Renal Changes in Hepatic Lesion: An Immunological Consideration on the Mechanism of its Development

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It is well known that a slight degree of histological and functional renal lesion may be accompanied in a majority of patients with liver disease and a clinical observation on this problem was previously reported from our clinic.[1)]

The main histological renal change in patients with liver cirrhosis is represented in glomerular changes, consisting of depositions located in the subendothelial space of the capillary wall and the mesangium, increase in the amount of mesangial matrix, thickening of the basement membrane and focal fusion of the foot processes, whereas the main renal change in patients with obstructive jaundice is represented in tubular degeneration. It seems, therefore, that there is some different mechanism in the development of renal lesion between both patients with liver cirrhosis and those with obstructive jaundice.

Much attention has, recently, been made on a concept that an autoimmune factor may be involved into the mechanism of development and prolongation of glomerulonephritis. Heyman et al.[2)] have produced renal lesion in rats by repeated intraperitoneal injections of rat liver suspension incorporated in Freund's adjuvant.

Having a postulation that an antigen-antibody reaction may be involved into the mechanism of the development of renal lesion in patients with liver cirrhosis, liver cancer and chronic hepatitis, the following animal experiments have been carried out.

The experiment was, firstly, designed on parabiotic rats to determine whether a humoral factor, which is toxic or antigenic to the kidney, can be liberated from injured tissue of the liver into the circulation and, if it is possible, whether it may induce a renal lesion. Heterologous and homologous immunization experiments with crude rat liver homogenate, hepatic gamma-globulin fraction and hepatic polysaccharide-protein fraction were undertaken, secondarily, to determine whether a renal lesion can be induced through these immunological processes. Thirdly, the fluorescent antibody technique and the Ouchterony's method were applied on the second experiment to demonstrate a common antigenic substance between both tissues of the liver and the kidney.

METHODS

I. Parabiosis experiment :

Male rats of Wistar strain weighing 150 to 200 gm were fed on a diet containing 0.06 per cent of p-dimethylaminoazobenzen (DAB) during one month, receiving repeated 5 to 8 minutes' inhalations of carbon tetrachloride gas twice weekly. The histological picture of the liver thus treated was cirrhotic as shown in Fig. 1.

Following several days' withdrawal of the toxic diet, peritoneal parabiosis was made between both rats with the cirrhotic liver and the normal liver. The parabionts were fed on normal diet during 8 weeks, then the kidney of the normal parabiont was examined histologically. As a control experiment, parabiosis was made between both normal rats and the kidney was examined at 8 weeks following the operation.

The normal diet used was composed of 75 per cent corn powder, 10 per cent casein, 4 per cent McCollum salts, 10 per cent vitamines powder and 1 per cent plant oil, as well. Ten gm of this dryed diet was fed to each rat daily.

The humoral transfer between both parabionts was ascertained by the intravenous injection of bromosulfophthalein and the intravenous pyelography.

II. Immunological experiments :

Immunological experiments were undertaken on rats in the following schedule.

A) Heterologous immunological experiments :

a) Experimental groups with the rabbit antiserum against homogenate of the rat whole liver :

1) A single injection of the normal rabbit serum (control group) :
2) A single injection of the rabbit antiserum against homogenate of the normal rat liver :
3) A single injection of the rabbit antiserum against homogenate of the cirrhotic rat liver :

b) Experimental groups with the rabbit antiserum against extract of the cirrhotic rat liver :

1) A single injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver :
2) Repeated injections of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver :
3) Administration of prednisolone to the animals in the experimental group of A-b-2 :
4) A single injection of the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver :
5) Repeated injections of the rabbit antiserum against gamma-globulin frac-

tion of the cirrhotic rat liver :

B) Homologous immunological experiments :

a) Experimental groups with homogenate of the rat liver :

1) Repeated intraperitoneal injections of homogenate of the cirrhotic rat liver combined with Freund's adjuvant :

2) Repeated intraperitoneal injections of homogenate of the normal rat liver combined with Freund's adjuvant :

3) Repeated intraperitoneal injections of Freund's adjuvant alone :

b) Experimental groups with the rat antiserum against homogenate of the rat liver :

1) A single injection of the rat antiserum against homogenate of the normal rat liver :

2) A single injection of the rat antiserum against homogenate of the cirrhotic rat liver :

3) A single injection of the homologous serum to the normal rats :

C) Experiments with the fluorescent antibody technique :

Both rabbit's antisera against the polysaccharide-protein fraction of the cirrhotic liver and that of gamma-globulin fraction were conjugated to fluorescein isothiocyanate. The kidney slices from the animals in both experiments in A-b-2 group and A-b-5 group were stained with these conjugated antisera. The conjugating and staining techniques used were essentially as same as that was described by Hamajima et al.[4]

D) Experiments with the Ouchterony's technique :

Using the Ouchterony's technique,[5] an immunological reaction between the rabbit antiserum against homogenate of the normal rat liver and either homogenate of the normal rat liver or kidney, between homogenate of the cirrhotic liver and either rabbit antiserum against homogenate of the normal rat liver or that of the cirrhotic rat liver and between homogenate of the cirrhotic rat liver and either the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver or the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver were examined.

E) Detail of the technique :

a) Technique for obtaining the cirrhotic liver :

Male rats of Wistar strain weighing 150 to 200 gm were received repeated 15 minutes' inhalation of carbon tetrachloride gas in every other day during about one month. The histological picture of the liver thus obtained was cirrhotic as shown in Fig. 2.

b) Technique for obtaining antigens :

1) Liver homogenate : Either blood-free normal rat liver or that of cirrhotic rat liver was sliced and washed with cold normal saline and homogenized with a Waring blender in cold 0.88 M sucrose solution. The homogenate was made

20 per cent sucrose suspension and then 0.5 per cent volume of phenol was added.

2) Hepatic polysaccharide-protein fraction: According to the same technique as described by Cole et al.,[6] the cirrhotic rat liver homogenate was digested with trypsin, heated at 60°C for 30 minutes and adjusted pH 7.0 with 1 N hydrochloride, respectively. Then the suspension was centrifused at 27,000 rpm for 60 minutes and the supernatant was dialysed against running water. The dialized solution was, finally, purified by the 68 per cent alcohol fractionation technique as was described by Raistrick and Topley.[7]

3) Hepatic gamma-globulin fraction: Homogenate of the cirrhotic rat liver was centrifused at 3,000 rpm for 10 minutes, the supernatant was re-centrifused at 35,000 rpm for 60 minutes, then gamma-globulin fraction was extracted according to the method as was described by Cohn et al.[8]

4) Antiserum: Equal volume of Freund's adjuvant and suspension of the liver homogenate or the liver extract were mixed. Rabbits were sensitized by the repeated intraperitoneal injections of 10 ml of the mixture twice weekly. When the antibody titer was increased up to 130 and 16 units, in the heterologous and homologous experiments, respectively, on the agar gel double diffusion technique,[9] the blood was drawn. The serum was inactivated by heating at 56°C for 30 minutes.

5) Injection of antisera: During a temporal unilateral ligation of renal artery, 0.5 ml per 100 gm body weight of the antiserum was injected into the inferior vena cava according to the technique as was described by Grabar et al.[10] In the repeated injection experiment, successive injections of the antiserum were given into the tail vein once a week during 3 months.

6) Routine examinations: Following the first injection of the antiserum proteinuria, serum protein concentration and paper-electrophoretical serum protein fractions were examined at 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months and 3 months, respectively.

III) Histological examination:

The renal tissue was fixed in 10 per cent neutral formalin for the light microscopy and 2 per cent buffered osmic acid for the electron microscopy. Formalin-fixed tissues were stained with hematoxylin-eosin and periodic acid-Schiff's reagent (PAS). The tissue for the electron microscopy was dehydrated in graded alcohol and embedded in Epon and stained with both uranyl acetate and lead citrate.

The degree of light and electron microscopic alteration were represented by the following criteria; (−) no abnormal finding, (±) partial, slight change, (+) diffuse, slight change, (±) partial, distinct change, (++) diffuse, moderate change and (+++) diffuse, distinct change.

The fluorescent antibody reactions were graded on a scale ranging from negative (−), slightly positive (±), positive (+) and to distinctly positive (++).

RESULTS

All the results obtained in the following experiments were listed in Tables 1～5.

I. Parabiosis experiment:

In the parabiotic group between both rats with the normal liver and with the cirrhotic liver, proteinuria in the normal parabiont was as much as about 300 mg per 100 ml from the beginning of the second week through the experimental period of eight weeks, while it was principally negative in the control group.

The histological changes of the kidney were indicated in Tables 1 and 2. In the control group, the renal alteration showed, if anything, a slight degree of swelling of the epithelial cell in the proximal tubulus, dilatation of the tubular lumina and the alteration of blood vessels on light microscope and, in the glomerulus, revealed increase in mesangial matrix, thickening and deposition of protein-like material in the basement membrane, blunted and/or fused foot processes in the epithelial cells, disappearance of slit pores in the endothelial cells and deposition of membraneous material located in the subendothelial space of the capillary wall and the mesangium on electron microscope. These renal changes in the control group were, however, far less prominent to assume that the "parabiosis" itself may induce a renal histological alteration.

In the parabiotic group between both rats with the normal liver and with the cirrhotic liver, the histological alteration of the kidney in the normal parabiont showed, light microscopically, more prominent alterations such as the increased cellularity of the glomerular tuft, thickening of the lobular stalk, glomerular capillary collapse, swelling of the epithelial cell in the proximal tubulus and dilatation of the lumina and existence of casts in the distal tubulus than observed in the control group (Fig. 3). Inflammatory cell infiltration in the interstitium of the kidney was noted, but it was limited to a slight degree of lymphocytic infiltration alone.

Electron microscopically, the renal glomerulus in the experimental group showed more prominent alterations such as the increase in the amount of matrix, mesangial proliferation and swelling, granular deposition in the mesangium, thickening and deposition of protein-like material in the basement membrane, blunted and/or fused foot processes in the epithelial cells, swelling of the podocyte and deposition of membraneous material and osmiophilic body located in the subendothelial space of the capillary wall and the mesangium than those observed in the control group (Fig. 4, 5).

II. Immunological experiments:

A) Heterologous immunological experiments:

a) Experimental groups with the rabbit antiserum against homogenate of

the rat whole liver

1) A single injection of the normal rabbit serum : (A-a-1)

As a rule, proteinuria and changes of serum protein and gamma-globulin concentration were not induced by a single injection of the normal rabbit serum. About two to three weeks following an injection of normal rabbit serum a limited number of the glomeruli revealed capillary collapse, increase in the amount of PAS-positive material, fusion of the epithelial foot processes and disappearance of the endothelial slit pores on light and electron microscopes. These histological alterations, however, were unusual and were not always prominent; it was assumed, then, as not abnormal change.

2) A single injection of the rabbit antiserum against homogenate of the normal rat liver : (A-a-2)

Following a single injection of the rabbit antiserum against homogenate of the normal rat liver, rats excreted progressively increasing amounts of protein and red cells, having the maximum peak at the second week. The concentration of serum protein was unchanged through the experimental period of three months, but that of serum gamma-globulin was slightly elevated on the third month.

On light microscope, the kidney showed partially scattered slight alterations such as swelling of the glomerulus, increase in the amount of PAS-positive material in the glomerulus, intra-capsular exudate of protein-like material and erythrocytes and swelling of the tubular epithelium at one week following the injection (Fig. 6) and diffusely scattered distinct alteration including casts in the tubular lumina besides the above-mentioned alterations at two or three weeks, whereas it showed no histological alteration on the third day. These renal alterations were more prominent than those observed in the control group. The degree of these renal alterations were subsided exceedingly, but the increase in the amount of PAS-positive material in the glomerulus was still noted at three months following the injection.

On electron microscope, the kidney showed such alterations as swelling of the endothelium and partially scattered disappearance of the epithelial cell slit pores as early as on the third day when no histological change was demonstrated on light microscope.

At one or two weeks following the injection of antiserum the kidney showed such changes as the increase in the amount of matrix in the mesangium, irregular density in the basement membrane and fusion of the epithelial foot processes (Fig. 7).

At three weeks or one month following the injection of antiserum, the capillary lumina was narrowed owing to the significant increase in the mesangial matrix, the epithelial cell was swollen, including various sizes of vacuoles in the protoplasma, and the epithelial foot processes were fused and flattened.

At three months following the injection of antiserum the degree of the

histological alterations above-mentioned were subsided except for the thickening and irregular density of the glomerular basement membrane.

3) A single injection of the rabbit antiserum against homogenate of the cirrhotic rat liver: (A-a-3)

From two weeks following a single injection of the rabbit antiserum against homogenate of the cirrhotic rat liver, rats excreted progressively increasing amount of protein through the experimental period of three months. The degree of proteinuria in this group was much abundant than that observed in the group whom the rabbit antiserum against homogenate of the normal rat liver was injected,but the degree of hematuria, serum protein level and serum gamma-globulin level were almost equal in both groups.

On light microscope, the kidney showed such alterations as swelling of the glomerulus, exudate of protein-like material into the Bowman's space, adhesion of the lobular tufts onto the Bowman's capsula and a slight degree of turbid swelling of the tubular epithelial cells from one week following the injection of antiserum (Fig. 8). On electron microscope, the glomerulus showed such alterations as disappearance of slit pores of the endothelial cells, a slight degree of increase in the amount of mesangial matrix, partial thickening of the basement membrane and partial fusion of the endothelial foot processes.

Concerning the degree of renal histological changes and the time when these changes appear and disappear, no difference was noted between both groups with homogenate of the normal liver and that of the cirrhotic liver. However, limiting on the second and third week when abundant proteinuria was observed, glomerular changes, including the thickening of the basement membrane, increase in the amount of the mesangial matrix, fusion of the epithelial slit pores, were observed more prominently in the group with the antiserum against homogenate of the cirrhotic liver (Fig. 9).

b) Experimental groups with the rabbit antiserum against extracts of the cirrhotic rat liver:

1) A single injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver: (A-b-1)

At two and three weeks following a single injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver, rats excreted the maximal degree of protein and large numbers of erythrocytes and leukocytes in the urine. The level of serum gamma-globulin was elevated slightly at the third month.

The renal histological change in this group was similar to that observed in the group whom the rabbit antiserum against homogenate of the cirrhotic rat liver was injected. The most significant renal change was noted at two and three weeks following an injection of the antiserum. The increase in the amount of PAS-positive glomerular material, thickening of the glomerular basement

membrane and fusion of the epithelial foot processes were remained for a long time.

2) Repeated injections of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver: (A-b-2)

Following the repeated injections of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver, rats excreted more abundant protein through the whole experimental period than that observed following a single injection of this antiserum. Erythrocytes, leukocytes and casts were observed in urinary sediment from the early stage of the experiment. The level of serum gamma-globulin was elevated during three months from the first to the third month of the experiment.

In the early stage of the experiment, renal histological alteration in this group was similar to that observed in the single injection group, but it became more distinct with lapse of the time from the second or the third week of the experiment, showing a slight degree of tubular alteration besides glomerular alteration.

At two months following the first injection of the antiserum, most of the Bowman's spaces were disappeared owing to the swelling of the glomerulus, nuclei of the lobular tufts were increased, a large number of the glomerular capillaires were collapsed and partial hyalinization was observed in a small number of the glomeruli (Fig. 10). Inflammatory cell infiltration and fibrosis in the interstitium and turbid and/or vacuolated swelling of the epithelial cells and casts in the tubulus were observed at this stage. In the glomerulus such remarkable electron microscopic changes as the diffusely thickened basement membrane, disappearance of the endothelial slit pores, swelling of the epithelial cells containing vacuoles, fusion and blunting of the foot processes and swelling of the mesangial cells were observed (Fig. 11, 12).

At three months following the first injection, the degree of these renal alterations was more increased. The deposition of protein-like material in the basement membrane, deposition of membraneous material located in the subendothelial space of the capillary wall and the mesangium and deposition of osmiophilic granules in the basement membrane and epithelial cells were furthermore observed.

3) Administration of prednisolone to the rat in the experimental group of A-b-2: (A-b-3)

Rats received an intramuscular injection of prednisolone, 1.5 mg per 100 gm of body weight, prior one hour to an injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver, then, the experiment was carried out in the same manner as in the group A-b-1.

Rats excreted protein as small as below 50 mg per 100 ml through the whole

experimental period and excreted small numbers of erythrocytes during the limited period from the third day to the second week following the injection of antiserum. Levels of serum protein and serum gamma-globulin were normal for three months.

On light microscope, the degree of renal histological changes in this group was less prominent than that observed in the group with a single injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver, (A-b-1). Namely, at three days and one week following the injection of antiserum no detectable renal changes were observed and, even at two and three weeks following the injection, merely slight degree of such glomerular changes as the swelling, collapse, increase in the numbers of nuclei in the lobular tufts and intracapsular exudate of protein-like material were observed sporadically ; at one month following the injection these glomerular changes were inclined to subside.

On electron microscope, the increase in the amount of mesangial matrix was observed within one month following the injection of antiserum but the endothelial and epithelial cells maintained normal figures. A slight degree of thickening of the glomerular basement membrane which was observed during the first month was subsided at two or three months following the injection of antiserum.

4) A single injection of the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver : (A-b-4)

Following a single injection of the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver, rats excreted far less protein and erythrocytes than that observed in the group with a single injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver. Levels of serum protein and serum gamma-globulin were normal.

On light and electron microscopes, the renal histological changes in this group were similar to that observed in the group with a single injection of the rabbit antiserum against the polysaccharide-protein fraction, but, in general, the degree of alteration was far less than that observed in the later group. Thickening of the glomerular basement membrane could not be observed at three months following the injection of antiserum.

5) Repeated injections of the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver : (A-b-5)

In spite of the repeated injections of the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver, the degree of proteinuria and hematuria during one month following the first injection of the antiserum was equal to that observed in the group with a single injection of the antiserum, whereas it was accompanied with an elevation of serum gamma-globulin level.

On light microscope, following one month after the first injection of the

antiserum, repeated injections of the antiserum induced more significant renal changes such as glomerular swelling, increase in the amount of glomerular PAS-positive material and increase in the number of glomerular nuclei than those observed in the group with a single injection of the antiserum. (Fig. 13). In addition, a slight degree of such alterations as degeneration of the tubular epithelial cell, casts in the tubular lumina and cell infiltration in the interstitium were observed.

On electron microscope, the degree of such glomerular changes as the deposition of protein-like material located in the subendothelial space of the capillary wall and the mesangium, the thickening of the basement membrane and the increase in the amount of mesangial matrix was observed significantly and such remarkable changes were maintained for a long period (Fig. 14).

B) Homologous immunological experiment:

a) Experimental groups with homogenate of the rat liver: (B-a-1,-2,-3)

The repeated injections of the homogenate of either normal (B-a-1) or cirrhotic (B-a-2) rat liver combined with Freund's adjuvant into rats resulted a slight degree of proteinuria on the second week, a relative high degree of proteinuria on the first month and an increase of serum gamma-globulin level on the third month.

On light microscope, a slight degree of such glomerular changes as the swelling, the increase in the amount of PAS-positive material, the increase in the numbers of nuclei and the exudate into the capsular space were observed on and after one month following the first injection of the liver homogenate.

On electron microscope, although a slight degree of some mesangial and endothelial changes were already observed on the second week, a significant degree of glomerular chanes, in particular, the thickening of the glomerular basement membrane and the fusion of the epithelial foot processes, were observed on the second month. No detectable renal histological differences were demonstrated between both groups with homogenate of the normal liver and with that of the cirrhotic liver.

The injection of Freund's adjuvant alone (B-a-3) could not result any notable renal histological changes except for a slight degree of the swelling of some of the mesangiums and the disappearance of some of the endothelial slit pores.

b) Experimental groups with the rat antiserum against homogenate of the rat liver: (B-b-1, -2, -3)

A single injection of the rat antiserum against homogenate of either normal (B-b-1), or cirrhotic (B-b-2) rat liver into rats resulted a slight or moderate degree of proteinuria and a slight degree of renal histological changes. In particular, the changes of the glomerular basement membrane and the mesangium were noted and they remained until three months following the injection.

No obvious renal histological differences were observed between both groups with the antiserum against the normal liver and that against the cirrhotic liver on light microscope, but, on electron microscope, the degree of swelling of the glomerular basement membrane was more distinct in the group with the antiserum against the cirrhotic liver.

No detectable renal histological changes were observed in the group whom normal homologous serum was injected intraperitoneally (B-b-3).

C) Experiments with the fluorescent antibody technique :

Sections from the kidney of the rat sensitized with a single injection of the rabbit antiserum against polysaccharide-protein fraction of the rat liver, when stained with rabbit antiserum against the rat liver polysaccharide-protein fraction conjugated with fluorescein, showed an abundant bright deposition of fluorescein on the glomerular basement membrane and mesangium before and after the injection of the antiserum (Table 6).

Sections from the kidney of the rat sensitized with the rabbit antiserum against polysaccharide-protein fraction of the rat liver, when stained with rabbit antiserum against the rat liver gamma-globulin fraction conjugated with fluorescein, showed a slight or a moderate degree of fluorescent deposition on the glomerular basement membrane and mesangium on and after three weeks following the antiserum injection and they were, furthermore, observed on the endothelium on and after one month following the injection.

In the kidney of the rat sensitized with a single injection of the rabbit antiserum against gamma-globulin fraction of the rat liver, when stained with the rabbit antiserum against the rat liver gamma-globulin fraction conjugated with fluorescein, showed a moderate degree of fluorescent deposition on the glomerular basement membrane, mesangium and endothelium on and after one month following an injection of the antiserum.

When the antiserum against the cirrhotic rat liver polysaccharide-protein fraction and the cirrhotic rat liver gamma-globulin fraction conjugated with fluorescein were absorbed with each corresponding antigen, no fluorescein was demonstrated on the sections of the rat kidney. Some degree of fluorescent deposition was, however, demonstrated when the antiserum against the cirrhotic rat liver polysaccharide-protein fraction was absorbed with the supernatant of the normal rat liver homogenate.

D) Experiments with the Ouchterony's technique :

The rabbit antiserum against homogenate of the normal rat liver gave a distinct precipitin band not only against homogenate of the normal rat liver but also against homogenate of the normal rat kidney with the Ouchterony's technique.

The precipitin bands between the rabbit antiserum against homogenate of the

cirrhotic rat liver and homogenate of the cirrhotic rat liver was somewhat wider and greater in number than those observed between the rabbit antiserum against homogenate of the cirrhotic rat liver and homogenate of the normal rat liver (Fig. 15).

The identical precipitation lines were demonstrated among the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver, the rabbit antiserum against cirrhotic rat liver homogenate, the cirrhotic rat liver homogenate and the rat kidney homogenate (Fig. 15).

DISCUSSION

In the parabiosis experiment between both rats with the normal liver and with the cirrhotic liver, the histological changes of the kidney in the normal parabiont was more severe in the glomerulus than in the tubulus. On electron microscope, the kidney showed glomerular changes such as thickening and deposition of protein-like material on the basement membrane, increase in the amount of mesangial matrix, granular deposition on the mesangium, fusion and flattening of the epithelial foot processes, disappearance of slit pores in the endothelial cell and depositions of osmiophilic substance, osmiophilic body and membraneous material in the subendothelial space of the capillary wall and the mesangium.

Although these glomerular changes were similar to those observed in the patients with liver cirrhosis,[11] it could not be concluded that the renal histological changes in the parabiotic rat is induced by liver cirrhosis itself, because they are not pathognomonic to liver cirrhosis. They were, however, quite different from the renal changes that observed in toxic nephrosis, because the major renal histological change induced by such nephrotoxin as DAB and carbon tetrachloride was observed in the tubulus.

The purpose of parabiosis in this experiment was to avoid renal lesion coincidental with hepatic lesion by DAB and carbon tetrachloride. The results of the parabiosis experiment suggest that a humoral factor, which is toxic or antigenic to the kidney, can be liberated from the injured tissue of the liver into the circulation, thus inducing secondarily a renal lesion on the normal parabiont.

It was demonstrated that nephrosis induced by an immunological procedure could be transfered through peritoneal parabiosis.[12] The fact suggests that a similar immunological mechanism may be involved in the development of renal lesion from the rat with liver lesion in the parabiosis experiment.

Heymann et al.[2] observed a slight degree of renal lesion among a few cases of rats after repeated intraperitoneal injections of an extract of the liver tissue incorporated with Freund's adjuvant, but other investigators[13][14] could not observe

any renal lesion in a similar experiment on guinea pigs. At all events, it seems very difficult to induce the renal histological lesion with the injection of an extract of liver tissue in the homologous sensitizing experiment. We could demonstrate, however, somewhat severe glomerular changes on electron microscope, though it was slight on light microscope, in the homologous sensitizing experiment, when the volume and the numbers of injections of the antigen from the cirrhotic rat liver were increased. Proteinuria was also observed in these rats.

In the heterologous sensitizing experiment, furthermore, a single injection of the rabbit antiserum against homogenate of the rat liver induced a distinct renal histological change in the glomerular basement membrane and mesangium. In this case, the antigen from the cirrhotic liver tissue induced much severe renal changes than those observed by the antigen from the normal liver tissue.

In addition to the results above-mentioned, the fact that a precipitin band was demonstrated between the antiserum against homogenate of the rat liver and both homogenates of rat hepatic tissue and renal tissue, suggests that an antibody against the hepatic tissue may react with the renal tissue.

The precipitin bands between the rabbit antiserum against homogenate of the cirrhotic rat liver and the homogenate of the cirrhotic rat liver was somewhat wider and greater in number than those observed between the rabbit antiserum against homogenate of the cirrhotic rat liver and the homogenate of the normal rat liver. It suggests that cirrhotic liver tissue contains some sorts of antigenic agent(s) differed from that contained in the normal liver tissue and that the same antigenic agents as contained in the normal liver tissue is increased in the cirrhotic liver tissue.

On the tissue culture experiment, Hill[15] has demonstrated that sera from patients with infectious hepatitis induce destruction of chimpanzee kidney cells and that the destructive agents are serologically cross-reactive with the serum in fluorescent antibody and neutralization tests. Using complement fixation technique, Wagner et al.[16] have demonstrated an anti-kidney antibody in the serum from patients with eclampsia and the cross-reaction of the serum with the liver tissue. These results are coincident with our experimental data which were obtained in the fluorescent antibody technique.

Okuda and Grollman[17] have recently reported renal lesions in rats following the injection of placental extracts and have suggested an immunological process in the development of renal lesions in patients with eclampsia.

It has been reported that the polysaccharide (-protein) of renal tissue seems to be the true antigenic agent rather than the protein itself in the development of nephrotoxic nephritis and that anti-kidney antibody is located in the glomerular basement membrane.[18]

For the purpose of clarifying the hepatogenous antigenic agent (s) against the

kidney, antigenicity of both polysaccharide-protein and gamma-globulin fractions of the cirrhotic rat liver was examined on heterologous immunological experiments.

An injection of the antiserum against the polysaccharide-protein fraction induced a more significant renal histological lesion than that observed after an injection of the antiserum against the gamma-globulin fraction. Repeated injections of the antiserum against the polysaccharide-protein fraction, furthermore, produced an aggressive renal histological changes from the early stage, while that of the antiserum against the gamma-globulin fraction produced it far later.

Sections from the kidney of the rat sensitized with the rabbit antiserum against polysaccharide-protein fraction of the rat liver, when stained with rabbit antiserum against the rat liver polysaccharide-protein fraction conjugated with fluorescein, showed an abundant bright deposition of fluorescein on the glomerular basement membrane and mesangium before and after the injection of the antiserum. In the kidney of the rat sensitized with the injection of the rabbit antiserum against the gamma-globulin fraction of the rat liver, when stained with rabbit antiserum against rat liver gamma-globulin fraction conjugated with fluorescein, however, showed no fluorescent deposition on the glomerular basement membrane and mesangium as long as one month following the injection of antiserum.

The results above obtained suggest that the polysaccharide-protein fraction of hepatic tissue may play an important role in the development of renal lesion in patients with chronic liver disease. The antigen in the polysaccharide-protein fraction of the liver may directly react with the glomerular basement membrane through an immunological specific activity, thus resulting an early lesion of the kidney. On the other hand, the gamma-globulin fraction which has no direct reaction on the glomerulus may deposit on the glomerular basement membrane through a biological filtration mechanism, then his own antigen which has secondarily been produced by glomerular deposits may induce the renal lesion. Further studies with more purified polysaccharide-protein, however, are necessary because the polysaccharide-protein fraction obtained in this experiment is so crude as to make a conclusion on this problem.

It has been reported that a single injection of anti-nephrotoxic duck serum into rabbits can induce persisting and aggraviating glomerular changes while duck serum gamma-globulin as a carrier of antibody had mostly disappeared from the glomerulus within two months following the injection.[19]

In our experiments some glomerular histological changes were remained on three months following an injection of the rabbit antiserum against homogenate of the cirrhotic rat liver, the rabbit antiserum against polysaccharide-protein

fraction of the cirrhotic rat liver or the homologous antiserum against homogenate of the cirrhotic rat liver, some degrees of hyper-gamma-globulinemia were observed in the course of renal lesion and prednisolone depressed the glomerular changes following the injections of antisera. These results suggest that an autoimmune mechanism may have some role in the development of the renal lesion in patients with chronic liver disease.

Heymann et al.[20] have reported that Freund's adjuvant itself is nephrotoxic. In the present experiment, however, rats treated with Freund's adjuvant alone have shown no detectable histological change.

CONCLUSION

For the purpose of clarifying the mechanism in the development of renal lesion in patients with chronic liver disease, the animal experiments on the standpoint of immunological aspect were carried out, obtaining the following results.

1) Rats were treated with the oral administration of DAB and the inhalation of carbon tetrachloride gas in order to produce liver cirrhosis. At two months following the peritoneal parabiosis of the rat with the cirrhotic liver and that with the normal liver, albuminuria and histological renal lesion were observed on the side of the rat with the normal liver.

2) A single intravenous injection of the rabbit antiserum against homogenate of the normal or cirrhotic rat liver into rats caused albuminuria and renal histological lesion. The degree of renal lesion was more prominent in the case of cirrhotic liver homogenate than that observed in the case of normal liver homogenate. Antisera were obtained by repeated intraperitoneal injections of liver homogenate simultaneously with Freund's adjuvant.

3) With the Ouchterony's technique a common antibody was suggested between the rabbit antiserum against homogenate of the cirrhotic rat liver and each homogenate of the normal rat liver, the cirrhotic rat liver or the normal rat kidney.

4) A single injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver induced a more prominent renal histological lesion than that observed after an injection of the rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver. Repeated injections of the antiserum against the polysaccharide-protein fraction caused an aggressive renal histological changes from the early stage, while those of the antiserum against the gamma-globulin fraction caused it far later.

5) Sections from the kidney of the rat sensitized with the rabbit antiserum against polysaccharide-protein fraction of the rat liver, when stained with the

rabbit antiserum against rat liver polysaccharide-protein fraction conjugated with fluorescein, showed an abundant bright deposition of fluorescein on the glomerular basement membrane and the mesangium before and after the injection of the antiserum. However, in the kidney of the rat sensitized with the rabbit antiserum against gamma-globulin fraction of the rat liver, when stained with the rabbit antiserum against rat liver gamma-globulin fraction conjugated with fluorescein, showed no fluorescent deposition on the glomerular basement membrane and the mesangium as long as one month following an injection of the antiserum.

6) Repeated intraperitoneal injections of either homogenate of the normal or cirrhotic rat liver, simultaneously with Freund's adjuvant, caused a slight degree of renal histological lesion in rats.

7) Prednisolone depressed the renal lesion following an injection of the rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver.

8) The results suggest that an auto-immune mechanism may have some role in the development of renal lesion in patients with chronic liver disease.

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Table 1. Light microscopic renal lesions in the parabiosis experiment.

Renal Lesions	Normal liver parabiont (9 rats)	Damaged liver parabiont (9 rats)	Control parabionts (10 pairs)
Glomerulus			
Exudate into capsular space	−	−	−
Swelling of capsular epithelium	±	±	±
Increased cellularity of tuft	±	±	±
Thickening of lobular stalk	±	±	±
Epithelial desquamation	−	−	−
Capillary collapse	+	+	−
Proximal tubules			
Dilatation of lumina	−	−	−
Pyknosis of nuclei	±	±	−
Casts	±	±	−
Epithelial desquamation	−	−	−
Swelling of epithelium	±	±	±
Distal tubules			
Dilatation of lumina	+	+	±
Pyknosis of nuclei	−	±	−
Cellular casts	−	−	−
Hyaline casts	+	+	−
Granular casts	−	−	−
Pigmented casts	−	−	−
Epithelial desquamation	−	−	−
Fatty vacuolation	±	−	−
Tubal rupture	±	±	−
Regeneration	−	−	−
Collecting tubules			
Casts	−	−	−
Blood vessels			
Angitis	−	−	−
Cortical collapse and medullary hyperemia	±	±	±
Interstitium			
Edema	−	−	−
Inflammatory cell infiltration	±	±	−
Granulomatous reaction	−	−	−

Table 2. Electron microscopic renal lesions in the parabiosis experiment.

Renal Lesions	Damaged liver parabiont (5 rats)	Control group (5 pairs)
Mesangium		
Proliferation	±	−
Swelling	±	−
Increased matrix	±	±
Granular deposit	+	±
Basement membrane		
Thickening	±	±
Deposit of protein-like material	±	±
Epithelial cell		
Blunted foot processes	+	±
Fusion of foot processes	±	±
Swelling of podocyte	±	±
Vacuolization	−	±
Endothelial cell		
Swelling	±	±
Desquamation	±	−
Disappearance of slit pores	±	±
Vacuolization	−	−
Bowman's capsula		
Thickening of basement membrane	±	−
Fusion with tuft	±	±
Swelling of epithelial cell	±	−
Narrowing of capsular space	±	±
Specific findings in mesangium and basement membrane		
Membraneous material	±	±
Osmiophilic material	+	±
Osmiophilic body	+	−

Table 3. Microscopic findings of the kidney in rats injected with rabbit antiserum against homogenate of the rat whole liver.

		Days after the first injection	A-a-1						A-a-3						A-a-1 (control)					
			3	7	14	21	30	90	3	7	14	21	30	90	3	7	14	21	30	90
Light microscopic findings	Glomerulus	Swelling	−	±	+	−	−	−	−	+	+	+	−	−	−	±	−	−	−	−
		Increased cellularity of tuft	−	±	+	±	±	−	−	+	++	±	±	−	−	−	−	−	−	−
		Thickening of basement membrane	−	±	±	+	+	±	±	+	++	+	+	+	−	−	±	−	−	−
		Protein-like exudate into urinary space	−	±	±	−	−	−	−	±	±	−	−	−	−	−	−	−	−	−
		Erythrocyte in urinary space	−	±	±	−	−	−	−	±	±	±	−	−	−	−	−	−	−	−
		Adhesion with Bowman's capsule	−	−	−	±	−	−	−	±	±	±	−	−	−	−	−	−	−	−
		Epithelial desquamation	−	±	±	−	−	−	±	±	+	+	−	−	−	−	−	−	−	−
		Collapse	−	+	+	±	−	−	±	+	+	+	±	−	−	−	−	−	−	−
	Tubulus	Cloudy swelling of epithelium	−	±	±	±	±	−	−	±	±	−	−	−	−	−	−	−	−	−
		Pyknosis of nuclei	−	±	±	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
		Vacuolation	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−
		Casts	−	−	+	−	−	−	−	−	±	+	−	−	−	−	−	−	−	−
	Interstitium	Cell infiltration	−	−	−	−	±	−	−	−	−	±	−	+	−	−	−	−	−	−
		Fibrosis	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−	−
Electron microscopic findings	Basement membrane	Thickening	−	−	±	±	±	±	−	±	++	++	++	+	−	−	−	−	−	−
		Loosing	−	±	±	±	±	±	−	±	+	+	±	−	−	−	−	−	−	−
		Deposit of protein-like material	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−	−
	Mesangium	Proliferation	−	−	±	±	±	−	±	−	+	±	±	−	−	−	±	−	−	−
		Swelling	±	±	+	−	±	−	±	+	+	±	−	±	−	±	±	−	−	−
		Increase of matrix	−	±	±	−	±	−	−	+	±	±	−	−	−	−	−	−	−	−
		Granular osmiophilic deposit	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Epithelial podocyte	Swelling	±	±	+	−	−	−	±	+	−	+	−	−	−	−	−	−	−	−
		Cytoplasmic change	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Vacuolation	±	±	+	−	−	−	±	−	+	+	−	−	−	−	−	−	−	−
	Epithelial foot process	Fusion	−	±	±	−	±	±	−	+	++	±	+	±	−	−	−	−	−	−
		Flattening	−	±	±	−	±	−	−	+	++	±	+	±	−	−	−	−	−	−
	Endothelium	Swelling	−	−	+	−	−	−	−	−	+	±	−	−	−	±	−	−	−	−
		Desquamation	−	±	+	−	−	−	±	+	++	++	±	±	−	±	−	−	−	−
		Disappearance of slit pore	±	+	+	−	−	−	±	+	++	++	±	±	−	−	±	−	−	−
		Vacuolation	−	−	±	−	−	−	±	+	++	++	±	±	−	−	±	−	−	−
	Thickening of basement membrane in Bowman's capsule		−	−	+	±	±	±	−	±	±	±	±	±	−	−	−	−	−	−
		Albuminuria	±	++	+	++	±	−	−	±	++	+	+	++	−	−	−	±	−	−
		Hematuria	±	±	+	±	−	−	±	±	±	−	−	−	−	−	±	−	−	−
		Serum gamma-globulin (%)	22	20	21	19	22	22	19	22	22	21	22	25	20	19	18	20	20	19

Table 4. Microscopic findings of the kidney in rats injcted with rabbit antiserum against extracts of the cirrhotic rat liver.

		Days after the first injection	A-b-1						A-b-4						A-b-2						A-b-5						A-b-3 (control)					
			3	7	14	21	30	90	3	7	14	21	30	90	3	7	14	21	30	90	3	7	14	21	30	90	3	7	14	21	30	90
Light microscopic findings	Glomerulus	Swelling	±	+	++	+	±	−	±	+	±	−	−	−	−	+	++	++	++	++	−	±	±	±	±	±	−	±	±	−	−	−
		Increased cellularity of tuft	−	±	+	±	−	−	−	±	±	−	−	−	−	±	+	+	++	++	−	±	−	±	±	±	−	±	±	−	−	−
		Thickening of basement membrane	−	±	+	+	±	±	−	±	±	−	−	−	−	±	+	+	++	+++	−	−	±	±	+	++	−	−	±	−	−	−
		Protein-like exudate into urinary space	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	+	+	−	−	−	−	±	±	−	−	−	−	−	−
		Erythrocyte in urinary space	−	±	−	−	−	−	±	−	−	−	−	−	−	±	+	+	++	++	±	−	±	±	+	++	−	−	−	−	−	−
		Adhesion with Bowman's capsule	±	±	±	±	−	−	±	±	−	−	−	−	±	±	+	+	+	±	±	±	±	±	±	±	−	±	±	−	−	−
		Epithelial desquamation	±	±	±	−	−	−	±	−	−	−	−	−	±	±	+	+	±	±	±	±	±	±	±	±	−	±	±	−	−	−
		Collapse	−	−	±	±	−	−	−	−	−	−	−	−	−	−	±	±	+	+	−	−	−	−	±	±	−	−	−	−	−	−
	Tubulus	Cloudy swelling of epithelium	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	±	±	−	−	±	±	±	±	−	±	±	−	−	−
		Pyknosis of nuclei	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Vacuolation	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Casts	−	−	±	−	−	−	−	−	−	−	−	−	−	−	±	±	+	±	−	−	−	−	±	±	−	±	−	−	−	−
	Inter-stitium	Cell infiltration	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	±	+	−	−	−	−	−	±	−	−	−	−	−	−
		Fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	±	−	−	−	−	−	−
Electron microscopic findings	Basement membrane	Thickening	−	±	+	±	±	±	−	±	±	−	−	−	−	±	+	++	++	+++	−	−	−	±	+	++	−	±	±	−	−	−
		Loosing	−	−	±	±	−	−	−	−	−	−	−	−	−	±	±	+	+	++	−	−	−	−	±	+	−	±	−	−	−	−
		Deposit of protein-like material	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	++	−	−	−	−	−	++	−	−	−	−	−	−
	Mesangium	Proliferation	−	±	±	−	−	−	−	±	−	−	−	−	±	−	±	+	++	++	−	±	−	±	+	++	−	±	−	−	−	−
		Swelling	±	+	++	+	±	−	−	±	±	−	−	−	±	±	±	±	±	±	−	±	+	±	+	+	−	−	−	−	−	−
		Increase of matrix	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	+	−	−	−	−	±	+	−	−	−	−	−	−
		Granular osmiophilic deposit	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	±	−	−	−	−	−	−
	Epithelial podocyte	Swelling	−	±	+	±	−	−	−	+	±	−	−	−	−	±	+	++	++	+++	−	±	+	+	+	++	−	±	−	−	−	−
		Cytoplasmic change	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+	−	−	−	−	−	+	−	−	−	−	−	−
		Vacuolation	−	±	±	−	−	−	−	−	−	−	−	−	−	±	±	+	++	++	−	±	±	−	±	+	−	±	−	−	−	−
	Epithlial foot process	Fusion	±	+	+	±	±	±	±	±	±	±	−	−	±	+	+	+	+	+	±	±	±	±	±	++	−	±	±	−	−	−
		Flattening	−	±	±	±	−	−	−	±	−	−	−	−	−	±	±	±	±	±	−	−	−	±	±	+	−	±	−	−	−	−
	Endothelium	Swelling	−	±	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	±	−	−	−	−	−	±	−	−	−	−	−	−
		Desquamation	±	+	±	−	−	−	−	+	−	−	−	−	±	+	+	+	±	±	±	±	±	±	±	+	−	±	−	−	−	−
		Disappearance of slit pore	±	+	±	±	−	−	±	±	±	±	−	−	±	±	+	+	+	++	±	±	±	+	±	+	−	±	−	−	−	−
		Vacuolation	−	±	−	−	−	−	−	−	−	−	−	−	±	±	±	±	±	+	±	−	−	−	±	+	−	±	−	−	−	−
	Thickening of basement membrane in Bowman's capsule		−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	±	+	+	−	−	−	−	±	+	−	−	−	−	−	−
		Albumiuria	−	±	++	+	+	±	−	±	+	+	±	±	±	+	++	++	++	++	±	+	+	±	+	+	−	−	±	±	−	−
		Hematuria	±	±	++	±	±	−	±	+	−	−	−	−	++	++	++	+	++	+	−	−	±	−	±	−	±	±	±	−	−	−
		Serum gamma-globulin (%)	20	19	17	18	20	22	19	19	18	29	20	23	15	18	15	19	25	31	18	15	16	16	22	26	18	16	19	19	20	21

Table 5. Microscopic findings of the kidney in rats injected with homogenate of the rat whole liver and rat antiserum against the rat whole liver.

		Days after injection	B-a-1		B-a-2		B-a-3		B-b-1						B-b-2						B-b-3 (control)					
			30	60	30	60	30	60	3	7	14	21	30	90	3	7	14	21	30	90	3	7	14	21	30	90
Light microscopic findings	Glomerulus	Swelling	−	±	−	−	−	−	−	±	±	−	−	±	−	±	±	−	−	±	−	−	−	−	−	−
		Increased cellularity of tuft	−	±	±	±	−	−	−	±	±	−	−	−	−	±	±	−	−	−	−	−	−	−	−	−
		Thickening of basement membrane	±	±	±	±	−	−	−	±	±	+	±	±	−	+	+	±	±	±	−	−	−	−	−	−
		Protein-like exudate into urinary space	±	±	−	−	−	−	−	±	−	−	−	−	−	±	−	−	−	−	±	−	−	−	−	−
		Erythrocyte in urinary space	−	−	−	−	−	−	−	±	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
		Adhesion with Bowman's capsule	±	−	−	−	−	±	±	±	±	−	−	−	−	+	±	±	−	−	±	−	−	−	−	−
		Epithelial desquamation	±	±	−	±	−	−	±	±	±	−	−	−	±	−	±	−	−	−	−	−	−	−	−	−
		Collapse	−	−	−	±	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−
	Tubulus	Cloudy swelling of epithelium	−	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	±	−	−	−	−	−	−
		Pyknosis of nuclei	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Vacuolation	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−
		Casts	−	−	−	±	−	−	−	−	−	−	−	±	−	−	−	−	−	±	−	−	−	−	−	−
	Interstitium	Cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Electron microscopic findings	Basement membrane	Thickening	+	±	±	±	±	±	−	±	±	±	±	±	−	±	+	±	±	+	−	±	−	−	−	−
		Loosing	±	±	−	±	±	−	−	−	±	±	−	−	−	±	−	−	−	−	−	−	−	−	−	−
		Deposit of protein-like material	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Mesangium	Proliferation	−	±	±	±	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
		Swelling	−	±	±	±	−	−	±	±	±	±	±	±	±	±	+	±	−	±	±	±	−	−	−	−
		Increase of matrix	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
		Granular osmiophilic deposit	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Epithelial podocyte	Swelling	±	±	−	±	−	−	−	±	±	±	−	−	±	±	−	−	−	−	−	±	−	−	−	−
		Cytoplasmic change	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Vacuolation	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−
	Epithelial foot process	Fusion	±	+	+	+	−	±	−	±	±	±	±	±	−	+	±	−	±	±	±	−	−	−	−	−
		Flattening	−	±	±	±	−	±	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
	Endothelium	Swelling	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Desquamation	−	−	−	±	−	−	−	±	−	−	−	−	−	±	±	−	−	−	−	−	−	−	−	−
		Disappearance of slit pore	±	±	+	±	−	−	±	±	±	−	−	−	±	±	±	±	±	−	±	−	−	−	−	−
		Vacuolation	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
	Thickening of basement membrane in Bowman's capsule		+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	±	−	±	−	−	−	−	−	−
		Albuminuria	+	±	+	+	−	−	−	−	+	±	±	±	±	±	±	+	+	+	−	±	±	−	−	−
		Hematuria							−	±	±	±	−	−	−	±	±	−	−	−	−	−	−	−	−	−
		Serum gamma-globulin (%)	21	25	24	27	20	20	16	21	21	20	22	22	20	18	19	23	23	24	18	21	20	21	19	18

Table 6. Fluorescent microscopic observations of the kidney in rats injected with rabbit antiserum against polysaccharide-protein or gamma-globulin fraction of the cirrhotic rat liver.

Tissue used	Staining solution		Before injection	Days after the first injection 3	7	14	21	30	60	90
Kidney from the rats of group A-b-2	Fluorescein-labeled rabbit antiserum to polysaccharide-protein fraction of cirrhotic rat liver (A)	Epithelium	−	−	−	−	−	−	−	−
		Basement membrame & Mesangium	++	++	++	++	++	++	++	++
		Endoththelium	−	−	−	−	−	−	−	−
Kidney from the rats of group A-b-2	Fluorescein-labeled rabbit antiserum to gamma-globulin fraction of cirrhotic rat liver (B)	Epithelium		−	−	−	−	−	−	−
		Basement membrane & Mesangium		−	−	−	±	+	+	+
		Endothelium		−	−	−	−	±	±	±
Kidney from the rats of group A-b-5	(B)	Epithelium	−	−	−	−	−	−	−	−
		Basement membrane & Mesangium	−	−	−	−	−	±	+	+
		Endothelium	−	−	−	−	±	±	+	+
Kidney from the rats of group A-b-2	(A) after absorption with polysaccharide-protein fraction of the cirrhotic rat liver		−	−	−	−	−	−	−	−
	(A) after absorption with suspension of the normal rat whole liver		−～±	±	−	±	±	±	±	±
Kidney from the rats of group A-b-5	(B) after absorption with gamma-globulin fraction of cirrhotic rat liver		−	−	−	−	−	−	−	−
	(B) after absorption with suspension of the normal rat whole liver		−	−	−	−	−	−	±	±
	Blocking test		−	−	−	−	−	−	−	−

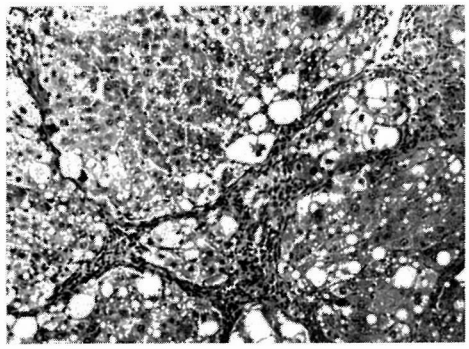

Fig. 1. × 240

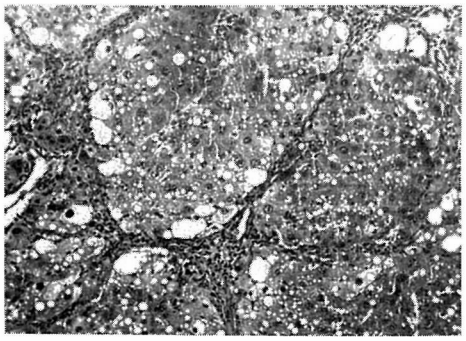

Fig. 2. × 240

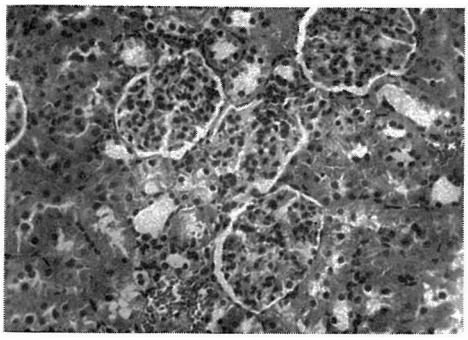

Fig. 3. × 240

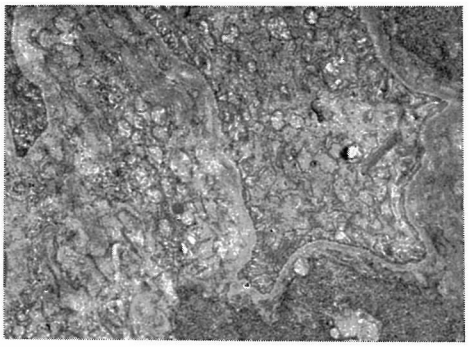

Fig. 4. × 5500

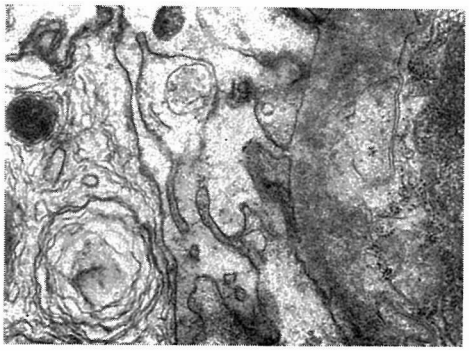

Fig. 5. × 16400

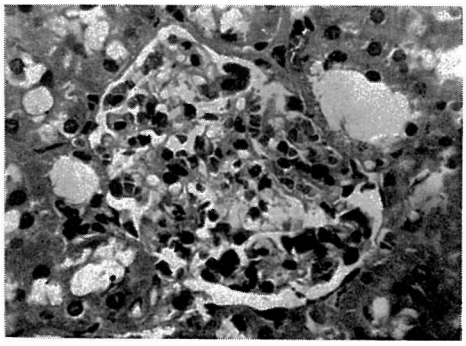

Fig. 6. × 480

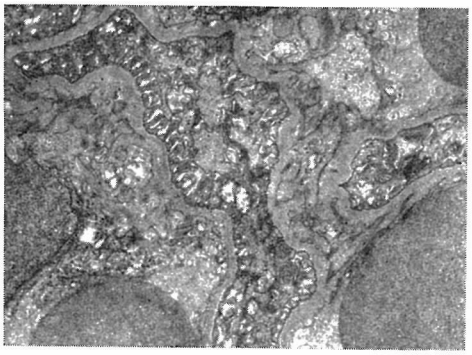

Fig. 7. × 5500

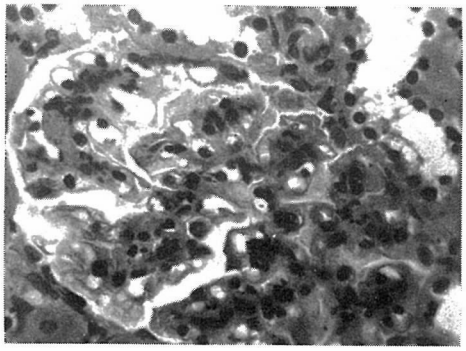

Fig. 8. × 960

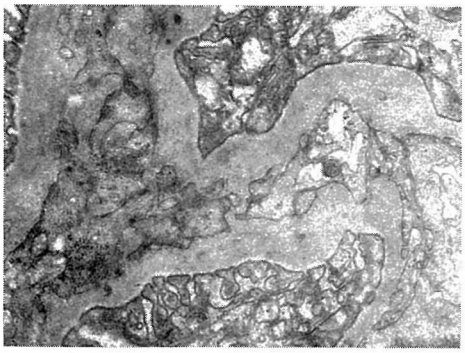

Fig. 9. × 11000

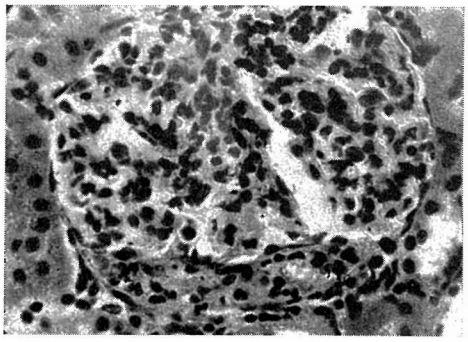

Fig. 10. × 720

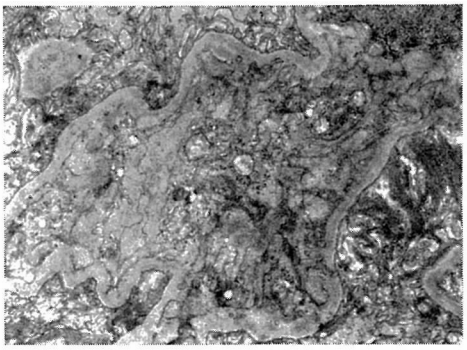

Fig. 11. × 5500

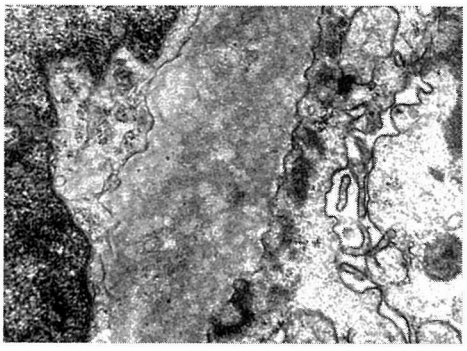

Fig. 12. × 13800

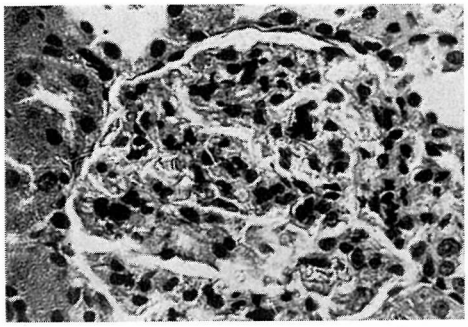

Fig. 13. × 720

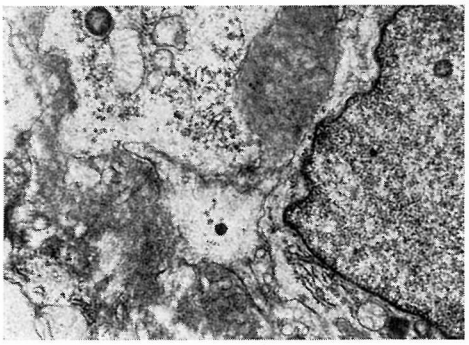

Fig. 14. × 11000

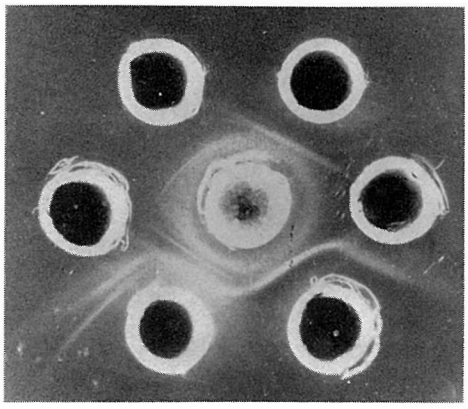

Fig. 15. Ouchterlony's agar plate
A: homogenate of the cirrhotic rat liver. B: rabbit antiserum against homogenate of the normal rat liver. C: rabbit antiserum against gamma-globulin fraction of the cirrhotic rat liver. D: homogenate of the normal rat kidney. E: rabbit antiserum against homogenate of the cirrhotic rat liver. F: rabbit antiserum against polysaccharide-protein fraction of the cirrhotic rat liver.