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Autoimmune Nephritis in Rats and its Influence on Subsequent Generations

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Summary. The purpose of this study was to determine whether autoimmune nephritis in rats affects subsequent generations. Moreover, it was planned to study the nature of the changes in the kidneys in subsequent generations of rats. In these experiments eight generations of rats were investigated. The first five generations were immunized with a mixture of β-*Streptococcus hemolyticus* with an emulsion of renal cortical substance; the rats were given from 4 to 8 injections. After the fifth generation the animals were not immunized, but their nephritis was considerably more pronounced than in their parents. In the 6th and 8th generations there was a sharp rise in the number of animals that died, mainly pregnant rats and newborns. In the course of the experiments, the animals were found to have hypertension, elevated blood nitrogen and proteinuria. A morphological investigation of the rats of the 6th, 7th, and 8th generations that were not immunized, but were born of immunized parents, revealed membranous-proliferative glomerulonephritis, which according to its clinical course and morphological data resembled the nephrotic syndrome the mixed type of human glomerulonephritis.

Zusammenfassung. Die Untersuchungen über den Generationswandel der Autoimmunnephritis der Ratte erstreckten sich über 8 Generationen. Bei den ersten 5 Generationen wurde durch eine 4—8malige Injektion einer Mischung aus beta-hämolytischen Streptokokken mit einer Emulsion aus Nierenrindengewebe eine Immunnephritis erzeugt. Die Ratten der 6. bis 8. Generation erhielten keine immunisierenden Injektionen. Trotzdem kam es in der 6. und 8. Generation zu einem akuten Anstieg der Spontantodesrate besonders unter den graviden und neugeborenen Versuchstieren. Klinisch führte die Immun-Nephritis zu einem Blutdruckanstieg, einer Proteinurie und Erhöhung des Harnstoffes. Histologisch entsprachen die Nierenbefunde der 6.—8. Generation einer membranösen proliferativen Glomerulonephritis.

For a number of years attempts have been made to create an experimental model of glomerulonephritis with a pathogenesis resembling that in human beings. With the help of the wellknown model of Musugi nephritis, the morphological picture of the changes could be well reproduced. Its weak point, however, is the administration of ready-made anti-kidney, anti-bodies and a large amount of foreign protein. It is generally recognized that acute glomerulonephritis often follows group. A hemolytic streptococcal infection (Rammelkamp and his colleagues, 1953, 1955a, b; Kushner *et al.*, 1961), and streptococcal antigens can be found in the blood of the patient (Longcope, 1938; Lyttle *et al.*, 1938; Seagul and Lyttle, 1933).

It is believed that immunological disorders play an important part in the pathogenesis of glomerulonephritis, as anti-kidney antibodies are detected in the blood of the patients (Lange *et al.*, 1949; Pfeiffer and Bruch, 1952; Kramer *et al.*, 1961; Vorlaender 1953, 1955; Michael *et al.*, 1964), and in the glomeruli fixation

of gamma-globulin is observed, apparently by antibodies (Freedman and Markowitz, 1962; Freedman, Peters and Kark, 1960; Kaffer and Paronetto, 1965; Mellors, Ortega and Homan, 1957; Seegal *et al.*, 1965).

Attempts to produce experimental autoimmune nephritis were made by P. and E. Cavelti (1945) who injected a mixture of Streptococcus B hemolyticus and emulsion of kidney tissue and observed anti-kidney auto-antibodies in the blood of experimental animals and typical morphological changes in the kidney. Later however, Humphrey (1948), Reck and Thomas (1948), Middleton and co-authors (1953) tried to repeat the Caveltis' experiment but failed to get data similar to theirs. It is noteworthy that Humphrey, Reck and Thomas did not adhere to the pattern of immunization that took place in the Caveltis' experiments, which might explain their negative results. In one of our previous works, we tried to produce Cavelti's nephritis (Bykovskaya and Vihert, 1965). In this experiment we strictly followed the pattern of immunication proposed by Cavelti and we managed to induce glomerulonephritis in almost 100 per cent of rats.

It is known also that autoimmune lesions of the kidney can be induced by injecting the animals with Freund's adjuvant and an emulsion of kidney tissue (Hayman *et al.*, 1959). Antikidney autoantibodies were detected in the blood of the animals, a clinical picture of the nephrotic syndrome developed and in the kidney membraneous glomerulonephritis could be found; the latter could be transferred to tolerant recipients by means of lymphocytes, but not by serum containing anti-kidney antibodies (Pfeiffer, Müller-Ruchholtz, and Federlin, 1962).

The possibility of transmitting renal autoimmune diseases, including the nephrotic syndrome, from parents to their progeny is another question that provokes considerable interest today. In human beings clinical observations of newborns from parents who suffered from renal diseases, conducted by Kouvalainen *et al.* (1962), Norio *et al.* (1964), and others, have shown that even at birth there are already signs of the nephrotic syndrome. One of the anatomical manifestations of the disease in the newborn is a big placenta, which sometimes exceeds the weight of the foetus. The life-span of the offspring with congenital nephrotic syndrome is extremely short, quite often a few months to one year. The urine of the affected newborns was found to contain protein and erythrocytes in large quantities. Morphological changes were observed in the glomeruli and tubuli. In the glomeruli there were proliferative changes, while in the tubuli there was fatty degeneration of the epithelium. An electron microscope study revealed a thickening of the basal membranes with the formation of unusual laminar structures.

In spite of the fact that considerable interest has been devoted to the study of the congenital nephrotic syndrome, many questions still remain unanswered, for instance, how the nephrotic syndrome is, passed on from the mother or the father. Perhaps the experimental way of investigation may, to a certain degree, throw light on the given problem.

Materials and Methods

In the experiment of transferring autoimmunenephritis from parents to subsequent generations of rats we chose the Cavelti nephritis pattern, as we believe that it demonstrates the participation of *Streptococcus B hemolyticus* in the etiology and pathogenesis of glomerulonephritis.

The experiment was carried out on sexually mature white rats (males and females), mated 3–4 weeks after a course of immunization with streptococcal-renal emulsion. The newborns were kept under strict clinical observation and the rats were allowed to mate again after immunization; the immunization pattern was continued in this manner up to the 5th generation. The 6th, 7th, and 8th generations were not subjected to immunization.

Preparing Emulsion Kidney Tissue for Immunization

Using a light ether anesthesia, the rats were sacrificed by decapitation, after which the kidneys were washed through the artery with a physiological solution warmed to 37° C. The cortical layer of the kidney was sliced off, weighed and ground in a homogenator with a physiological solution in a ratio of 1:5. The 20% emulsion of renal cortical substance so prepared was ready for mixing with the streptococcal emulsion.

Streptococci Cultivation

A culture of *β-Streptococcus hemolyticus* (*S. pyogenes*), strain N° 291, was grown from the mucus of the pharynx of a scarlet fever patient. The *β-Streptococcus hemolyticus* was cultivated in Marten agar with the addition of 5% rabbit blood and 2% glucose solution. This bacterial mass was centrifuged, washed in a physiological solution, then treated with 96° alcohol, after which it was dried with acetone.

The dried *β-Streptococcus hemolyticus* was diluted in a physiological solution (3 mg of dry bacteria per 1 ml of physiological solution), after which it was mixed with the emulsion of the renal cortical substance (tissue antigen) in a ratio of 1:1.

Immunization Pattern

The mixture prepared by the above method (*β-Streptococcus hemolyticus* and 20% emulsion of renal cortical substance) was injected intra-abdominally 0.6 ml twice a week; each rat received from 4 to 8 injections. The animals were allowed to mate 3–4 weeks after the final injections of the streptococcal-renal emulsion, providing they displayed clinical signs of the disease. The newborns were reared and subjected to the above-mentioned procedures once again (Fig. 1). The number of animals in each generation and the number of injections received can be seen in Fig. 1.

Histological Investigations

The rats were sacrificed at different ages from 40 to 730 days. At the moment of sacrificing, heart, and kidneys were weighed and their weight was calculated per 100 g of body weight.

The renal tissue was fixed in 10 per cent formalin, imbedded in paraffin, and sections 3 μm thick were stained with haematoxylin-cosin; the trichrome Masson method, Johns-Mowery silver method and the PAS-reaction were also employed; sections of the spleen were stained by the Brushe method.

In order to determine the fixation of γ-globulins in the renal glomeruli in the 6th and 8th generations, we employed the direct Coons method: rabbit serum against γ-globulin of the rats (the fluorescent serum was received from the Gamaleya Institute of the AMS of the USSR).

Results of the Investigations

In the given experiment, we succeeded in obtaining 8 generations of animals from rats with autoimmune nephritis induced by intra-abdominal injections of streptococco-renal emulsion. Pronounced clinical signs of the disease usually appeared a month after the last injection. Hypertension, azotemia, and proteinuria were corded in animals of all generations. Clinical signs of glomerulonephritis intensified gradually, growing from generation to generation, and were accompanied by a high percentage of stillbornyoung. The animals from the 1st to the 5th generations were found to have increased arterial pressure, on the average by 25–30 mm Hg (Fig. 2). In addition stable proteinuria was observed in practically all the animals, while the protein content in the urine varied from 0.66 to 1.32, and in some animals was as high as $2‰$ (Fig. 3). A study of the urinary sediment revealed erythrocytes, hyaline, and granular casts. In the first generation of rats we registered quite a high percentage of deaths among the young rats (6 out of 20, Fig. 1).

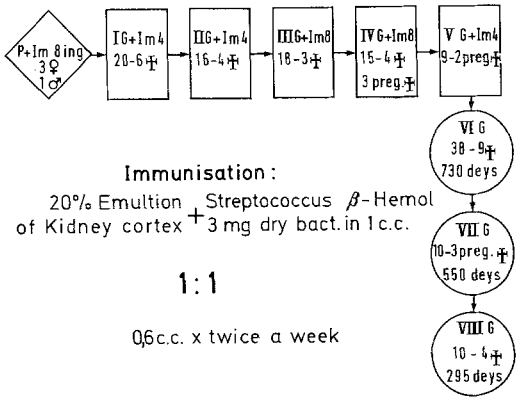

Fig. 1. The rectangular columns show the generations of animals immunized and the number of injections (*Im-4, Im-8*). The figures below give the number of animals in each generation; those that died (+) and the number of dead pregnant rats (*preg.* +). The circles denote the generations without immunization, the number of animals and those that died, and the maximum duration of the experiments

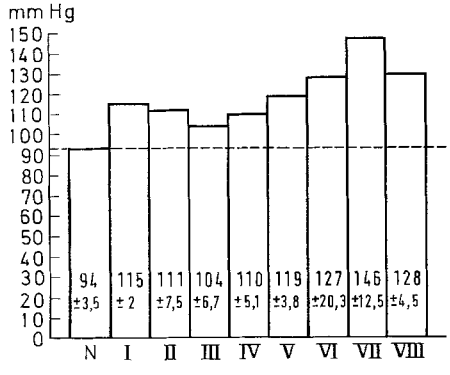

Fig. 2

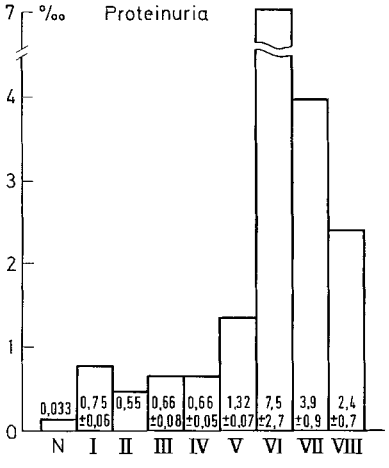

Fig. 3

Fig. 2. Mean value of the blood pressure for 8 generations of animals. Statistically significant differences were observed between control (*N*) and the 2nd, 5th, 6th, 7th and 8th generations with P 0.01

Fig. 3. Mean value of proteinuria in animals of the 8th generation

In the 2nd generation, the animals received 4 injections, as in the 1st generation; 25% of the newborns died (4 out of 16) before reaching the weaning stage. The surviving animals were found to have a slightly increased arterial pressure while the protein content in the urine was somewhat lower. This was why the number of injections in the subsequent generations was increased (Fig. 1).

The condition of the animals of the 3–4th generations that each received 8 injections of streptococco-renal emulsion (4.8 ml) became considerably worse.

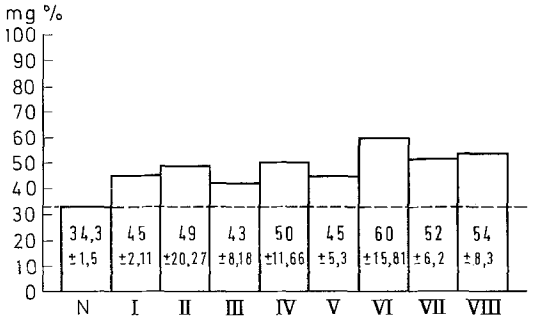

Fig. 4. Mean value of residual nitrogen in the blood plasma of 8 generations of animals. Statistically significant differences were observed between control (N) and the 2nd, 5th, 6th, 7th and 8th generations (P 0.01)

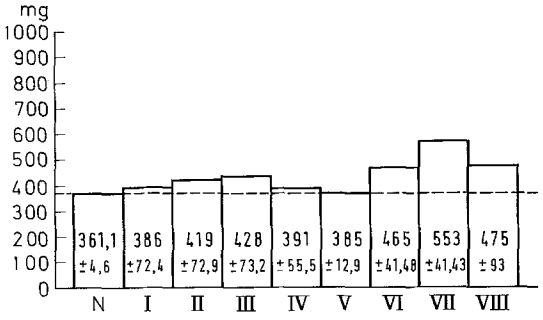

Fig. 5. Average weight of the heart in 8 generations (per 100 g of body weight). Statistically significant difference was observed only in the 7th generation (P 0.01)

The progeny were weak, with signs of edema. Both in the 3rd and 4th generations, there was a high persentage of stillborns. In the 3rd generation, 3 out 18 animals died, in the 4th generation, 4 out of 15, including 3 pregnant females.

In the 5th generation, the number of injections was again reduced to 4. In spite of this, however, the progeny was weak, with thin fur and of a low vitality. A high percentage of the newborns died during the suckling period, and two females perished at the end of their pregnancy. All the surviving rats were found to have increased arterial pressure and increased residual nitrogen in the blood serum (Fig. 4), and the protein content in the urine was up to $2^0/_{00}$. The weight of the heart and kidneys increased considerably compared to that of the control group. On the average the weight of the heart increased by 16%, which may be attributed to hypertrophy of the heart muscles owing to the increased arterial pressure throughout the experimental period (Fig. 5). The kidneys of the animals were swollen and pale and their weight 30–40% more than those of the control group (Fig. 6).

A microscopic investigation revealed that renal changes were present in all five generations with only a slight difference in the degree of damage. In the main, the morphological changes revealed a picture of proliferative glomerulo-

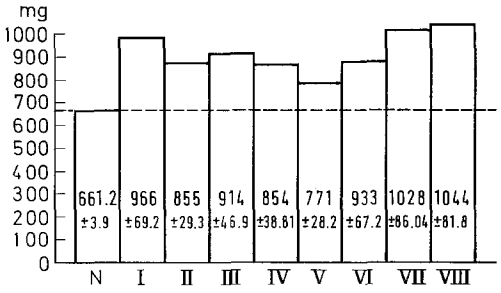

Fig. 6. Average weight of kidneys (per 100 g of body weight) statistically significant difference between the control and all 8 generations with P 0.01

nephritis. There was a certain increase in the size of the glomeruli, and there was division into separate loops and thickening of the basal membranes and proliferation of mesangial cells. In the central lobular areas of the glomerulum it was possible to observe a deposition of PAS-positive substance. The damage to the glomeruli was not uniformly pronounced; mostly the changes were moderate or mild.

The 6th, 7th, and 8th generations were of particular interest; they were not immunized, but it was possible to observe clinical signs typical of glomerulonephritis, while morphologically the membranous nephritis resembled that of man.

Clinical signs of the disease in animals of the 6th generation appeared soon after birth. The newborns were weak, their fur was thin and they were sluggish, and as a result some of them (9 out of 38) perished in the first few days or weeks after their birth. In the 7th generation 3 pregnant rats died. In the 8th generation 4 out of 10 newborns died, while in the 9th generation all the newborns died, thus terminating the experiment. The surviving animals showed a considerable increase in arterial pressure, it was possible to observe azotemia, and proteinuria was as high as 15–20‰, being especially pronounced in the 6th generation. During the life-span of the animals it was possible to observe periodically pronounced edema of the muzzle and paws, and the disease proceeded with remission and relapses. The weight of the heart and kidneys increased considerably compared to that of the control group (Fig. 5).

In the 6th, 7th, and 8th generations described above, the morphological changes were more severe than in the animals of the 5 preceding generations. The morphological changes in these animals corresponded to the most pronounced clinical symptoms, depending on their life-span. The changes were of a proliferative membranous type of nephritis. It was possible to observe a significant increase in the size of the glomeruli. The basal membranes became considerably thicker and rougher, while the lumina of the capillaries narrowed somewhat, as a result of which they resembled thick-walled rings with narrow lumina (Fig. 7). There was also a proliferation of mesangial cells, especially in the early stage of the disease, though not sharply pronounced, and there was a slight polymorphism of the nuclei. As the pathological process developed, the glomeruli devided into separate loops, which steadily became tougher, and some of them adhered to

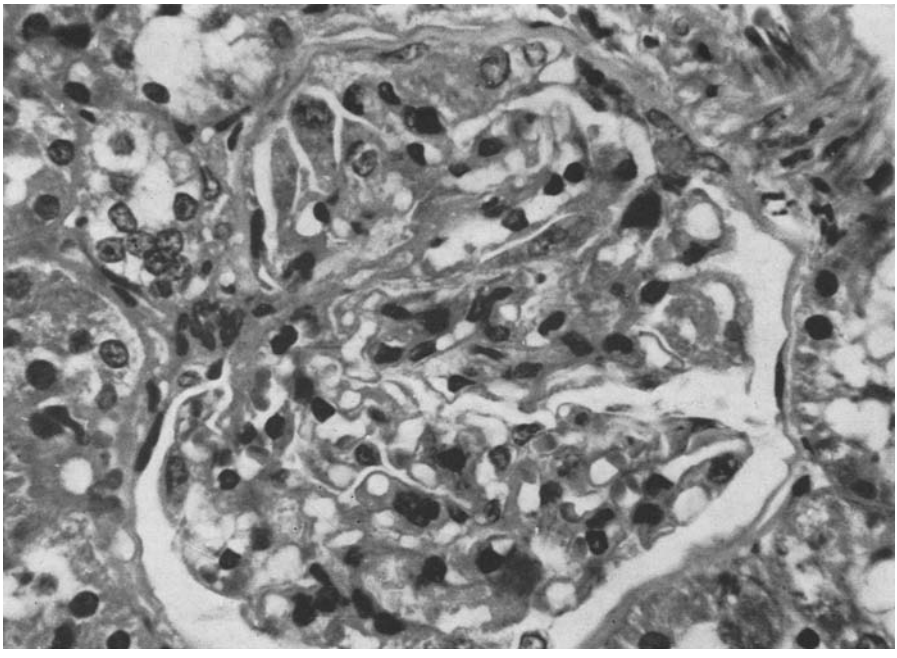

Fig. 7. Rat of the 7th generation with proliferative-membranous nephritis; pronounced thickening of the membrane, narrowing of the lumina of the capillaries of the loops, proliferation of mesangial cells; at the top adhesion of one of the loops with the capsule. Vacuolar and granular degenerations of epithelium of the proximal tubules. G.-E. × 420

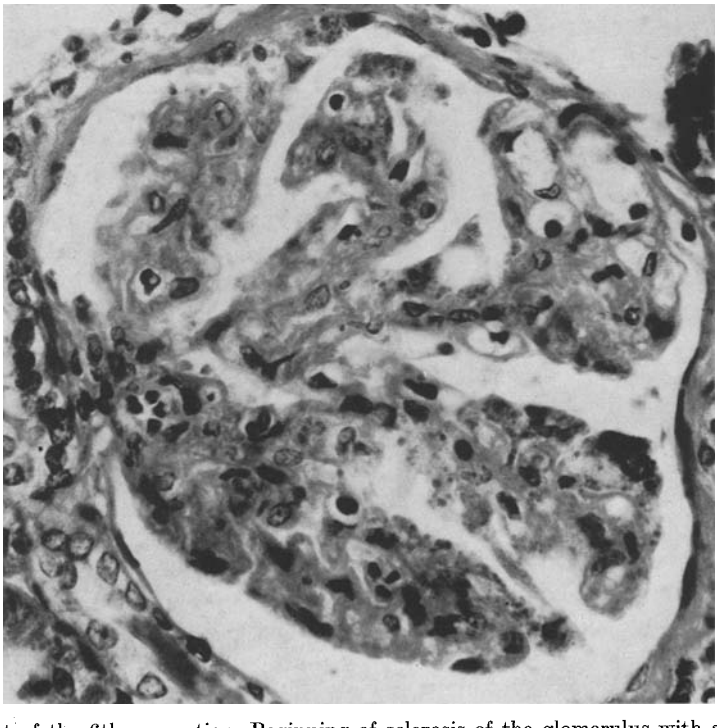

Fig. 8. Rat of the 6th generation. Beginning of sclerosis of the glomerulus with adhesion of the loops to the capsule. Trichrome Masson. × 600

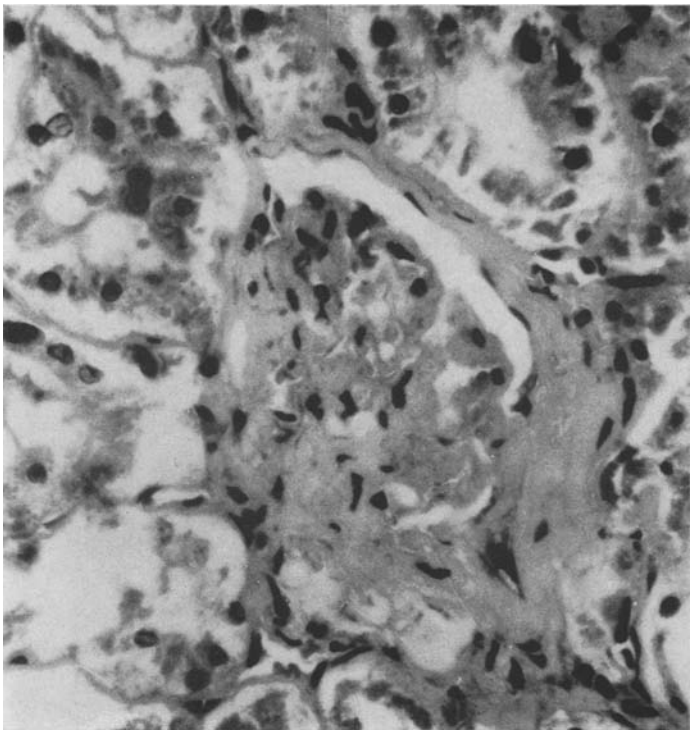

Fig. 9. Rat of the 6th generation. Complet sclerosis of the glomerulus. Severe degenerative changes (vacuolar and hydropic degeneration), desquamation, and necrobiosis of the epithelium of the proximal tubules. Trichrome Masson. × 600

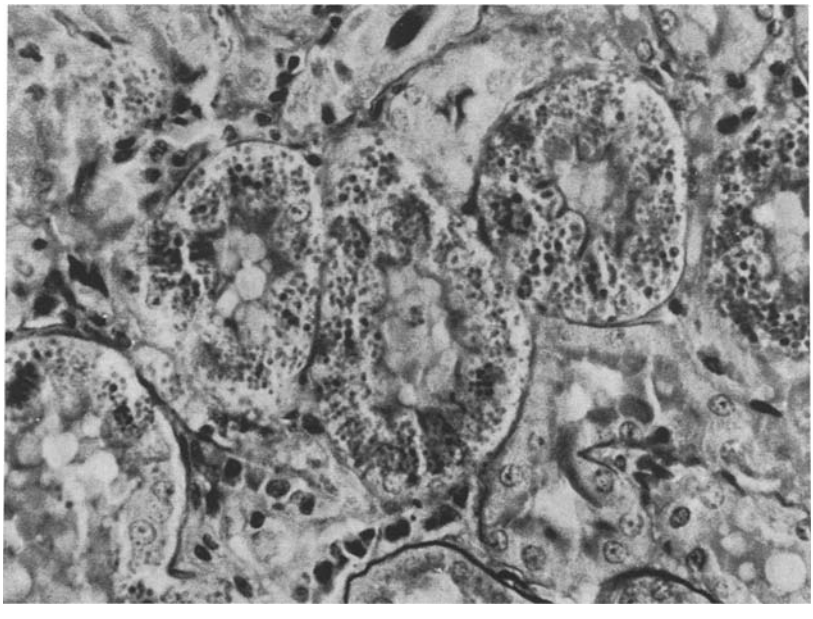

Fig. 10. Rat of the 7th generation. Vacuolar and hyalinedrop degeneration of the epithelium of the proximal tubules, protein casts in their lumina. PAS. × 700

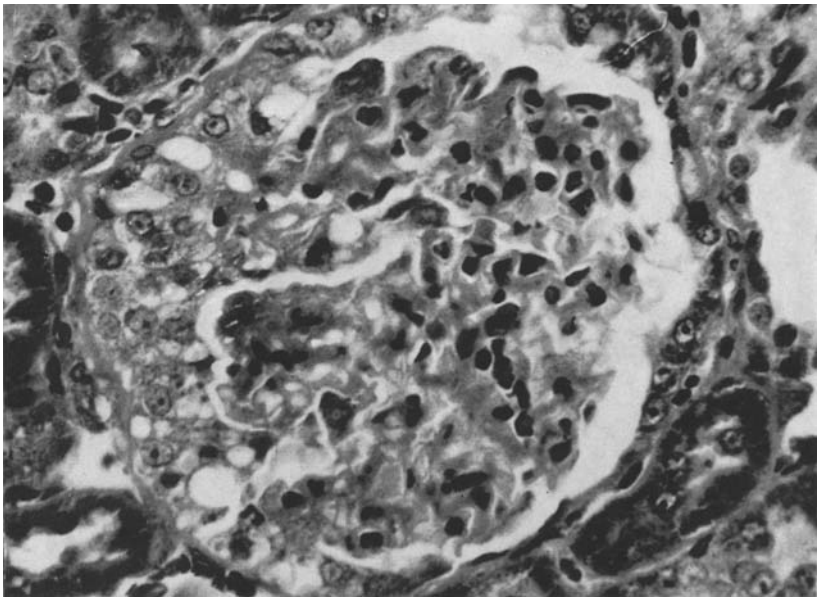

Fig. 11. Proliferation and degeneration of the epithelium of the Bowman capsule. Trichrome Masson. × 500

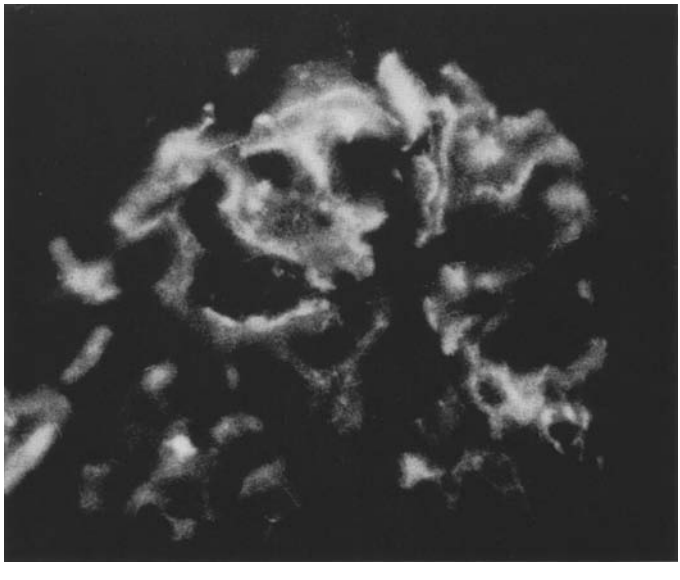

Fig. 12. Rat of the 8th generation. Fixation of the fluorescent anti-γ-globulin serum in the glomerulus. Coons'direct method. × 500

the capsule (Fig. 8); there was partial obliteration of the capsule, partial or total sclerosis, and hyalinization of the glomeruli (Fig. 9). In the mesangium it was frequently possible to observe hyalin drops, which were also found in the cyto-

plasm of the podocytes, and sometimes in large quantities in the cytoplasm of the epithelium of the proximal tubules (Fig. 10). A proliferation of the epithelium of the Bowman capsule was observed, sometimes with almost complete blocking of its lumen. The proliferating epithelium was vascularized with numerous inclusions (Fig. 11). It was also noted that the tabular epithelium was subjected to vacuolar degeneration; protein and casts were visible in their lumina. In advanced cases it was possible to observe atrophy of a small group of proximal tubules surrounded by interstitial fibrosis.

The application of Coon's direct method with fluorescent antigamma-globulin serum, revealed a large deposition of gamma-globulin on the basal membranes of the glomeruli (Fig. 12).

In the spleen and lymph nodes a diffused plasmocellular reaction was observed. The sinuses were filled with plasmoblasts and plasmocytes of varying degrees of maturity.

Discussion

The experiments described showed that, in the offspring of rats which had received 4–8 injections of a mixture of killed culture β-hemolytic streptococci and an emulsion of the renal cortex, experimental glomerulonephritis was induced siderably earlier, and from a smaller number of injections than in our previous experiments reproducing Cavelti's nephritis. Besides that, the nature of the damage differed somewhat from that of the initial experiments, in which the disease developed primarily as a hypertonic type, while in these experiments a pronounced nephrotic syndrome developed.

In the first 5 generations the intensity of the changes gradually increased, so that by the 5th generation 4 injections sufficed to induce severe disease. Since the progeny of the 6th generation was very weak, it was decided to postpone the beginning of their immunization, and then there was no need for immunization as clinical signs of the disease developed spontaneously. The 7th generation was the first and the only litter and was obtained with great difficulty and in small number from 5–6 month old rats, since there were many stillborns. After that, the rats of the 6th generation had no progeny though they lived for about 2 years and, with time, the clinical picture of the disease gradually became more pronounced—proteinuria reached a high level (up to $20^0/_{00}$ in certain animals, with an average protein level for the 6th generation of $7.5^0/_{00}$; hypertension, azotemia, and edema were observed (Figs. 2–4). In the 7th and 8th generations it was also possible to observe a gradual aggravation of the clinical manifestations throughout life. In the 7th generation, progeny was also obtained only from 5–6 month old rats (first litter), and in the 8th generation the young animals gave birth to stillborns, after which 4 gravid females died and the experiment came to an end. As the animals of the 6th generation lived the longest, the morphological picture of their changes was the most pronounced (Fig. 9); pronounced sclerotic changes in the kidneys were observed only in the 6th generation (Fig. 9). We tried to improve the condition of the animals of the 6th generation by hormone therapy, since in our previous work (Bykovskya *et al.*, 1966) we obtained positive results with prednisolone; however, hormone therapy produced only a temporary clinical effect. The animals soon died and a morphological investigation yielded no positive results compared to those of the untreated animals. Our data correspond to the

investigations of Hallman *et al.* (1959), who claim that inherent renal diseases are very resistant to treatment, in particular, hormone therapy.

The disease was of an autoimmune nature. This was confirmed histologically, the lymph nodes and the spleen being found to have undergone changes typical of acute immunological processes, i.e. sharply defined proliferations of the immunocompetent cells (plasmoblasts, and plasmocytes of different degrees of maturity). Coon's direct method with antiglobulin fluorescent serum revealed intensive deposition of γ-globulin on the basal membranes, which is typical of glomerulonephritis.

Since the 6th generation was not immunized directly, we can assume that the damage to the kidneys in the animals of this generation was due to transplacental immunization during the interuterine period from the 5th generation parents whose nephritis was induced by injecting renal tissue emulsion and β-Streptococcus *hemolyticus*. The 7th and 8th generations also showed transplacental transmission of the disease, but no longer due to external immunization. Unfortunately, we did not conduct an investigation of the placenta. If changes confirming this hypothesis had been observed in the placenta, this could have confirmed this suggestion.

Apparently, it is possible to consider the above-mentioned facts as a kind of explanation of the congenital nephrotic syndrome, especially in those cases when renal pathology was observed in the parents.

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