EXPERIMENTAL NEPHRITIS IN RATS INDUCED BY INJECTION OF ANTIKIDNEY SERUM

V. CHRONIC NEPHRITIS OF INSIDIOUS DEVELOPMENT FOLLOWING APPARENT RECOVERY FROM ACUTE NEPHROTOXIC NEPHRITIS

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Plates 14 and 15

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Previous experiments have demonstrated that the acute glomerulonephritis induced in rats by the injection of anti-rat-kidney serum prepared in rabbits will, under proper conditions, develop into a chronic progressive disease with ultimate renal failure and death (1). Early in the work we believed that the principal factor influencing the development of chronic progressive nephritis was the quantity of nephrotoxin administered. Thus, the injection of an appreciable quantity of a weakly nephrotoxic serum, or of a small amount of a more potent antikidney serum, resulted in a mild nephritis that rapidly subsided. Continued renal irritation was originally observed only in rats that had survived a severe acute disease induced by sublethal amounts of nephrotoxin. Later, however, it was shown that the course of the nephritis could be markedly influenced by diet (2). For example, almost all of the rats fed a basal diet after receiving adequate amounts of nephrotoxin developed chronic nephritis and the majority died within a year with renal insufficiency. On the other hand, the feeding of a high protein diet to similarly injected animals uniformly resulted in progressive kidney involvement; and all but a few died within a matter of months. Finally, rats fed a low protein diet almost invariably recovered promptly from the acute process. More recently (3), an additional factor, i.e., one inherent to differing degrees in certain inbred lines of rats, was found to affect the course of the induced disease. The purpose of the present study was to amplify the observations on the role of heredity in the experimental glomerulonephritis that follows injection of antikidney serum.

Materials and Methods

Strains of Rats.—Whelan Strain: This inbred strain of animals was used exclusively throughout the earlier studies. Its origin remains obscure. The animals, practi-
cally all of which are hooded, were raised as pets for a number of generations. They were healthy, easy to handle, free of scabies and had a low incidence of infection with *Salmonella muris*; in the beginning, these were the principal reasons for employing this strain of rats.

**Long-Evans Strain:** Rats of this breed were descended from animals left at the Institute by Dr. Herbert M. Evans. These animals, like the Whelan rats, were generally hooded, but the adults were definitely heavier and of greater stature than Whelan animals.

**Wistar Strain:** Animals employed in this work were raised from a group of rats of the “Experimental Colony Strain” obtained from the Wistar Institute in 1937.

**Anti-Rat-Kidney Serum.**—Nephrotoxic serum was obtained from rabbits immunized with suspensions of perfused rat kidney (4). Antiserum pool I was prepared by injecting renal tissue from animals of the Whelan strain and was administered intravenously to rats of the three strains when they attained a fasting body weight of 60 to 80 gm.

**Feeding and Care of Rats.**—Two isocaloric diets were employed. These have been previously described in detail and designated basal and high protein diets (2). They contained, respectively, 18 and 40 per cent of protein and 51 and 29 per cent of carbohydrate; fats, minerals and vitamins were present in each diet mixture in similar amounts. Animals were kept in individual glass cages with food and water constantly available. The methods used for collecting and examining urine have been described in earlier publications. Rats were weighed and their urine specimens were analysed on alternate days during the first few weeks after injection of antikidney serum; subsequently these data were collected once or twice a week.

Rats were sacrificed when moribund or at the end of designated periods of observation. Organs were fixed in acetic Zenker’s solution and paraffin sections prepared and stained by the usual methods (1 b).

**EXPERIMENTAL**

Throughout most of our work on nephrotoxic nephritis, rats of the Whelan strain have been employed. However, in recently reported experiments (3), animals of the Whelan and Evans strains were injected with comparable amounts of anti-rat-kidney serum. Evans rats, in contrast to the Whelan animals, showed a marked tendency to recover from the acute renal injury even when maintained on a high protein diet. This unforeseen development, in an experiment designed for the elucidation of another point and terminated at the end of 3 months, seemed to warrant further investigation.

1 Dr. Evans, in a personal communication, informs us that this strain of rats originated in 1910 in the laboratory of Professor Joseph A. Long of the Department of Zoology, University of California, Berkeley. Two albinous females of unknown origin were mated with a wild gray male rat which was trapped in the vicinity and probably was of the Norwegian strain. Brother-sister inbreeding of the two litters which resulted gave the Long-Evans strain. Throughout this paper we shall designate these animals as Evans rats.
Course of Nephritis in Rats of the Whelan, Evans and Wistar Strains.—The response of rats of three different inbred lines to injections of nephrotoxin and the subsequent course of the resultant nephritis were studied in the following experiment.

Groups of twelve young rats, of comparable weight and sex, of the Whelan, Evans and Wistar strains, respectively, were injected with a total of 1.0 cc. of antikidney serum, pool I, per 100 gm. body weight, in divided doses over a period of 3 days. This antiserum, which had been prepared by immunizing rabbits with renal tissue from Whelan rats, was capable of inducing moderately severe acute nephritis in Whelan animals injected in this manner. One-half of the members of each group was fed a basal diet, the remainder received a high protein diet. Observations were continued for 14 months when the surviving rats were sacrificed. One of the Whelan animals was discarded because of improper injection of nephrotoxin; a Wistar rat, which died following an accident on the 90th day of observation, is not included in the group. A difference in the severity of the chronic nephritis was noted in animals of the Whelan strain fed the basal and high protein diet. However, the response of rats of the other two strains was similar on both diets. For simplicity, the data presented in Text-fig. 1 have been arranged to portray graphically the average excretion of protein and of casts by animals of each of the three strains without regard to diet.

The acute nephritis induced in Whelan rats of the present group was somewhat less severe than that observed earlier in animals of this strain that received a smaller amount of a more potent antiserum (2). None of the eleven Whelan animals died from renal failure associated with the acute nephritis but all developed anasarca during the 1st week after injection. The milder degree of initial renal injury undoubtedly contributed to the prolonged course of the chronic nephritis, e.g., six of the eleven Whelan rats lived through the 14 months of the experiment and these survivors were evenly divided between the two diet groups. Albuminuria and cylindruria, which were 4+ during the week following injection of nephrotoxin, diminished thereafter but remained between 3+ and 2+ throughout the period of observation (Text-fig. 1). None of the animals recovered permanently: One rat in the basal group excreted normal urine between the 2nd and 4th months but during the remaining months had urinary changes indicative of low grade to moderate renal involvement. Five rats died with chronic nephritis; their average life span after injection was 8 months.

Rats of the Evans strain responded to nephrotoxin with an acute renal injury almost as severe as that of Whelan rats; excretion of albumin and casts was comparable in the two groups but anasarca was not observed among the Evans animals. Moreover, urinary abnormalities rapidly diminished after the 2nd week in members of this group. Indeed, from the 30th to the 70th days, the urines of the majority of the rats were free of albumin and casts, and in the remainder only relatively slight amounts of these constituents were
Following this period of apparent recovery or of low grade nephritic activity, one after another of these rats began to excrete progressively increasing amounts of albumin and casts. By the end of the 6th month, the average urinary abnormalities of the Evans group were comparable to those of the Whelan group; this was in spite of the fact that two animals recovered shortly after injection and remained free of signs of nephritis thereafter. Only two of the Evans rats died with chronic nephritis; these animals, a male and a female were both fed a basal diet (see Text-fig. 1).

Wistar animals behaved even more atypically than Evans rats in their response to nephrotoxin when compared with the behavior of members of the Whelan strain as a standard. In the first place, the excretion of protein and formed elements during the 1st week after injection, while marked, was less intense than in the other two groups. Furthermore, subcutaneous edema was not observed during the acute phase of the induced nephritis. Finally, the urinary abnormalities diminished so rapidly during the 1st month after injections that at the end of this period, half the animals had normal urines and the remainder excreted only traces of albumin and a few casts. During the 3rd month, eight of the animals were considered to have recovered completely.
while two were regarded as being in a latent phase; the final animal regularly
excreted small amounts of albumin but no casts during this period. The two
rats with latent nephritis gave evidence of increasing renal irritation during
the 4th month. Furthermore, like the Evans rats, the Wistar rats which had
been considered cured also developed recurrences, until by the end of the 7th
month all but two of the animals were suffering from active chronic nephritis.
The two rats just mentioned remained normal throughout the remainder of the
period of observation. Recurrence of kidney disease, once established, pro-
gressed slowly but unremittingly (Text-fig. 1). The general appearance of the
rats remained good and none died during the 14 months of study.

Rats of the Evans and Wistar strains irrespective of the diet consumed,
followed the same general course: a rather rapid amelioration of the acute neph-
ritis followed by a period of several weeks or months during which the nephritis
appeared to be cured or in a state of latency; and finally, a recrudescence of
renal disease which was slowly progressive. It may be mentioned, although
the interpretation is not immediately obvious, that the two Evans and two
Wistar rats (a male and a female of each strain), which recovered without re-
currence of nephritis were all fed a high protein diet. Furthermore, recurrences
appeared somewhat later and were slightly less severe in the animals fed a high
protein diet. This is in contrast to the observation on Whelan rats in which
signs of renal injury were greater in the animals given a high protein diet.

Nephritis in Rats of the Wistar and Whelan Strains Induced by Anti-Wistar-
Rat-Kidney Serum.—Observations
on the severity of acute nephrotoxic ne-
phritis and the apparent recovery from the initial disease which are recorded
in the previous section are subject to at least two interpretations. In the first
place, animals of the several inbred lines may differ in their susceptibility to
nephrotoxin and in their tendency to recover from its injurious effects because
of inherited characteristics. On the other hand, the variable responses might
depend primarily upon differences in the effective potency of the nephrotoxin
rather than upon the susceptibility of the animals. For example, if the renal
tissues of each inbred line were of slightly different antigenic structure, then
the nephrotoxic antibody obtained by immunization with tissue from one line
might be most active against the kidneys of animals of the homologous strain.
This idea was investigated by preparing anti-Wistar-rat-kidney serum and
observing its effect on animals of the Wistar and Whelan strains.

Preliminary titrations indicated that antiserum 4733, which was prepared by im-
munizing rabbits with suspensions of perfused kidney tissue from Wistar rats, in-
duced only a mild and transient renal disease in young rats of the Wistar strain that
received 1.0 cc. per 100 gm. of body weight, whereas, the administration of 1.4 cc.
was followed by severe kidney damage. Groups of twelve Wistar and Whelan rats
of comparable age, weight and sex were injected with antiserum 4733 in the following
manner: twelve Wistar rats received intravenously on consecutive days, 0.2, 0.4 and
0.8 cc. of antiserum per 100 gm. body weight; six Whelan rats were similarly treated; and finally, six other Whelan rats were injected with 0.2, 0.4 and 0.4 cc. of antiserum per 100 gm. body weight during the same period. All the animals were fed a high protein diet. Survivors were observed in the usual manner for 3 months. The degree of albuminuria displayed by individual animals in the Wistar group is graphically portrayed in Text-fig. 2. The average excretions of protein and formed elements by members of the first and last groups are presented in Text-fig. 3.

All animals of the Wistar strain receiving 1.4 cc. amounts of serum 4733 developed severe acute nephritis. On the day following the third injection of antikidney serum marked oliguria was observed; the urines coagulated solidly when heated with dilute acetic acid or when mixed with 10 per cent trichloroacetic acid, however, they contained only a few epithelial cells and cellular casts and failed to give a positive guaiac test. During the ensuing week
the degree of proteinuria diminished slightly but all the rats developed marked cylindruria and anasarca. Three animals died of renal failure during the first 2 weeks. The clinical course of the induced disease in surviving animals was irregular, as is apparent from the data presented in Text-fig. 2. Two rats displayed signs of severe renal involvement throughout the period of observation. Three animals improved considerably after the 1st month but continued to show evidence of moderate kidney disease until sacrificed. Three rats had apparently recovered completely by the end of the 1st month. The final animal in the group improved rapidly but intermittently excreted small amounts of albumin and casts. In this group of Wistar animals that survived the acute injury, sex apparently influenced the course of nephritis for all of the males.

![Urinary abnormalities shown by two strains of rats after injection of anti-Wistar-rat-kidney serum](image)

Text-Fig. 3

developed severe or moderate chronic nephritis while three of the four females apparently recovered and the other presented only slight urinary abnormalities during the latter part of the experiment. It is doubtful whether any significance can be attached to the observation that two females, but only one male, succumbed with acute nephritis.

The same dose of nephrotoxin 4733 that had been administered to the Wistar rats, i.e., 1.4 cc. per 100 gm. body weight, was uniformly lethal for members of the Whelan strain: all animals developed severe nephritis and succumbed between the 6th and 12th days following the third injection of antikidney serum. Even the injection of 1.0 cc. of this serum in Whelan animals resulted in nephritis of such severity that two of the six rats died during the 2nd week. Each of the surviving animals continued to excrete highly abnormal urine throughout the 3½ months of observation. The results of urinalysis, summarized in Text-fig. 3, indicate the severity of the chronic nephritis in this group of animals and bring into contrast the degree of average involvement in Whelan rats receiving
1.0 cc. of nephrotoxin with that of Wistar animals injected with a larger amount of antiserum.

It is apparent from the evidence so far presented that the difference in intensity of acute nephrotoxic nephritis induced in animals of the Wistar and Whelan strains by a given dose of antikidney serum is dependent primarily on the relatively different vulnerability of the kidneys of members of each inbred line and not on strain specificity of the nephrotoxin. The experiment brings out an additional fact, namely, the difference in the ability of rats of the two lines to recover, temporarily at least, from the injury induced by nephrotoxin. Thus, Wistar rats with severe acute nephritis tended to recover even while being fed a high protein diet. Whelan animals with a correspondingly intense acute nephritis have never recovered while being maintained on a high protein diet. Finally, for the first time in our experience with nephrotoxic nephritis, sex appears to have had a consistent influence on the course of the disease. It is evident from the data presented in Text-fig. 2, that female Wistar rats recovered more promptly and completely from the acute injury than did male Wistar rats.

Pathological Changes in the Kidneys of Different Strains of Rats.—Histopathological lesions observed in sections of kidney tissue from Whelan rats used in the current experiments were similar to those described earlier (1, 2). Moreover, essentially identical renal lesions were encountered in Wistar and Whelan rats that died of acute nephritis 1 to 2 weeks after injection of anti-Wistar-rat-kidney serum. In animals of both strains, the glomerular tufts were comparatively large but relatively anemic: marked uniform thickening of the glomerular capillary basement membranes was mainly responsible for the enlargement; in addition, some increase in number of endothelial nuclei and swelling of epithelial cells contributed to the size of the tufts. Tubules observed in the kidneys of Whelan and Wistar rats with acute nephritis were moderately dilated; their lumina contained albuminous material and their epithelial cells showed various stages of degeneration. Fig. 1 illustrates the typical severe acute renal lesion in a Wistar rat.

The renal lesions encountered in Wistar rats, Nos. 1 and 2, that showed clinical signs of moderately severe nephritis throughout the 3 months of observation, were practically indistinguishable from those of the four Whelan rats that received the same anti-Wistar-kidney serum and survived the 3 months period of the experiment (Figs. 4 and 5). Sections from these animals showed extensive glomerular and tubular lesions. Varying degrees of distortion and scarring of the glomerular tufts were brought about by irregularly thickened glomerular capillary basement membranes, which were not infrequently frayed and sometimes had given rise to intracapillary fibrillae; distortion had also been induced by ingrowth of connective tissue cells from the stalk or from crescents that had partially obliterated the capsular space.
Groups of tubules with thickened basement membrane, some with collapsed lumina and others with dilated empty lumina, were enmeshed in connective tissue which often contained foci of lymphocytes. Still other tubules were moderately dilated and filled with precipitated protein, or occasionally with hyalin casts. Hypertrophy of epithelium such as occurs in normal rats fed a high protein diet was present in many tubules but, here and there in a proximal segment, hyperplasia of epithelial cells had resulted in partial or complete occlusion; frequently epithelial cells in such areas had undergone necrobiotic changes and occasionally were even necrotic.

Of special interest were the histological findings in the kidneys of Wistar rats that had apparently recovered from the effects of anti-Wistar-kidney serum and that were sacrificed 3½ months after injection. Examination under low magnification revealed few abnormalities except a rare renal unit which was fibrotic (Fig. 3). Residual glomerular changes were discernible, however, in all of the Malpighian bodies in sections stained by the Mallory-Heidenhain technique and examined under higher magnification. These were uniform moderate dilatation of glomerular capillaries and widely distributed irregular thickenings of moderate degree of the capillary basement membranes (Fig. 2). Usually each tuft contained one or more strands of new formed connective tissue which had grown into the lobules from the stalk. Occasionally slight thickening of the capsular basement membrane was observed; and rarely adhesions joined the tuft and capsular epithelium, but crescent formations were not seen. None of these glomerular changes was conspicuous in sections stained with hematoxylin and eosin. Some hypertrophy of tubular elements was encountered such as is characteristically present when the high protein diet is fed. It may also be pointed out that lesions similar to those just described were also found in the renal glomerular tufts of Evans rats which had made a complete or nearly complete clinical recovery while consuming either high or low protein diets, and which were sacrificed 3 months after receiving markedly toxic doses of antikidney serum (3). In brief, clinical recovery from nephrotoxic nephritis, whether occurring in Whelan or Evans rats fed a low protein diet (2, 3) or in Evans (3) or Wistar rats maintained on a high protein diet, was characterized histologically by almost complete return to normal of tubular structures but by the persistence of definite though minor glomerular fibrosis.

Histopathological studies on Whelan, Evans and Wistar animals that survived 14 months after receiving anti-Whelan-rat-kidney serum contributed relatively little to an understanding of the pathogenesis of the recurring nephritis which developed after a period of apparent latency or recovery. The renal lesions in rats of all three strains with chronic nephritis were so alike that it was often impossible to tell from which group a given section had originated. In general, however, the pathological process was more severe in members of the Whelan line (compare Figs. 7 to 9); this was not unexpected, since the dis-
ease terminated fatally in almost half the animals in this group. It is also apparent from Figs. 11 to 13, that while qualitatively the glomerular and tubular scarring were similar in animals of the three groups, the number of completely fibrosed glomeruli and the amount of crescent formation were slightly greater in the Whelan rats. It seems reasonable to assume that the chronic nephritis observed 11 to 14 months after injection of antikidney serum represents the late result of a progressing disease of the type observed in rats 3 months after injection and illustrated in Figs. 4 and 5. Nevertheless, it is evident from the protocols that the animals whose kidneys are illustrated in Figs. 11 and 12 excreted essentially normal urines 3 months after injection; hence they may be considered to have had at that time mild lesions corresponding to those illustrated in Figs. 2 and 3. Is it not possible that the comparatively mild damage present in kidneys that have apparently recovered on clinical grounds may at a future time serve as a basis for the development of a slowly progressing lesion? Indeed, the glomerular changes did increase in intensity even in those Evans and Wistar rats that remained clinically free of disease for as long as a year after recovery. It is obvious that the amount of intraglomerular scarring is greater in Fig. 10 (14 months) than in Fig. 2 (3 months). In fact, the tuft lesions observed in the former are almost as severe as those found in many of the Malpighian bodies of rats with clinically demonstrable chronic progressive nephritis sacrificed 3 months after injection (Figs. 4 and 5); nevertheless, significant tubular changes are not seen (Figs. 6 and 10).

DISCUSSION

The various types of renal disease that are caused directly by antikidney serum or that take their origin from the injury induced by nephrotoxin are about as manifold as are those types grouped under the term "Bright's disease," in man. For, in addition to an acute glomerulonephritis which can be directed toward either permanent clinical recovery or progressive chronic nephritis terminating in uremia (2), we have also induced acute renal injury which quickly subsides, as shown by urinary examination, leaving the rats apparently physically normal and with only slight residual histopathological changes in their kidneys. Nevertheless, after a few months time, animals of this latter sort develop by insidious onset a chronic nephritis which is slowly progressing. These several forms of the experimental disease have been obtained by varying two factors; namely, heredity (Whelan, Evans and Wistar strains of rats) and diet (low, basal and high protein diets). There is some indication in the present work that sex also may influence the course of the disease.

It seems justifiable in light of the present experiments to differentiate between two phases in the response of animals of the several inbred lines to neph-
rotoxin. These are, first, relative susceptibility to nephrotoxic injury and, second, comparative capacity to recover from such an induced injury. The order of increasing susceptibility of the three strains to nephrotoxin was Wistar, Evans and Whelan, with only a slight difference between the last two, but an appreciable disparity between them and the Wistar strain. Similarly, the decreasing order of the three as regards their capacity to recover from the initial nephrotoxic trauma was Wistar, Evans and Whelan. Thus, under similar environmental conditions, Whelan rats were most susceptible to nephrotoxic injury and least capable of repairing the damage once it was established; the converse was true for Wistar rats. It would appear that both aspects of response to nephrotoxin, i.e., vulnerability and capacity to recover are conditioned by some trait inherited by rats of different inbred lines. Furthermore, the latter aspect can be influenced by an environmental condition such as diet; or rather by the comparative amount of functional injury that probably results from varying diet. Whether relative vulnerability to nephrotoxin can also be altered by diet has not been studied. The importance of inherited factors in affecting resistance is well established from work with cancer and with infectious agents. Furthermore, Chase has recently shown that susceptibility to sensitizing chemical agents is also affected by heredity (5). The influence of heredity on the capacity of damaged tissue to undergo restoration is not so clearly understood. It is interesting to recall the experiments of MacNider (6) which show a divergent susceptibility to injury of tissues of animals of different age groups and which, furthermore, demonstrate the tendency of young animals to replace injured cells with normal ones, whereas older animals either fail to initiate repair or restore the structure with atypical cells.

The period of recovery from acute nephritis followed by the gradual development of chronic nephritis in Evans and Wistar rats fed a basal or high protein diet suggests strongly that different mechanisms may be responsible for the acute and chronic renal disease that develop after injection of antikidney serum. It was and still is difficult to understand how antibody from a heterologous species, i.e., nephrotoxic rabbit serum, could persist in the rat's body for 3 to 12 months and contribute to the progression or recurrence of renal disease. Because of this, we spoke in an earlier paper (1b) of "chronic nephritis that originates in the acute damage induced—by nephrotoxin" and merely said that "renal lesions of the early phase merge into scarring of the glomeruli and tubules." It seems probable that nephrotoxin causes only the acute renal damage and that subsequent pathological changes (except simple connective tissue replacement of structures totally destroyed by acute injury and some residual thickening of glomerular membranes) arise as a result of a slightly diseased organ attempting to function under conditions which are unfavorable. If such is the case, then it might be expected that in Whelan rats which are most susceptible to nephrotoxin and least able to recover from its effects, the
two processes responsible for the acute and chronic nephritis respectively might so overlap that the limits of each become indistinguishable. In Evans and Wistar animals, on the other hand, the two processes seemed to be separated by an interval of apparent clinical recovery, and hence each process is clearly distinguishable from the other.

A question of considerable interest is concerned with the fundamental damage caused by acute nephrotoxic nephritis, which usually leads sooner or later to the development of chronic nephritis. There is ample histopathological evidence of slight scar formation in the glomerular capillaries of most rats that have suffered from the acute disease. Moreover, this small amount of scarring in each glomerulus multiplied by the total number of glomeruli undoubtedly results in a diminution in total functioning glomerular tissue, notwithstanding the fact that function, as estimated by urea clearance, may be normal during the period of recovery (2). In this connection one might recall the opinions of Medlar and Blatherwick (7), whose description of dietary nephritis in partially nephrectomized Wistar rats conforms closely on clinical and pathological grounds with our observations of recurrent nephritis in Evans and Wistar rats. These authors have suggested that the development of progressive nephritis "hinges on the production of irreparable damage to the filter bed of the glomerulus—and that the etiological factors initiating this primal damage may be multiple and diverse in character." In agreement with this point of view is the fact that practically all histopathological alterations which are detectable in the kidneys of Evans and Wistar rats during the period of clinical recovery from acute nephrotoxic nephritis are limited to the glomerular filter bed. It is indeed tempting to assume that subsequent progressive glomerular lesions develop entirely from such unique areas of lowered resistance and that the tubular lesions evolve secondarily.

While the importance of the changes in the glomerular capillaries should not be underestimated, other possibilities must be considered. For example, similar glomerular tuft changes have also been found in several instances in which clinical recovery has been complete for a year or more, i.e., in a few Evans and Wistar rats that failed to develop chronic recurring nephritis while being fed high or basal protein diets and in those Whelan animals which were maintained on a low protein diet (2). Therefore, it would seem that the pathogenesis of this type of secondary chronic nephritis may depend in part on the response of some other tissue, probably the tubular structures. That tubular lesions may differ in character even in the presence of comparable glomerular scarring has already been observed in Whelan rats with unremitting progressive nephritis (2). In these animals, maintained on a basal protein diet, the distal convoluted tubule was more obviously involved than the proximal, whereas in those Whelan rats fed a high protein diet the converse was true. Furthermore, the recent work of Addis (8) with rats fed high or low protein diets indi-
cates that the amount of osmotic work required to be performed by the tubules in reabsorbing water during the concentration of glomerular filtrate strikingly influences the degree of compensatory renal hypertrophy that follows partial nephrectomy; this varying amount of work also markedly affects the course of an established nephritis of the type that develops in rats following subtotal nephrectomy.

Therefore, it appears that evolution of the chronic nephritis which develops in rats subsequent to recovery from acute nephrotoxic nephritis depends not alone on residual scarring in the glomerular filter bed, but also, on excessive or abnormal stresses and strains to which the tubules are subjected either as a direct result of glomerular damage or because the tubules are forced to function constantly in an abnormal manner.

SUMMARY

1. Three different inbred lines of rats were found to vary in their response to antikidney serum: rats of the Whelan strain were most susceptible to nephrotoxin and most prone to develop chronic glomerulonephritis immediately following the acute injury induced by this agent; animals of the Evans strain were almost as vulnerable to the acute effects of nephrotoxin; Wistar rats were the least affected.

2. Both Evans and Wistar rats usually recovered quickly from the acute injury, and between the 2nd and 5th months after injection they excreted normal or only slightly abnormal urines. During this period of absence of clinical signs of disease, histopathological examination of their kidneys revealed only minor scarring in the glomerular tufts.

3. Most of these apparently recovered rats subsequently developed a slowly progressing chronic glomerulonephritis irrespective of whether they were fed a basal or high protein diet.

4. Histopathologically similar renal lesions were present in all three strains of rats with active chronic nephritis regardless of whether the chronic disease followed immediately the acute nephrotoxic injury or was separated from it by an interval of months. These lesions were somewhat more severe, however, in Whelan rats.

5. Some intraglomerular scarring was present in the kidneys of all rats which survived acute nephrotoxic nephritis. It was especially prominent in those animals that remained clinically cured for as long as a year.

6. The permanent clinical recovery of certain animals, which were found to have moderate glomerular fibrosis on postmortem examination, suggests that factors other than this residual scarring contributed to the development of the recurrent nephritis observed in most of the Evans and Wistar rats. These unknown factors may produce varying degrees of renal functional trauma affecting both glomeruli and tubules.
BIBLIOGRAPHY


EXPLANATION OF PLATES

PLATE 14

Fig. 1. Kidney of Wistar rat, No. 10 in Text-fig. 2, which died 10 days after initial injection of anti-Wistar-rat-kidney serum. Proteinuria 2.7 to 5.1 per cent with numerous casts of all types found on urine analysis. Anasarca present at post-mortem examination. Glomerular capillary basement membranes are greatly swollen. Tubules are slightly dilated with granular material in lumina and with epithelium in various stages of degeneration. Mallory-Heidenhain stain. × 400.

Fig. 2. Kidney of Wistar rat, No. 8 in Text-fig. 2, which was sacrificed 3½ months after injection. Average proteinuria per 18 hours was 121 mg. during 1st month and 7 mg. during final 2 months. Irregularly thickened capillary basement membranes and several thick strands of connective tissue are distinguishable in the large tuft. Contrast with uniform extensive thickening of capillary basement membranes and lack of connective ingrowth in Fig. 1. Mallory-Heidenhain stain. × 300.

Fig. 3. A lower magnification of the slide illustrated in Fig. 2. Glomerular changes are less apparent at this power. Tubular lesions are minimal. × 60.

Fig. 4. Kidney of male Whelan rat which received a total of 1.0 cc. of anti-Wistar-rat-kidney serum and was sacrificed 3 months later. Average proteinuria for entire period was 56 mg. per 18 hours. Scarring in varying amounts is present in all the glomeruli. Nests of atrophic tubules with thickened basement membranes surrounded by fibrous tissue are present but most tubules are slightly dilated and lined by hypertrophic epithelial cells. Mallory-Heidenhain stain. × 85.

Fig. 5. Kidney of Wistar rat, No. 2 in Text-fig. 2, which was sacrificed 3 months after injections of nephrotoxin. Average proteinuria for entire period was 73 mg. per 18 hours. The lesions are similar to those illustrated in Fig. 4. Mallory-Heidenhain stain. × 85.
PLATE 15

Fig. 6. Kidney of female Wistar rat, T-28. Three injections of antikidney serum were administered on successive days. Proteinuria of 75 mg. per 18 hours occurred during 1st week, 7 mg. during 2nd week, and less than 1 mg. thereafter. Numerous casts were found in 1st week, they declined during next 3 weeks and were absent subsequently. Animal was fed a high protein diet and sacrificed 14 months after injections. Few abnormalities can be seen at this power. Mallory-Heidenhain stain. × 20.

Fig. 7. Kidney of female Wistar rat, T-27. Injection period and 1st week similar to rat T-28. Urinary abnormalities subsided more slowly but by 5th week animal excreted only 4 mg. of protein per 18 hours and no casts were found. Urine analyses revealed normal findings throughout 2nd and 3rd months. Average proteinuria during 4th month was 7.5 mg. and rare hyalin casts were seen. Slow steady increase in both constituents was as follows: 6th month, 30 mg. and a few casts; 8th month, 55 mg. and numerous hyalin, granular and cellular casts; 10th month, 75 mg. and numerous casts; 12th month, 71 mg. and numerous casts; 14th month, 130 mg. and numerous casts. Animal was fed a high protein diet and sacrificed during the 14th month. Some distortion of glomerular tufts and scarring of tubules are discernible. Several dilated tubules contain large hyalin casts. Mallory-Heidenhain stain. × 20.

Fig. 8. Kidney of male Evans rat, T-32. During the 2 weeks following injections of nephrotoxin this animal excreted overnight, 1 to 2 cc. of urine which contained between 3 and 4 per cent protein, average 49 mg. per 18 hours. The disease subsided rapidly; average proteinuria per 18 hours was 8.5 mg. for 2nd month and only rare casts were observed. Toward end of 3rd month, protein and casts increased. Average proteinuria in succeeding months was: 4th month, 26 mg.; 6th, 40 mg.; 8th, 86 mg.; 10th, 108 mg.; 12th, 101 mg.; 14th, 120 mg. The number of casts was generally proportional to the degree of proteinuria. The rat was maintained on a basal protein diet and sacrificed at the end of the 14th month. Histological changes are similar to those shown in Fig. 7. Mallory-Heidenhain stain. × 20.

Fig. 9. Kidney of male Whelan rat T-43. 1 to 1.5 cc. of urine was excreted per 18 hours during the 1st week after the series of injections of nephrotoxin; it contained 2.3 to 5.1 per cent protein (average excretion per 18 hours 41 mg.) and very many casts. An increase in urine volume with only moderate reduction in proteinuria during the remainder of 1st month raised the average 18 hour excretion for the period to 83 mg. 69 mg. per 18 hours was the average excretion for the ensuing 7 months with the lowest monthly average, 59 mg., occurring during the 7th month post injection and the highest, 87 mg., occurring during the 8th month. The urinary volume gradually increased in the final 3 months of life and terminally reached 10 to 14 cc. per overnight period; despite the lower concentration of protein in this dilute urine, the excretion per 18 hours was 120 to 130 mg. The animal was fed a high protein diet and sacrificed 11½ months after injection while in the terminal phase of nephritis but before it became moribund.

Scarring of glomeruli and tubules is more apparent in this illustration than in Fig. 8. Nests of dilated hypertrophic tubules separated by areas of connective tissue replacement are seen. Mallory-Heidenhain stain. × 20.

Figs. 10 to 13. A higher magnification of the sections illustrated in Figs. 6 to 9, respectively.

It is evident from Fig. 10, that the glomeruli of the recovered Wistar rat show some residual changes which consist principally of irregular thickening of glomerular capillary basement membranes. The process is more severe than that illustrated in Fig. 2. The tubular structures have been restored to an essentially normal state. × 85.

Extensive glomerular and tubular lesions are illustrated in Figs. 11 to 13. × 85.
(Smadel and Swift: Nephrotoxin and strains of rats)