Twenty-six days after the primary inoculations and twenty days after operation these dogs were subjected to intravenous injections of egg-white and horse serum.

Dog 12 received two doses of horse serum and three of egg-white. There were no marked symptoms after the injections of horse serum. Severe symptoms developed after the second and third injections of egg-white, and the dog died within twenty-four hours after the last injection. During these injections, the urine, which before had been normal, showed increasing amounts of albumin with epithelial cells, mononuclear cells, and granular casts. At autopsy the kidneys were rather large and pale. Microscopical examination showed edema of the interstitial tissue with scattered round cells particularly in the papilla and intermediate zone. There was extensive degeneration of the epithelium of Henle's loops and of the collecting tubules. Through the cortex areas of extensive degeneration involving the convoluted tubules were seen and the lumina of many tubules contained granular casts.

Dog 13 received four doses of egg-white and four of horse serum intravenously. During this period the urine, which before had been normal, showed traces of albumin, great numbers of epithelial cells, and small round cells and later many hyaline and granular casts. After each dose of foreign protein there were severe symptoms. He was killed thirty-four days after the first injection. The kidneys at autopsy were rather large and showed grayish markings in the intermediate zone. Microscopically all sections showed patches of degeneration of the tubular epithelium in the cortex surrounded by small round cells with diffuse small round cell infiltration of the intermediate zone.

Dog 14 received one injection of horse serum and two of egg-white, dying acutely from the second injection. At autopsy the kidneys were congested, and on microscopical examination showed extensive diffuse degenerative changes in the tubular epithelium

with a few foci of small round cell infiltration that in one instance surrounded many glomeruli (figure 14).

These three experiments demonstrate definitely that sensitized dogs whose kidneys are normal may, within a few weeks, develop nephritis from repeated injections of small amounts of horse serum and egg-white. On account of the constancy of the lesions described in the kidneys in the various animals, therefore, the involvement of the glomeruli, the development of urinary signs of nephritis, as well as the three experiments just described, it is justifiable to exclude spontaneous changes as a cause of the nephritis occurring in these animals and to conclude that it is a direct result of the injections of foreign protein.

A more difficult question is to determine definitely whether the lesions in the kidney really depend upon a sensitization of the animals to foreign protein and represent an effect of anaphylaxis, or whether the foreign protein itself, irrespective of sensitization, acts as a toxin, such as uranium nitrate, and brings about the nephritis in the same way that a chemical irritant or poison might. Though horse serum is almost harmless for rabbits, guinea pigs, and dogs, Schultz[32] states that it may produce grave symptoms or death in young cats when injected intravenously in doses of 0.0025 of a cubic centimeter per gram of body weight. Doses of this size, which would mean six to eight cubic centimeters for the cats employed were rarely given intravenously in my experiments. Moreover, the possibility that the serum might be eliminated as such through the kidneys and thus cause some irritating effect or injury is highly improbable. Though there has been some discussion as to the possibility of an animal's utilizing the protein of foreign serum injected intravenously, Lommel's[33] statement that the injection of horse serum in dogs increases the total nitrogen output in the urine 50 to 90 per cent., is not upheld by the recent experiments of Austin and Eisenbrey[34] which show that as much as eighty cubic centimeters of horse serum may be injected intravenously in dogs which are in nitrogen equilibrium without the appearance of albumin

[32] Schultz, W. H., *loc. cit.*

[33] Lommel, F., *Arch. f. exper. Path. u. Therap.*, 1907–08, lviii, 50.

[34] Austin, J. H., and Eisenbrey, A. B., *Arch. Int. Med.*, 1912, x, 305.

or increased incoagulable nitrogen in the urine. I have not observed an albuminuria in normal animals following the injection of horse serum in the doses used in the experiments. With egg-white, however, the results were different. This substance is in itself toxic to rabbits when injected in large doses, and Vaughan[35] has shown that repeated small doses may cause fever and other untoward symptoms. Egg-white, unlike horse serum, when introduced parenterally is eliminated unchanged through the kidneys, as was first shown by Stokvis.[36] Among others, Friedemann and Isaac[37] and Van Alstyne and Grant[38] have studied this question and conclude that native protein as well as the egg albumin is eliminated under these circumstances. That large quantities of albumin appear in the urine of dogs, cats, and rabbits very shortly after an intravenous injection of egg-white, I have observed repeatedly, and though no accurate investigations were made as to the exact time of appearance, albumin was in a few instances found in the urine within thirty minutes of the intravenous injection of egg-white. The following experiment shows definitely that the albumin found in the urine was in part egg-albumin.

Dog 5.—Weight 15,000 gm. Urine containing much albumin was collected for eighteen hours after injection of egg-white intravenously, and filtered through a Berkefeld filter. 3 c.c. of this filtered urine were injected intraperitoneally into each of two guinea pigs, 1 and 2, weighing 220 and 235 gm.; at the same time 0.5 c.c. of pure egg-white were injected intraperitoneally into guinea pig 3, weighing 222 gm.

After 3 weeks:

Guinea pig 1 was given 2.5 c.c. of egg-white intraperitoneally. Death in 15 min.
Guinea pig 2 was given 1.5 c.c. of egg-white intraperitoneally. Death in 5 min.
Guinea pig 3 was given 2.5 c.c. of egg-white intraperitoneally. Death in 4 min.
Normal control 4 was given 2.5 c.c. of egg-white intraperitoneally. No symptoms.

It is evident that under the conditions of these experiments egg-albumin was eliminated as such by the kidneys, and it is possible that the passage of this substance through the cells of the kidney might in itself cause some injury.

[35] Vaughan, V. C., *loc. cit.*

[36] Stokvis, J. B., *Centralbl. f. d. med. Wissensch.*, 1864, ii, 596.

[37] Friedemann and Isaac, S., *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 513; 1906, iii, 209.

[38] Van Alstyne, E. V. N., and Grant, P. A., *Jour. Med. Research*, 1912, xxv, 399.

In order to investigate this matter I have injected a certain number of normal animals with single doses or closely spaced doses of horse serum or egg-white or both. One dog which had received two cubic centimeters of egg-white intravenously and five cubic centimeters of horse serum subcutaneously and was killed five days later at the beginning of an attack of distemper, showed a few patches of small round cells in the cortex. Another dog injected in the same manner showed normal kidneys. None of the four sensitized guinea pigs dying acutely from the first dose of protein showed chronic lesions. Of ten rabbits, three inoculated with two to four consecutive doses of egg-white, one with casein, and six with three consecutive doses of egg-white and three of horse serum, five showed distinct changes in the kidneys after three to sixteen weeks. The most marked lesions were seen in three rabbits that received one dose of 1.5 cubic centimeters of egg-white intravenously, one of 2.5 cubic centimeters intraperitoneally, and one of 5 cubic centimeters subcutaneously on three successive days, and one week later two successive intravenous doses of 2.5 cubic centimeters of horse serum. These animals all received excessive doses and though the lesions did not compare in severity with many of the experimental series, it is evident that under these circumstances, particularly when one of the doses is very large and given subcutaneously, nephritis may develop. It will be necessary, however, to investigate this point further before any very definite conclusions may be drawn as regards the primary toxicity of egg-white for the kidneys of laboratory animals.

In the majority of experiments, however, advanced nephritis was obtained only after the frequent repetition of injections of small doses of egg-white or horse serum in sensitized animals, and though it is impossible at the present time to say definitely that the development of nephritis depends exclusively upon the previous sensitization of the animal to these substances, it seems probable that at least any primary toxicity of the protein for the kidney cells was heightened by this procedure, and that the lesions in the kidney as well as those in the liver, myocardium, and peritoneum are to be interpreted as a generalized reaction analogous to the local Arthus phenomenon in the skin of the rabbit.

CONCLUSIONS.

The repeated injection of small doses of horse serum and egg-white in dogs, cats, rabbits, and guinea pigs that have been sensitized to these proteins, causes injury to the cells of various organs and tissues with resulting inflammatory reactions.

The changes are especially marked after intraperitoneal injections in the peritoneum and after intravenous injections in the livers of rabbits and cats, and in the myocardium and kidneys of all groups of animals.

In dogs and rabbits, especially, there develops a well marked nephritis characterized by degeneration and necrosis of the epithelium of the loops of Henle, of the collecting tubules, and less frequently of the convoluted tubules. This is accompanied by an extensive small round cell infiltration of the interstitial tissue and later the formation of connective tissue. Together with these changes there are acute and chronic alterations in the glomeruli of all groups of animals.

Egg-white in large doses is itself injurious to the kidney of animals, but this slight primary toxicity is probably greatly enhanced through previous sensitization of the animal.

EXPLANATION OF PLATES.

PLATE 56.

FIG. 1. Rabbit 16. Fibrous thickening and round cell infiltration of the omentum, after three intraperitoneal injections of horse serum and six intraperitoneal injections of egg-white in a sensitized animal.

FIG. 2. Rabbit 16. New formation of cellular connective tissue of portal spaces of liver. Note the number of cells in the capillaries of the periportal zone, after three injections of horse serum and six of egg-white in a sensitized animal.

PLATE 57.

FIG. 3. Rabbit 24. Necrosis and round cell infiltration of the myocardium of the right ventricle, after twelve injections of egg-white in a sensitized animal.

PLATE 58.

FIG. 4. Rabbit 21. Area in the papilla of a kidney showing early necrosis and desquamation of the cells of the collecting tubules, after ten injections of egg-white in a sensitized animal.

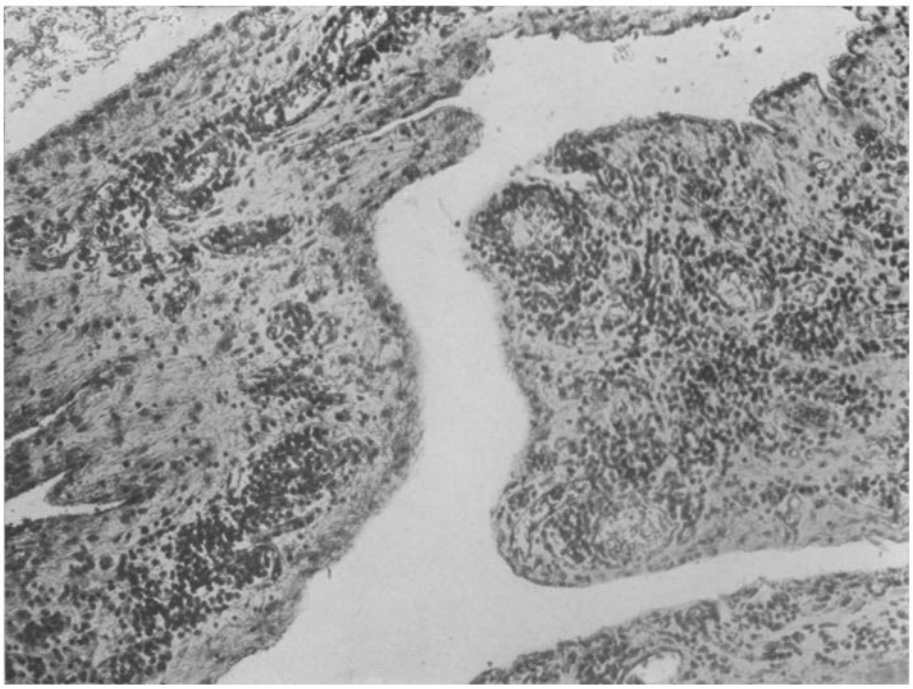

FIG. 1.

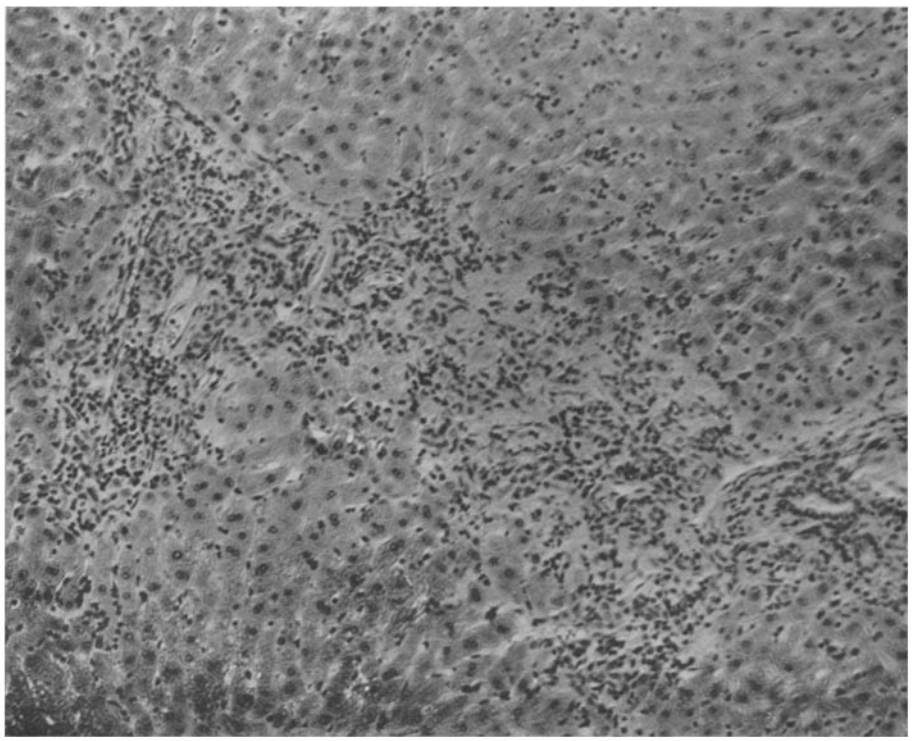

FIG. 2.

(Longcope: Production of Experimental Nephritis.)

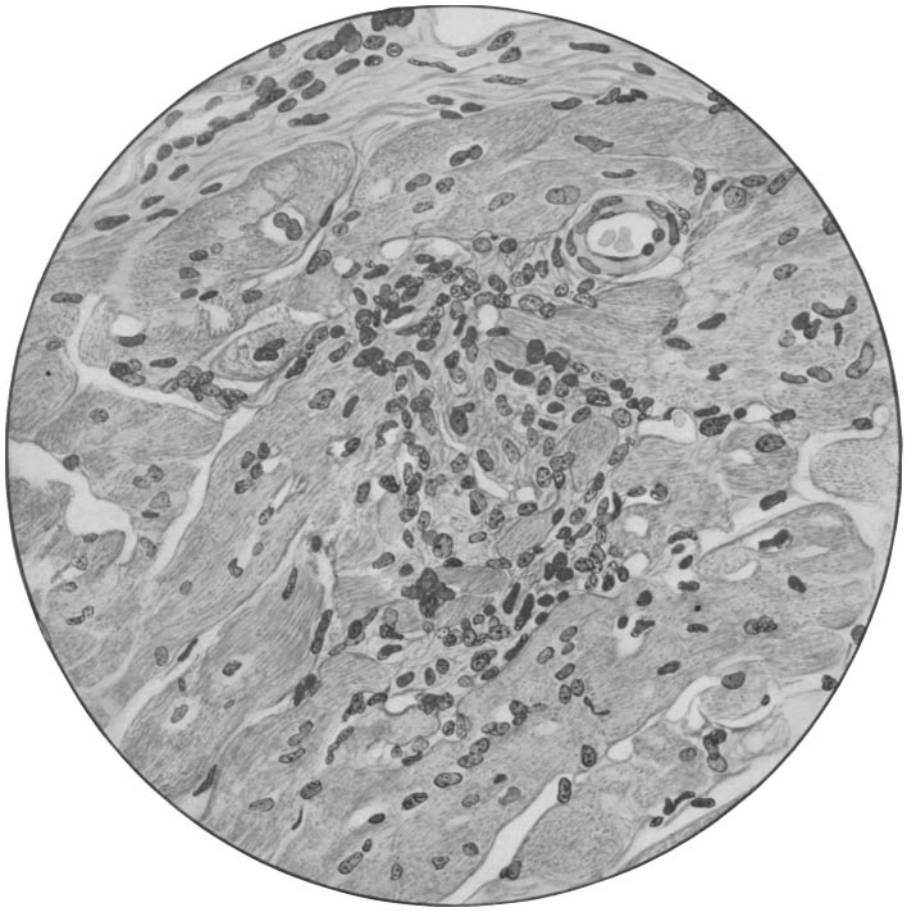

FIG. 3.

(Longcope: Production of Experimental Nephritis.)

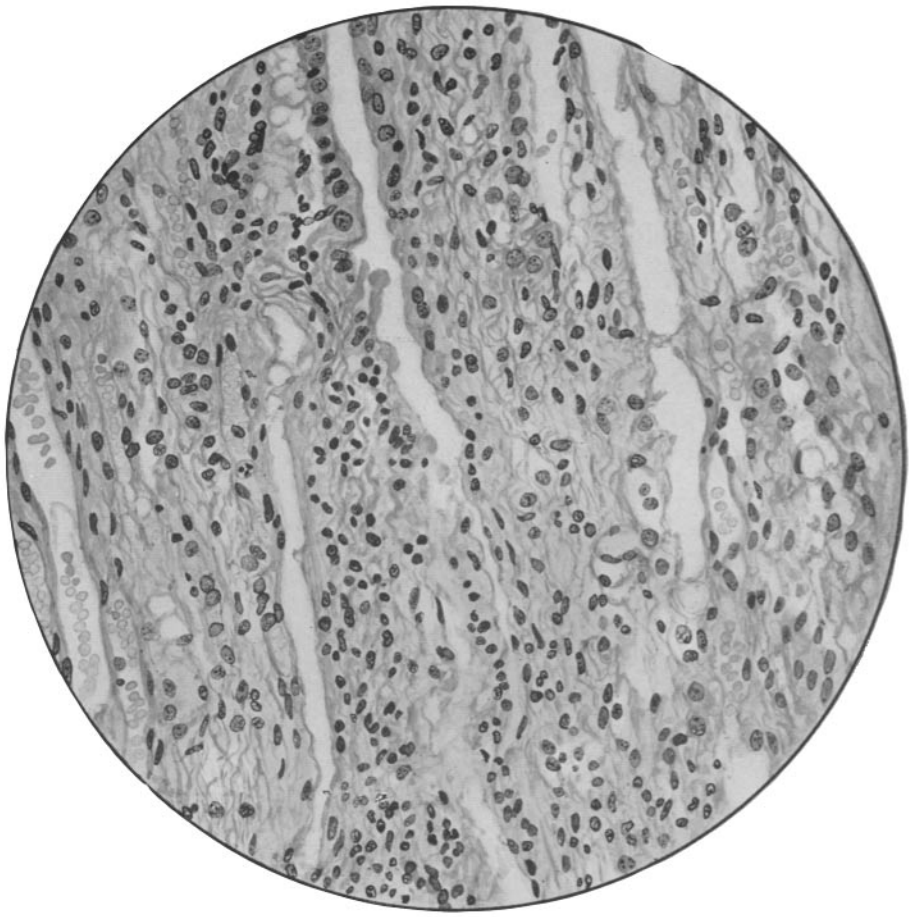

FIG. 4.

(Longcope: Production of Experimental Nephritis.)

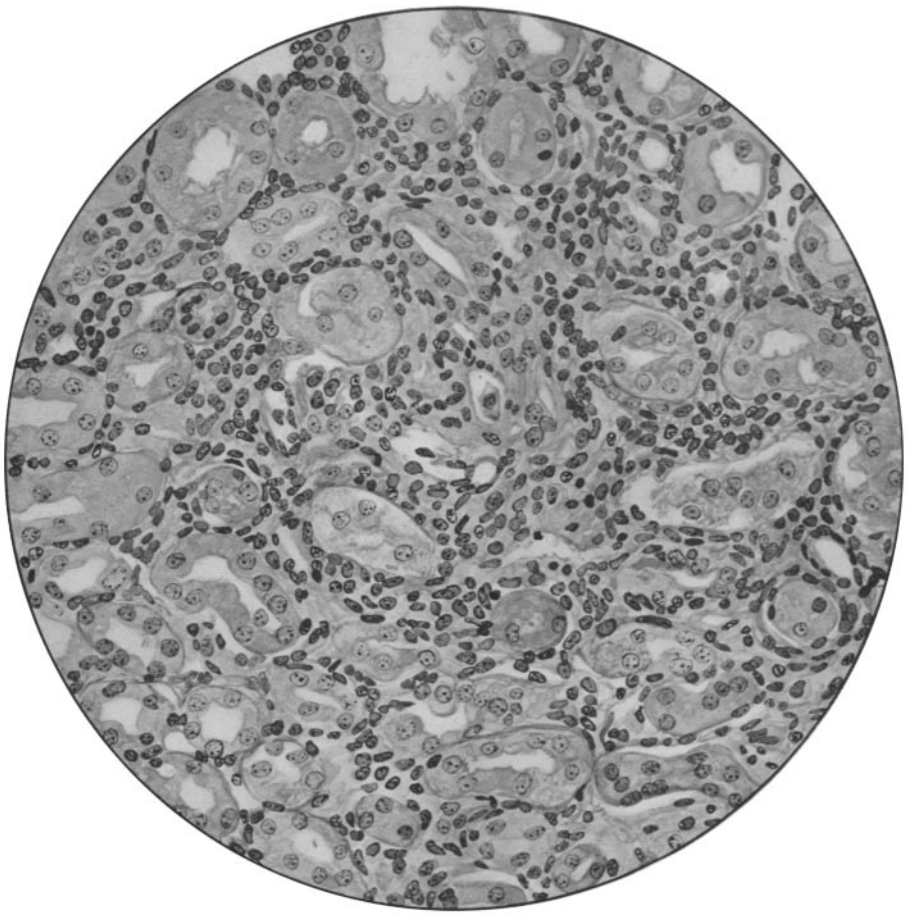

FIG. 5.

(Longcope: Production of Experimental Nephritis.)

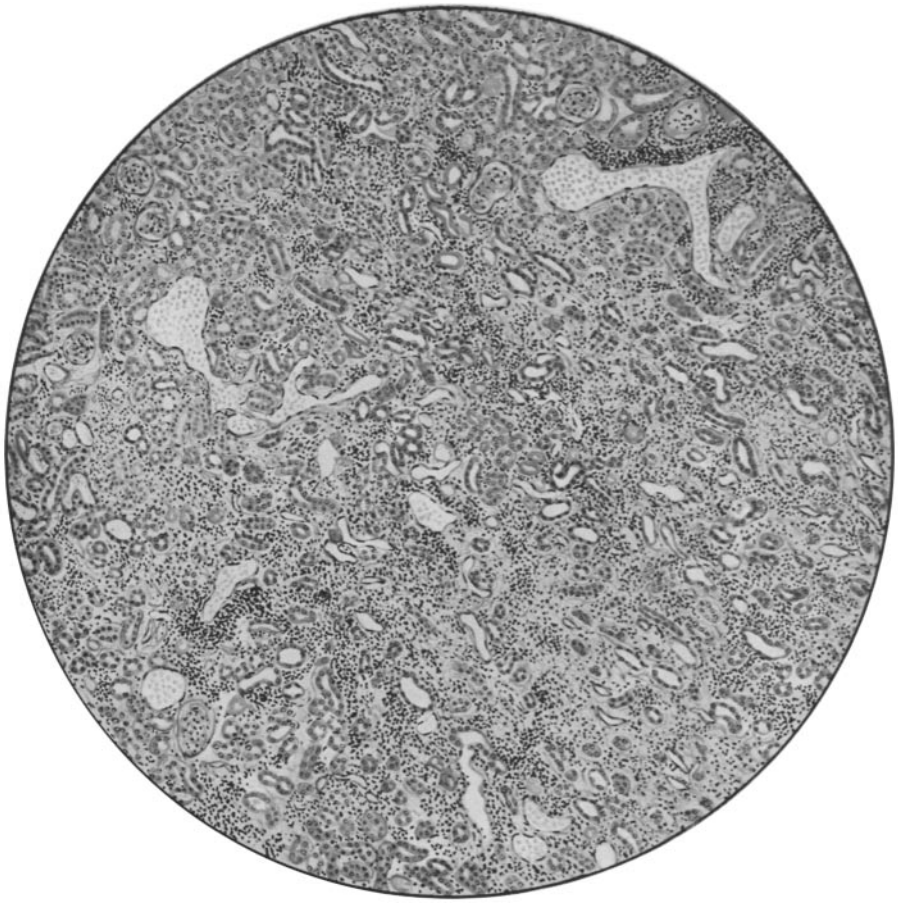

FIG. 6.

(Longcope: Production of Experimental Nephritis.)

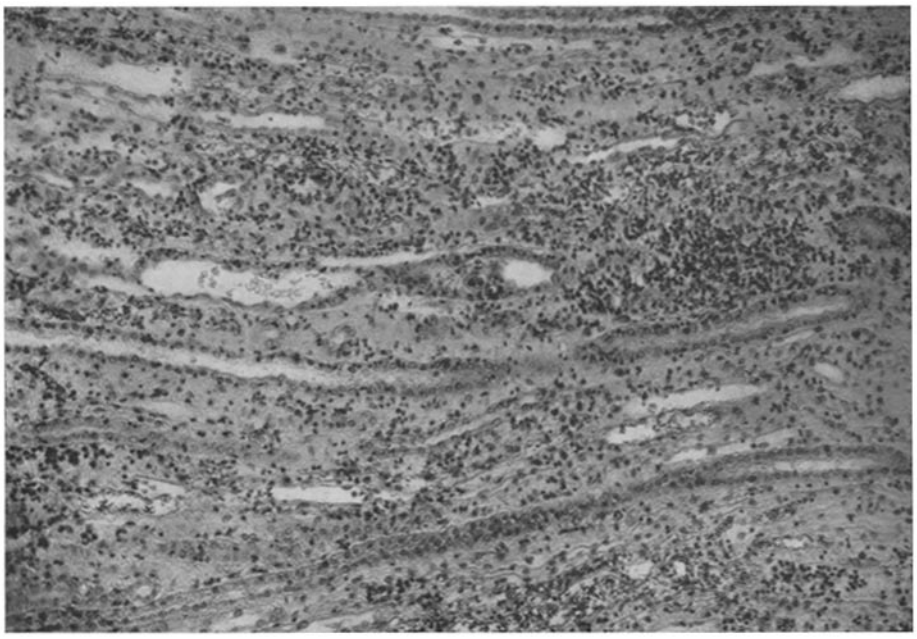

FIG. 7.

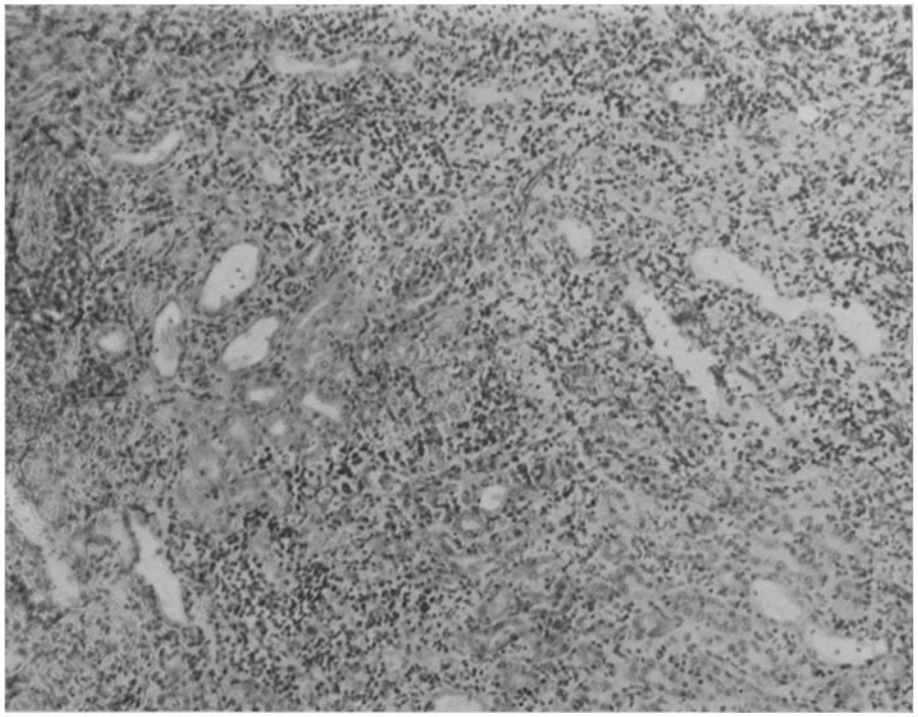

FIG. 8.

(Longcope: Production of Experimental Nephritis.)

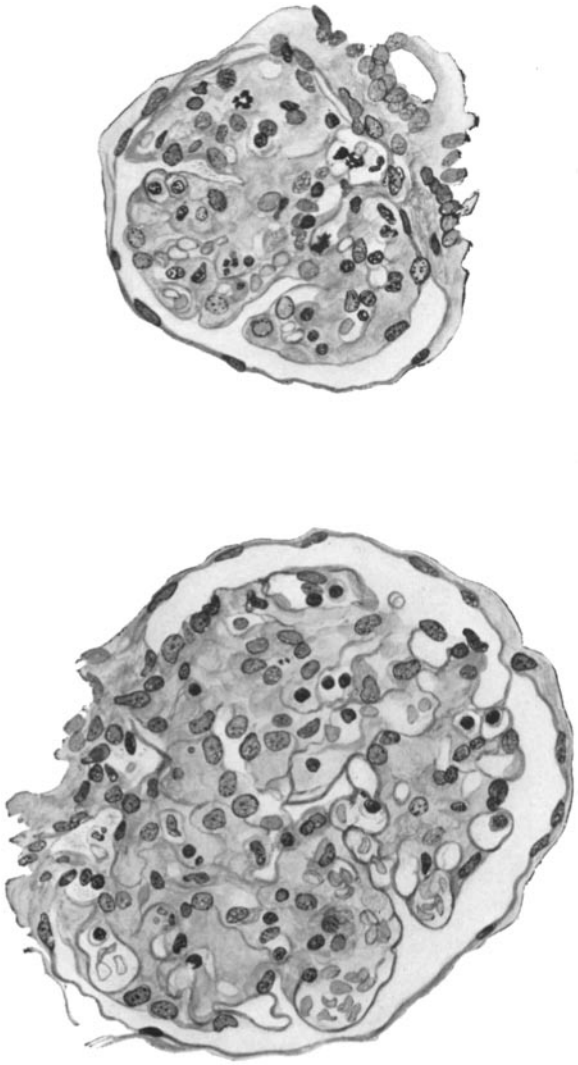

FIG. 9.

(Longcope: Production of Experimental Nephritis)

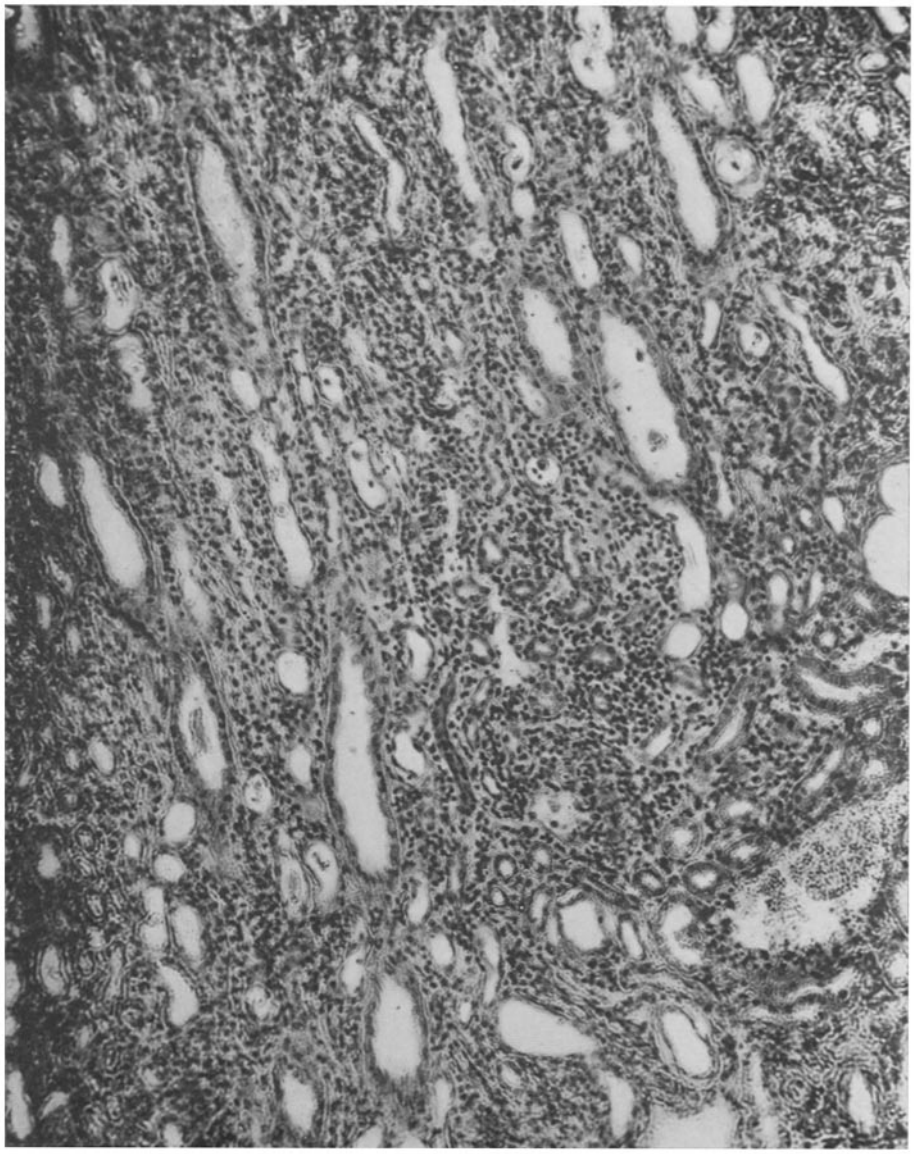

FIG. 10.

(Longcope: Production of Experimental Nephritis.)

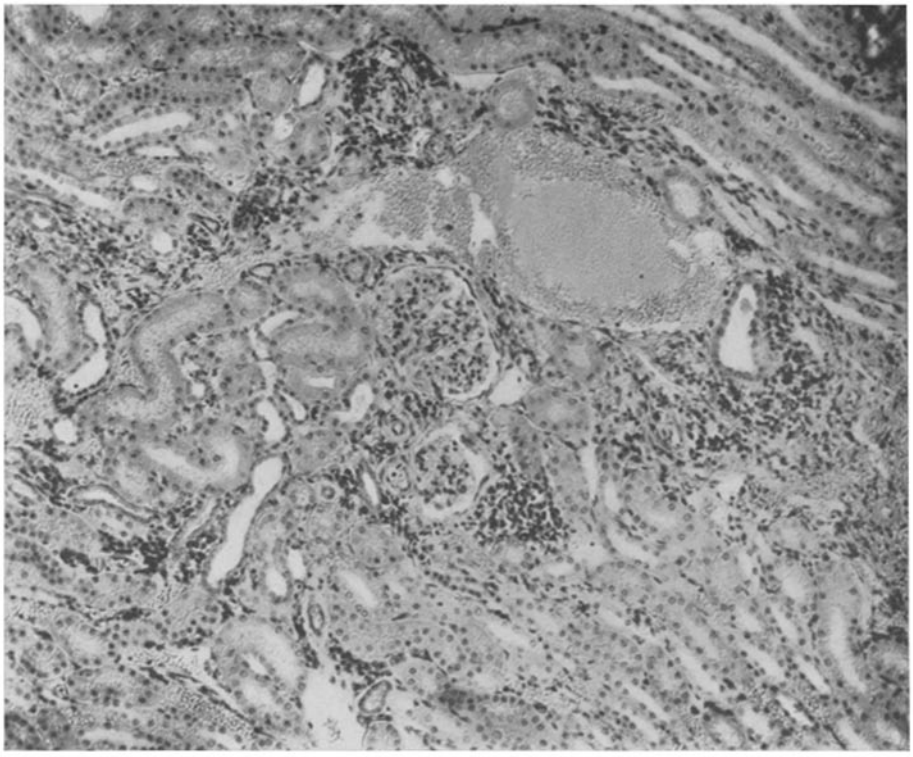

FIG. 11.

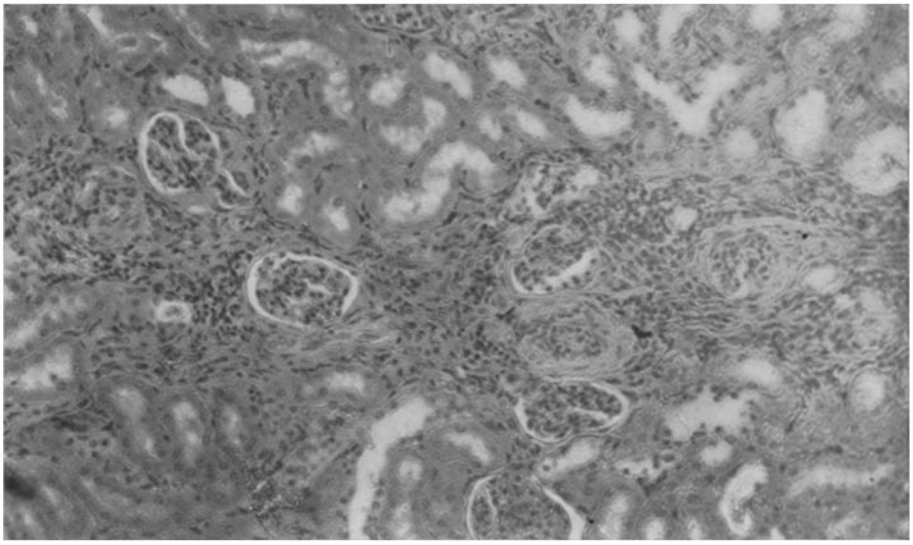

FIG. 12.

(Longcope: Production of Experimental Nephritis.)

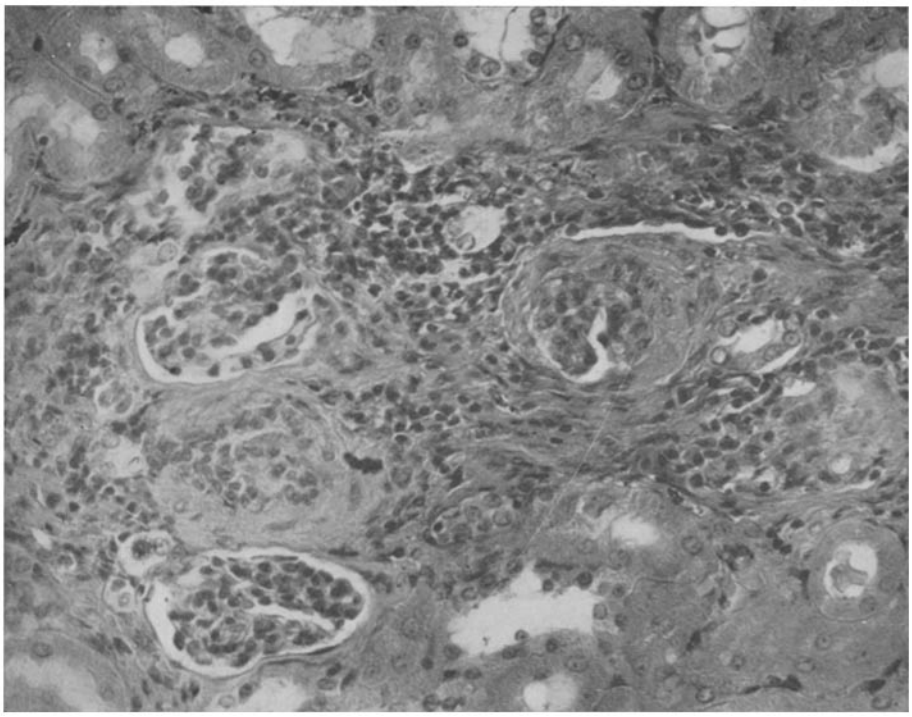

FIG. 31.

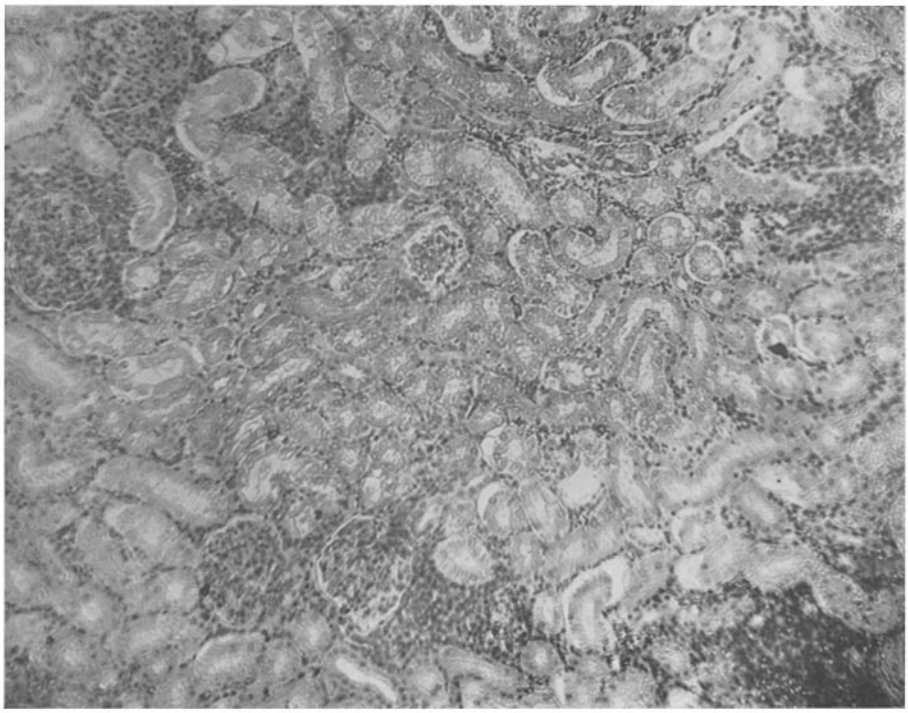

FIG. 14.

(Longcope: Production of Experimental Nephritis.)

PLATE 59.

FIG. 5. Rabbit 9. Early necrosis of the tubular epithelium of the kidney with round cell infiltration, after fourteen injections of horse serum in a sensitized animal.

PLATE 60.

FIG. 6. Rabbit 15. Intermediate zone and cortex of the kidney showing extensive round cell infiltration, degeneration of the tubular epithelium, and early connective tissue formation, after three injections of horse serum and five of egg-white in a sensitized animal.

PLATE 61.

FIG. 7. Rabbit 15. Area in the papilla of the kidney showing necrosis of the epithelium of the convoluted tubules with round cell infiltration.

FIG. 8. Rabbit 15. Another area in the cortex of the kidney.

PLATE 62.

FIG. 9. Rabbit 25. Glomeruli showing swelling of the endothelial cells, infiltration by mononuclear cells and karyorrhexis of nuclei, after seven injections of horse serum and ten of egg-white in a sensitized animal.

PLATE 63.

FIG. 10. Rabbit 16. Area of round cell infiltration and connective tissue formation in the intermediate zone of the kidney, after three inoculations of horse serum and six of egg-white intraperitoneally in a sensitized animal.

PLATE 64.

FIG. 11. Dog 4. Area of connective tissue increase and round cell infiltration in the intermediate zone of the kidney, after thirteen intravenous injections of horse serum and four intravenous injections of egg-white in a sensitized animal.

FIG. 12. Cortex of rabbit 5 showing fibrous area with extensive involvement of the glomeruli, after eleven injections of horse serum over a period of 130 days.

PLATE 65.

FIG. 13. Area in the cortex of rabbit 5, showing the same change as figure 12.

FIG. 14. Dog 14. Cortex of kidney showing round cell infiltration, especially about the glomeruli, after three intravenous injections of egg-white in a sensitized animal. A portion of kidney, removed at operation before injection, was normal.