EXPERIMENTAL NEPHRITIS IN RATS INDUCED BY INJECTION OF ANTI-KIDNEY SERUM

III. PATHOLOGICAL STUDIES OF THE ACUTE AND CHRONIC DISEASE*

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Antiserum prepared by immunizing rabbits with a suspension of perfused rat kidney contains an antibody relatively specific for rat kidney. Data supporting this conception were recorded in the first article of this series (1). The purpose of this communication is two-fold: to give in greater detail the histopathological picture of the developing lesions and to correlate the type of lesions with the clinical and renal functional changes previously reported (2).

Materials and Methods

Young black and white hooded rats weighing from 50 to 100 gm., of an inbred strain, were injected with unabsorbed anti-rat-kidney serum, No. 4138. The preparation and action of this serum are detailed in another communication (1).

The clinical and functional observations on most¹ of the animals mentioned below were reported in the preceding paper (2), and the rats are designated in the same way in both communications. The blood urea determinations were done by the micro method of Farr (3) and the urea clearance values were obtained by the technique of Farr and Smadel (4).

Rats were sacrificed at intervals after injection of anti-kidney serum or when moribund. Autopsy was performed immediately and portions of organs were fixed in 5 per cent Zenker's acetic solution and in 10 per cent neutral formalin. Paraffin sections of various organs were stained as routine with methylene blue-

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¹ Chemical determinations in a few of the animals, not recorded in the previous paper, were obtained through the kindness of Dr. Lee E. Farr.

eosin. In addition, Mallory's connective tissue stain and McGregor's modification (5) of the Mallory-Heidenhain stain were applied to sections of all kidneys. Fatty changes, when present, were shown in frozen sections stained with Scharlach R-hematoxylin. Other methods were occasionally used.

Acute Nephritis Induced by the Injection of Anti-Kidney Serum

The syndrome induced by the relatively specific nephrotoxin, was characterized clinically by marked and persistent albuminuria, cylindruria, and transient anasarca; significant hematuria did not occur (1). Macroscopic observation of the kidney at this stage revealed a moderately pale mottled smooth surface. The cortex bulged slightly on section, the glomeruli were visible as tiny pin-point elevations. Microscopically, the glomerular tufts were relatively anemic, yet they appeared large and almost filled the capsular spaces. This fullness of the tuft depended principally upon thickening of the capillary walls, which stained brightly with eosin and gave a refractile effect. The thickening of the glomerular capillary walls was best seen in sections stained with azan carmine (Mallory-Heidenhain technique) which McGregor (5) considered specific for the glomerular capillary basement membrane. This thickening of the capillary walls was roughly proportional to the amount of nephrotoxin administered, and varied to some extent throughout each tuft and to a greater extent among different tufts. This variation was most noticeable after a single moderate sized dose of nephrotoxin, and was least apparent when a relatively large amount of anti-kidney serum was given in several fractional doses. A number of the tuft nuclei and capsular cells were swollen. Proliferative changes in the glomeruli were inconspicuous, and infiltration of inflammatory cells into the tuft was practically non-existent. Fatty degeneration did not occur in the cells of either glomerular tuft or capsule during the first few days. In the latter part of the acute stage, however, a few cells in each glomerulus, usually epithelial cells, contained fat droplets.

The epithelial cells of the majority of the convoluted and straight tubules, especially the former, were the seat of cloudy swelling and of hyalin droplet degeneration. The lumina of many tubules were moderately distended with a granular protein precipitate. Scattered groups of tubules in cross section, apparently belonging to a single nephron, had widely dilated lumina often filled with granular material

or casts, and were lined by low cuboidal epithelium with slightly basophilic cytoplasm. Fatty degeneration was not observed in the tubular cells during the 1st week after injection, but usually occurred in a moderate degree later in the acute stage of the disease.

Collections of cells were frequently present about small vessels in the kidney, generally in the cortex. These were composed of mononuclear cells, lymphocytes, and eosinophilic polymorphonuclears; they appeared about the 4th day of the induced disease and increased somewhat thereafter. Indefinite changes occurred in the small arteries and arterioles; there was some swelling of the endothelial nuclei and slight hyalinization in the muscle coat. Microscopic alterations in other organs were limited to a perivascular reaction similar to that in the kidney, but less extensive.

Rat N-35, male, weighing 87 gm., was injected intravenously with 0.25 cc. per 100 gm. body weight of the nephrotoxic serum, No. 4138. A slight anaphylactoid reaction developed within a few minutes and subsided after a quarter of an hour. Urine collected during the first 18 hours contained over 4 per cent protein but no blood. Numerous casts appeared the next day. A blood urea nitrogen value of 34 mg. per cent was obtained on a specimen taken when the animal was sacrificed 48 hours after injection. Postmortem examination revealed 4.0 cc. of clear straw-colored ascitic fluid. The appearance of the kidney was as described above. Abnormalities of both glomeruli and tubules were less intense than in rat N-34 which received the same treatment but was sacrificed 2 days later. The clinical course of this second animal resembled that of the first but its terminal blood urea nitrogen reached 49 mg. per cent. Figs. 1, 2, and 3 depict the renal lesions of rat N-34.

Had these two animals (N-34 and N-35) not been sacrificed they would undoubtedly have survived the acute phase of the disease, as the dose of nephrotoxic serum was not large. Indeed, two or three times this dose was usually required to cause death during the acute phase. When this larger amount of serum was administered in divided doses, so that none induced a severe anaphylactoid reaction, then the clinical picture was as described above except that the blood urea rose to greater heights. The pathological alterations were qualitatively similar but quantitatively greater.

Rat N-74 received a total of 0.65 cc. per 100 gm. body weight of serum 4138 in four divided doses given at 3 day intervals. When moribund on the 16th day

its blood urea nitrogen value was 144 mg. per cent. Fig. 4 demonstrates the marked uniform swelling of the glomerular intercapillary material throughout the tuft.

When a severe anaphylactoid reaction was induced with anti-kidney serum, either as a result of giving a comparatively large amount of a relatively pure nephrotoxic serum or a smaller amount of serum rich in non-organ specific anti-rat-tissue antibodies as well as in the more specific nephrotoxin, the clinical and pathological picture differed (1) from that just described. Hematuria with death, occurring from a few hours up to 8 days, were the principal clinical differences. The animals thus affected showed many fibrin thrombi in the glomerular tuft capillaries; and often in addition there were lesions of vascular origin in other organs similar to those observed in anaphylactic shock in rats (6). The protocol given below (rat N-18) is that of an animal which had a rather severe anaphylactoid reaction after injection of a considerable amount of relatively pure nephrotoxic serum.

Rat N-18, weight 106 gm., received 0.51 cc. of serum 4138 in a single injection. Marked albuminuria and gross hematuria appeared within 12 hours. Albuminuria was constant until death on the 8th day, whereas hematuria decreased after the 1st day and was then made evident by a faintly positive guaiac reaction for the next 4 days. Numerous casts appeared on the 3rd day, when slight subcutaneous edema and ascites were noted. The anasarca became marked and the body weight rose to 134 gm. on the 6th day. The terminal blood urea nitrogen value was 156 mg. per cent. At autopsy extensive anasarca was noted. The kidneys macroscopically were moderately swollen, edematous, and pale.

Microscopic study revealed that portions of the capillary loops in the majority of the glomerular tufts were filled with thrombi (Fig. 5). These thrombi, colored purple by Weigert's fibrin stain, varied in size from a thin strand along one side of a capillary to a large sausage-shaped mass completely filling the space between the walls. In some instances the thrombus extended out into the afferent arteriole. The extensively thrombosed glomerular tufts showed necrosis of cells in patchy areas and in some instances granular protein precipitate or erythrocytes were seen in the capsular space. Thickening of the glomerular capillary basement membranes was best seen in tufts without thrombi. The tubular changes were similar to those observed when the effect was induced by the nephrotoxic action of the serum alone, uncomplicated by the added anaphylactoid reaction.

Chronic Nephritis Occurring after the Injection of Anti-Kidney Serum

The transition from the acute to the chronic phase of the nephritis induced by anti-kidney serum was indefinite clinically. If not too

severely injured, the majority of animals recovered from the edematous state and continued to show a moderate to marked cylindruria and albuminuria until they became moribund or were sacrificed. The urea clearance determination in these animals (2) usually did not indicate decreased renal function until several months after injection of the nephrotoxin. By histological criteria the kidneys had generally become extensively damaged before the urea clearance fell to what we have considered as a significantly low range. None of the rats that passed through the acute phase of the experimental nephritis (arbitrarily set at 21 days (2)) became moribund before the 84th day.

In order to study the pathological changes during this intermediary period, seven rats were sacrificed between 30 and 40 days after receiving an amount of nephrotoxic serum adequate to induce severe acute nephritis. There was little divergence in the clinical course or the pathological findings of these animals. The findings in rat N-25 were typical.

Microscopic Examination of Kidney.—Rat N-25. The histological lesions observed in kidneys of rats with the acute disease were present together with certain additional features. Many of the glomerular tufts were distorted; a few had club-shaped loops, and occasionally a significant increase in nuclei was noted in a portion of a tuft. Proliferation of capsular epithelial cells, forming a small crescent, was occasionally encountered. This was usually accompanied by a thickened hyalinized glomerular capsular membrane which was surrounded by lymphocytes and fibroblasts. In several areas the tubular basement membranes were thickened and the interstitial connective tissue was increased. Changes in the arteries were indefinite.

The chronic stage was studied in eleven rats that survived a severe acute nephritis. These animals were followed for periods varying from 84 to 313 days after injection; the average for the group was 208 days. Four of the eleven were killed when moribund. The others looked healthy when sacrificed, although all but one still showed albuminuria and casts. The most significant histological findings in these chronically affected animals were those indicating definite progression of the disease process as shown by glomerular crescent formation and scarring of different ages which occurred in the same microscopic field. In addition, cystic dilatation, atrophy, and hyalin droplet degeneration of the tubules were quite extensive.

Detailed studies are given of several of the eleven animals followed for from 3 to 10 months after treatment.

Rat N-21 received a single injection of 0.25 cc. per 100 gm. body weight of nephrotoxic serum 4138. At autopsy on the 84th day it had some subcutaneous edema and ascites. Another animal, N-36, received a total of 0.3 cc. of the same serum, given in fractional amounts over a period of 7 weeks, and was killed on the 98th day after the last injection, when markedly prostrate and while having intermittent generalized convulsions and muscular tremors. Both of these rats had developed slight edema during the acute phase and showed persistent albuminuria and cylindruria. The terminal blood urea nitrogen determinations were 55 and 97 mg. per cent, respectively. Cultures of the blood and of the kidneys taken at autopsy were negative in both animals; moreover, serum of neither contained agglutinins for *Bacillus enteritidis*. The macroscopic and microscopic descriptions of the organs of the two animals varied in minor respects. The protocol of rat N-36 is given below, since more complete chemical studies were made on the blood and urine of this animal (2).

Autopsy.—Rat N-36, female, weight 128 gm. The kidneys appeared enlarged and weighed 1.1 gm. each. The capsule stripped easily, revealing a pale surface which was roughly granular with many elevations of yellowish tan kidney substance as well as tiny cysts. On section, the cortex was of the normal thickness but was poorly demarcated from the medulla. The pale medulla contained many markedly dilated tubules filled with coagulated material, which in cross section gave a honeycomb appearance, and in longitudinal section a marked radial streaking. The other organs showed no significant macroscopic changes.

Microscopic Examination of the Kidney.—Most of the glomeruli were abnormal. Some had widely dilated glomerular spaces and small tufts, but the majority were large with tufts that almost entirely filled the spaces. The glomerular capillary walls were hyalinized and irregularly thickened; the Mallory-Heidenhain stain again showed this to be due principally to thickening of the intercapillary material. Clubbing of capillary loops occurred, and usually one or more loops appeared to have an increase in nuclei. Pyknosis and karyorrhexis of tuft cells were present but not conspicuous. The basement membrane of the capsular epithelium was generally thickened and hyalinized. The majority of the glomeruli were the seat of varying degrees of crescent formation and scarring. A number showed the same type of tuft changes observed above, with the addition of proliferation of the capsular epithelial cells. Mallory's connective tissue stain failed to show blue fibrils in these particular crescents, while in other glomeruli, usually with more extensive capsular proliferation, an ingrowth of connective tissue fibrils into the crescent was visible. Still other glomeruli were in more advanced stages of scarring; the oldest had completely lost their normal architecture and were represented in the Mallory stained sections by concentric whirls of blue fibrils at the periphery and interlacing fibrils in the center. With Scharlach R stain numerous fat laden cells were visible in most of the glomeruli; those with marked crescent

formation often showed many fat droplets in the epithelial cells of Bowman's capsule as well as in the tuft; and rarely, in the latter position, large masses of fat occurred.

The tubular changes were extensive. The most prominent feature microscopically was the great number of large cyst-like tubules. These were in groups, most abundant in the corticomedullary region; in the cortex they often extended to the capsule, producing elevations. The dilated tubules were lined by flat, or occasionally low cuboidal, epithelium in good state of preservation and filled with hyalin material which took either acidophilic or basophilic stain. Thickening of the basement membranes of these dilated tubules was not demonstrable, nor was there any increase of interstitial connective tissue in these areas.

Another type of atrophic change was frequently met with in groups of convoluted tubules. These were smaller than normal; some had narrow empty lumina, but in most, the lumina were obliterated. The epithelial cells had prominent vesicular nuclei and relatively scanty basophilic cytoplasm. Thickening and hyalinization of the tubular basement membrane was present. Between the tubules was a varying amount of cellular interstitial tissue containing connective tissue cells, lymphocytes, and an occasional eosinophilic polymorphonuclear leucocyte. These scarred areas were scattered throughout the cortex, but were most common near the surface, adjacent to the elevations produced by the cystic tubules.

Finally, enlarged convoluted tubules were seen in nests in the outer cortex or in elongated radial groups in the inner cortex; these were lined by hypertrophic epithelial cells which projected in a dentate fashion into the moderately dilated lumina. The coarse cytoplasmic granules of these cells took a bright pink with methylene blue-eosin and brick red with Mallory's stain. The lumina were empty or loosely filled with pink granular material in which degenerated epithelial cells were sometimes seen. Thickening of the tubular basement membrane was inconstant and never extensive, nor was the interstitial tissue in these areas appreciably increased. Occasionally fat droplets were seen in the epithelial cells, usually in those of the neck of the tubules.

In addition to the alterations already described in the interstitial tissue, numerous eosinophilic polymorphonuclear leucocytes were scattered singly or in loose foci throughout the section. Vascular changes consisted of cellular collections in the adventitia of certain interlobular and arcuate arteries, swelling or pyknosis of nuclei in the media of larger arteries, and some thickening of the walls of arterioles.

Other Organs.—A similar picture was observed in the vessels of other organs, especially in the heart, brain, pancreas, and spleen. The perivascular infiltrations were less frequent and when encountered were much less marked, but the changes in the media of arteries and in the arterioles were about as definite. Several areas of scarring of different ages were present in the myocardium surrounding or adjacent to affected small vessels; moreover, in the cerebral cortex an area of typical encephalomalacia was found. Eosinophilic polymorphonuclears were

observed in all the organs. They were found in the mediastinal tissue, about the pulmonary vessels, and in the splenic pulp with greater than normal frequency. The only other significant finding was a moderate amount of chronic interstitial pneumonitis. Figs. 6 to 11 illustrate lesions in the kidney and heart of this animal.

Rats with even more slowly progressing nephritis, as indicated by a still later decline in the urea clearance, had lesions in the kidney and elsewhere similar to those observed in animals dying after 4 months, but showed more connective tissue replacement.

One such animal (N-39) in the chronic group became moribund 6½ months after the last injection of anti-kidney serum, and another (N-37) 9 months after. These followed much the same clinical course as N-36, and had terminal blood urea values of 72 and 312 mg. per cent respectively. The macroscopic organ changes of N-39 were practically identical with those observed at 3 to 4 months. Rat N-37 had a number of additional findings. The kidneys were more markedly enlarged, weighing 2.1 gm. each and measuring 30 x 15 x 7 mm. The cortex was more granular, with depressed scars approximately 1 mm. deep alternating with areas of kidney tissue several millimeters in width. The heart appeared definitely enlarged, with thickening of the left ventricular wall. Furthermore, the heart and liver gave macroscopic evidence of fatty change, and the testicles were much atrophied.

The microscopic picture of the kidneys of both these animals differed from rat N-36 essentially only in the greater extent of connective tissue replacement. Each of the lesions described in detail as occurring at 4 months was present at 6½ and 9 months. The glomeruli, in general, showed more extensive connective tissue ingrowth into crescents and tufts; still, fields were easily found in which the various stages of the process were represented, *i.e.* (a) thickening of the glomerular capillary basement membrane; (b) proliferation of the epithelial cells of Bowman's capsule with the tuft changes; (c) connective tissue ingrowth into the crescent; and finally (d) conversion of the entire glomerulus into a scar. There was a greater increase in interstitial connective tissue than at 4 months. Focal collections of cells were less frequent as the age increased; moreover, the eosinophilic polymorphonuclear foci had disappeared. Rat N-37, which had a terminal anemia of rapid onset, showed a greenish yellow pigment in the epithelium of the atrophic collapsed tubules in scarred areas, as well as in the spleen both intra- and extracellularly.

Another type of glomerular lesion, seen rarely in animals dying in 3 months, but consistently in those succumbing later, consisted of fibrin-like material in the crescents and tufts. This substance was often plentiful and appeared as thick homogeneous masses or finely granular clumps. It stained as does fibrin with methylene blue-eosin and Mallory connective tissue stains, but with Weigert's technique the appearance was less typical of fibrin. Glomeruli that were ap-

parently heavily laden with fibrin, as judged by the first two stains, showed with Weigert's stain a meager amount of purplish blue material arranged in loose thin strands with very fine blue dots scattered between. The impression was gained that this represented a degenerative change and not true fibrin deposit.

The arterial and arteriolar alterations described in rat N-36 were present in the kidneys of these older rats in about the same degree and severity. The vascular lesions in other organs, however, were more striking. The heart particularly was affected. The coronary arteries showed thickened walls with hyalinization, and in some instances calcium deposit in the media, as well as fraying of the internal elastic membrane. Fatty degeneration of the wall was demonstrable. The arterioles were also thickened. A focal fibrous myocarditis, secondary to the vascular lesions, was observed in rats N-37 and N-39, in all stages from fresh necrosis of groups of muscle fibers to the final complete replacement by fibrous connective tissue. The brain of rat N-37 had an area of encephalomalacia in the cortex, and in addition a large area of gliosis in the midbrain with diminution of ganglion cells. Figs. 12 to 14 depict lesions in the kidney, heart, and pancreas of rat N-39.

Another group of these chronically affected animals showed albuminuria and casts throughout the period of observation, but gave no evidence of progressive disease, such as failure to gain weight, development of hypertension, or significant depression of the urea clearance. These animals, N-22, N-24, N-38, N-41, N-43, N-57, were sacrificed from 190 to 260 days after injection. Most of the kidney abnormalities described in the animals N-39 and N-37 were demonstrable in sections from these rats, but the damage was less extensive, and actively progressing disease was not histologically evident except in rat N-57. This animal had the most markedly involved kidney of any in this group, with lesions almost as severe and as varied as in the severely affected rat N-39; even young glomerular crescents were present. It is probable that had this animal not been sacrificed so soon it would shortly have developed clinical signs of progressive nephritis. Rat N-79, on the other hand, which after going through a severe acute phase to a complete clinical recovery, had an almost normal histological picture when sacrificed 6 months later, and showed only very old scars involving occasional nephrons. In none of these animals was the vascular change outside the kidney of any import, and even in the kidney it was never as notable as in animals with clinically progressive disease. Myocarditis and encephalomalacia were not encountered, nor was there any other significant lesion except a frequent chronic pneumonitis.

DISCUSSION

The present study demonstrates that rats, injected with anti-rat-kidney serum, develop a glomerulonephritis which is, in certain animals, a progressive disease. During the acute phase there is microscopic evidence of injury to both the glomeruli and tubules. The former are enlarged and anemic, due principally to swelling and thickening of the glomerular intercapillary substance. The tubular epithelial cells undergo necrobiotic changes of which hyalin droplet degeneration is the most common. A number of tubules are dilated with hyalin casts. Thrombosis of glomerular capillaries does not result from nephrotoxin alone; but fibrin thrombi are conspicuous in the glomeruli when the effects of a severe anaphylactoid reaction (1) are added to the action of nephrotoxin.

The histopathological lesions of the chronic stage of the pure nephrotoxic nephritis develop gradually and take their origin from those occurring during the acute phase. Proliferation of the cells of Bowman's capsule is followed by connective tissue ingrowth and finally by complete replacement of the glomerulus by scar tissue. In animals with progressive decline of the urea clearance (2) different individual glomeruli are found, in the same microscopic field, with scarring of apparently different duration. The tubules are severely damaged; extensive dilatation with large hyalin casts, atrophy in areas of interstitial scarring, and finally hyperplasia of the remaining functioning units are all present. Generalized vascular lesions with secondary areas of degeneration in various organs occur only in the animals with progressive chronic nephritis.

The chief literature dealing with this type of induced nephritis has already been reviewed (1). While the present investigation discloses no new kidney lesions, it does differentiate the early histological effects of nephrotoxin from those of the non-organ specific tissue antibodies. It traces, in addition, the progressive nature of the chronic nephritis that originates in the acute damage induced by so called nephrotoxin. The fibrin thrombosis of glomerular capillaries, as described by Wilson and Oliver (7), Takeda (8), Masugi (9, 10), and Hemprich (11) in animals dead shortly after receiving anti-kidney serum is, in the case of rats, attributable to factors other than the relatively organ specific antibody nephrotoxin. Moreover, the prolif-

eration of glomerular endothelium in rabbits during the acute phase recorded by certain authors (12, 11, 16) has occurred only occasionally in our hands and then only after massive doses of anti-kidney serum. The data available are insufficient to determine whether the endothelial proliferation is attributable to the nephrotoxic or to the anaphylactoid effect. The acute tubular lesions are similar to those described by others and are considered to be caused by the nephrotoxin. The swelling of the glomerular intercapillary substance in the kidneys of rats with acute nephrotoxic nephritis, brought out so clearly by the Mallory-Heidenhain stain, is apparently the same lesion that Masugi interprets as "condensation of the tuft wall" (9) or as "edema of the capillary wall" (12) and that Weiss (13) considers to be "serous exudate between the capillary walls."

The first convincing evidence of the induction of chronic nephritis with nephrotoxic serum was presented by Masugi (12), although others (14, 15, 8) noted glomerular crescent formation in a few animals observed for several months. Masugi (12) as well as Arnott and his coworkers (16) describe or give photomicrographs of rabbit kidneys, the seat of chronic experimental nephritis, in which individual glomeruli vary in the extent of their scarring; neither author, however, discusses differences in the duration of the disease processes which would connote progression of the nephritis. The development of chronic progressive nephritis is illustrated in the present paper by the records of two rats (N-21 and N-57) which received single injections of nephrotoxic serum, and also by three rats (N-36, N-37, and N-39) each of which received a series of several small injections of the same serum over a period of 7 weeks.

Worthy of special notice were the well marked generalized vascular lesions with areas of secondary degeneration in the heart, brain, and testes of rats with progressive chronic nephritis. That this vascular injury was dependent upon extensive renal damage is indicated by its failure to occur in normal rats of the same age, in rats receiving other antisera, or in rats which failed to develop clinical evidence of progressive nephritis after receiving anti-kidney serum. No mention of this type of generalized vascular lesion has been made by other authors. Cardiac hypertrophy, such as existed in rat N-37, has been occasionally noted by others (14, 12, 11).

A spontaneous glomerulonephritis occurring in rats has been regarded by Jaffé as a focal involvement (17). In our experiments approximately 460 rats have received either anti-rat-kidney serum or an antiserum against other rat tissues. In addition, 50 untreated animals were observed for from 2 months to a year. Among half of this last group, which was kept on a diet of bread and milk, several developed albuminuria and hematuria; at autopsy pyelonephritis was encountered and from the kidneys *B. enteritidis* was isolated. Certain untreated rats, without urinary abnormalities, had focal areas of chronic inflammation and scarring in the kidneys; and from the kidneys of these animals *B. enteritidis* was usually cultured. This type of infectious nephritis was encountered most frequently in the rats maintained on a poor diet and in rats obtained from one particular animal breeder. The black and white hooded strain of rats used in these experiments remained practically free from infectious nephritis when fed an adequate diet. The renal lesions attributable to anti-kidney serum in this communication were distinctly different from those observed in spontaneous infectious nephritis.

The clinical and functional studies recorded in the previous paper of this series (2) and the histopathological observations presented here indicate that a genuine diffuse glomerulonephritis is induced in rats by the injection of anti-rat-kidney serum, and that this experimental disease simulates, in most respects, glomerulonephritis in man. At present, however, it is hazardous to postulate that the mechanism responsible for nephrotoxic nephritis in laboratory animals is closely related to the etiology of human nephritis, and it is also unprofitable to compare too minutely the pathological changes in the acute experimental nephritis of laboratory animals with the lesions of the early human disease. Nevertheless, the close approximation of these two diseases offers an opportunity to obtain certain information from studies in animals that may be applicable to the clinically similar disease of man.

CONCLUSIONS

Administration of the relatively organ specific antibody, so called nephrotoxin, present in anti-kidney serum, is followed by a diffuse glomerulonephritis. This is characterized early by swelling of the

intercapillary substance of the glomerular tuft and by tubular degeneration. Fibrin thrombi are only present in the glomerular capillaries when the injection of anti-kidney serum results in a severe anaphylactoid reaction, and are due to factors other than nephrotoxin.

The urinary abnormalities which develop in all rats after a suitable injection of nephrotoxin usually continue until the animal dies or is sacrificed. Microscopic renal lesions of the early phase merge into scarring of the glomeruli and tubules. Histological study of those animals which die from 3 to 11 months after treatment reveals a chronic progressive glomerulonephritis with generalized vascular lesions.

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EXPLANATION OF PLATES

PLATE 20

FIG. 1. Rat N-34, male, weighing 87 gm., sacrificed 4 days after receiving 0.25 cc. per 100 gm. body weight, anti-rat-kidney serum (from rabbit 4138). Corticomedullary region. Methylene blue-eosin stain. The convoluted tubules about the glomerulus show hyalin droplet degeneration. Other tubules are dilated and have flattened epithelium; some have empty lumina while others are filled by hyalin casts or debris. Occasional epithelial cells are necrotic. $\times 125$.

FIG. 2. Rat N-34. Glomerulus. Methylene blue-eosin stain. The glomerular capillaries are almost free of erythrocytes, yet the tuft is large, due to thickening of the glomerular capillary walls and, to some extent, swelling of the tuft nuclei. $\times 450$.

FIG. 3. Rat N-34. Glomerulus. Mallory-Heidenhain stain. The deeply staining glomerular intercapillary substance or capillary basement membrane is clearly demonstrated. This thickening varies in different loops. $\times 450$.

FIG. 4. Rat N-74, female, weighing 108 gm., received 0.65 cc. per 100 gm. body weight of nephrotoxic serum (No. 4138) given in four divided doses at 3 day intervals. Animal died on 16th day. Glomerulus. Mallory-Heidenhain stain. The swelling of the glomerular capillary basement membrane is greater and more uniform than that in Fig. 3. $\times 450$.

FIG. 5. Rat N-18, male, weighing 106 gm., severe anaphylactoid reaction after receiving a single injection of 0.5 cc. per 100 gm. body weight of nephrotoxic serum (No. 4138). Died on 8th day. Glomeruli. Methylene blue-eosin stain. One glomerulus has numerous fibrin thrombi in the tuft capillaries, and cells in portions of loops are degenerated. The other glomerulus resembles Fig. 2. $\times 450$.

PLATE 21

FIG. 6. Rat N-36, received a total of 0.3 cc. per 100 gm. body weight of serum 4138 in four divided injections over a period of 7 weeks. Moribund 98 days after last injection. Corticomedullary region. Mallory connective tissue stain. Extensive tubular destruction of various types and interstitial scarring are evident. $\times 48$.

FIG. 7. Rat N-36. Cortex. Mallory-Heidenhain stain. Glomeruli in different stages of scarring are present. The several types of tubular lesions described in the text are illustrated. $\times 125$.

FIG. 8. Rat N-36. Glomerulus. Mallory-Heidenhain stain. A large glomerulus is shown with distorted tuft, irregularly thickened glomerular capillary basement membranes, and proliferated capsular epithelium into which connective tissue is beginning to infiltrate. $\times 450$.

FIG. 9. Rat N-36. Heart. Methylene blue-eosin stain. The upper vessel shows thickened and increased cellularity of the media and a loose infiltration of the cells in the adventitia. $\times 400$.

PLATE 22

FIG. 10. Rat N-36. Kidney. Scharlach R-hematoxylin stain, frozen section. Two glomeruli have dark staining masses representing fat, while a third tuft contains no fat. $\times 110$.

FIG. 11. Rat N-36. Heart. Scharlach R-hematoxylin stain, frozen section. The dark areas in the media of the coronary vessel represent fat. $\times 110$.

FIG. 12. Rat N-39. Received the same treatment as rat N-36, but became moribund 204 days after the last injection of anti-kidney serum. Heart. Meth-

ylene blue-eosin stain. The dark material in the muscle coat of the medium sized branch of the coronary artery is calcium. Scarring is present about the vessel and in a neighboring area of myocardium. Thickened arterioles are observed in the focus of fibrous myocarditis. $\times 195$.

FIG. 13. Rat N-39. Pancreas. Methylene blue-eosin stain. Changes in and about two small arteries are illustrated. A somewhat thickened arteriole lies below them. $\times 210$.

FIG. 14. Rat N-39. Kidney cortex. Mallory-Heidenhain stain. Connective tissue replacement is more extensive here than in Fig. 7. $\times 240$.

Photographed by Louis Schmidt

(Smadel: Nephritis induced by anti-kidney serum, III)

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