CHRONIC ALLOGENEIC DISEASE

I. Development of Glomerulonephritis*

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The type of allogeneic disease that occurs after the administration of foreign lymphoid cells to either neonates or X-irradiated recipients is usually acute and rapidly fatal. In both laboratory animals and humans, its characteristic manifestations are wasting (runting), dermatitis, diarrhea, and atrophy of lymphoid tissue (1–3). Another, more chronic form of the disease can develop when mature hosts are given relatively low doses of allogeneic lymphoid cells (4). These experimental conditions, if applied to a system in which inbred animals donate lymphoid cells to their F_1 hybrids, can result in prolonged confrontations between intolerant (donor) immunocompetent cells and indigenous (host) antigens. The ensuing chronic allogeneic disease represents a model of certain types of autoimmunization (5).

In the present experiments, glomerulonephritis was induced by transplanting sublethal doses of normal BALB/c spleen cells to mature (BALB/c $\times$ A/Jax)F_1 mice. The renal lesion, which was often accompanied by a severe nephrotic syndrome, was membranous glomerulonephritis. As judged by light, immunofluorescent, and electron microscopy, its morphology was typical of an immune complex–induced nephritis. This disorder is proposed as a model of glomerulonephritis provoked by intolerant immunocytes, since in all likelihood antigens native to the host were involved.

Materials and Methods

Animals.—BALB/c, A/Jax, and (BALB/c $\times$ A/Jax)F_1 (hereafter referred to as CAF_1) mice were obtained from Jackson Laboratories, Bar Harbor, Maine. CAF_1 mice bred in our own laboratory from Jackson parental stocks were used in many experiments. The animals were housed in disposable plastic cages and fed standard mouse diets.

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Preparation of Spleen Cell Suspensions.—The BALB/c donors were killed by cervical fracture and their spleens were removed and processed aseptically. Pooled spleens were minced in Ringer's solution and then gently pressed through tantalum gauze with a small test tube. Cell viability was checked with trypan blue, and the number of living cells in the suspension was adjusted to the desired concentration. The dose of cells ranged from 10×10^6 to 200×10^6. The cells were given intraperitoneally; except for the group of mice given 10×10^6 cells, each injection contained 50–80×10^6 cells and the total amount was achieved by weekly injections. For example, mice to receive 150×10^6 spleen cells were given two injections of 75×10^6 cells a week apart.

Experimental Design.—The CAF_1 recipients were 6 wks old when the first injection of BALB/c spleen cells was given. Various groups of these mice were organized so that the time elapsed between the last dose of parental spleen cells and the day of autopsy ranged from 1 month to 1 yr. Mice with an overt nephrotic syndrome were killed when moribund.

Chemical Pathology.—The degree of proteinuria was estimated with a Uristix®. Blood urea nitrogen was measured colorimetrically using the Hyland micromethod (UN-Test®). Electrophoresis of serum was carried out on cellulose acetate strips, which were stained with Ponceau-S.

Histological Studies.—Tissues were fixed in 10% buffered formalin and sections of kidney were cut at 6 μ and stained with hematoxylin and eosin and periodic acid–Schiff (PAS). Sections representative of all stages of the disease were also stained with Congo red.

Immunofluorescent Studies.—

Rabbit anti-mouse gamma globulin: Commercial mouse gamma globulin (Pentex, Inc., Kankakee, Ill., Lot No. 8) was used to immunize rabbits. The antiserum obtained after multiple injections of this antigen produced a single precipitin arc in the zone of IgG when reacted against whole mouse serum in immunoelectrophoresis. A crude gamma globulin fraction of this reagent was obtained by fractionation in 50% ammonium sulfate. The rabbit globulins were further purified by DEAE-cellulose chromatography using 0.02 M phosphate-buffered saline (PBS), pH 6.3 (6).

Rabbit anti-rat β_{1C} globulin: The 7S gamma globulin fraction of rabbit anti-mouse β_{1C}-globulin was prepared in the manner of Mardiney and Müller-Eberhard (7). Immunoelectrophoresis of this reagent revealed a single precipitin arc when reacted against whole mouse serum.

Goat anti-rabbit 7S gamma globulin: The globulin fraction of goat serum containing antibodies to rabbit globulins was obtained from Microbiologic Associates, Bethesda, Md. (Lot No. 64011). Upon immunoelectrophoresis, it developed three bands in the gamma globulin region when reacted against whole rabbit serum.

Conjugation of antisera: The 7S gamma globulin fractions of antisera were adjusted to a concentration of 10 mg of protein/ml and conjugated to fluorescein isothiocyanate by the dialysis method of Clark and Shepard (8). Conjugated antisera were twice absorbed with lyophilized mouse kidney powder (50 mg of powder/ml of conjugate) before staining.

Preparation of tissue: Tissue was prepared for fluorescent antibody studies by snap freezing in a dry ice–acetone bath. Cryostat sections were cut at 4 and 6 μ, placed in a humid chamber, and refrigerated at 4°C until ready for use. Long-term storage of tissue sections (weeks) was possible if the sections were air-dried for 2 hr at room temperature and stored dry at 4°C. Cryostat sections were washed in 0.15 M PBS, pH 7.2, for 5 min, fixed in equal volumes of absolute alcohol and ether for 10 min at room temperature, fixed in 95% ethanol for 20 min, and twice washed in PBS prior to staining.

Fluorescent staining: Standard direct and indirect staining techniques were employed. The period of staining was 45 min in all procedures, followed by two 10 min washes in PBS. Examination of the tissue sections was performed with a Leitz Ortholux microscope utilizing an

HBO 200 W mercury vapor high pressure lamp, a B6 38 red-suppressing filter, and a UG-1 ultra violet filter. Photomicrographs were recorded on Kodak high speed Ektachrome film.

Control procedures used with the fluorescein staining techniques were as follows: (*a*) sections of normal mouse kidneys were stained concurrently with sections of test kidney; (*b*) fluorescent staining was blocked by prior incubation of the purified, conjugated reagents with their corresponding antigens; (*c*) fluorescent staining was diminished by incubation of the unconjugated reagent on the slide before staining with fluorescein conjugate of the same antiserum.

Electron Microscopy.—Fragments of kidney were fixed for 30 min in 6.5% glutaraldehyde (9) and postfixed with 1% osmium tetroxide buffered with 0.1 M phosphate buffer (pH 7.2). The tissues were then dehydrated in graded alcohols and embedded in epon (10). Thick sections (0.5 μ) were cut on a Porter-Blum microtome with glass knives and stained with azure and methylene blue (11). After identification of glomeruli under the light microscope, ultrathin sections were cut, mounted on uncoated grids, and stained with lead citrate (12). The sections were examined with an RCA 3 C electron microscope (50 kv, objective aperture 50 μ).

TABLE I

Clinical Abnormalities in CAF_1 Mice following Administration of BALB/c Spleen Cells

Time after spleen cell injection	No. of mice	Body weight	Urine protein (mg/100 ml)		Ascites	"Nephrotic" ELP*	Lactescent serum
			30–100	300–1000			
months		*g*	%	%	%	%	%
1–2	27	23.4 ± 8.5‡	0.0	0.0	0.0	0.0	0.0
3–5	56	29.7 ± 3.7	54.8	45.2	31.0	40.5	26.2
6–9	32	28.4 ± 4.3	59.5	40.5	9.5	17.0	3.2
10–12	45	28.5 ± 3.9	89.7	10.3	0.0	10.0	0.0

*By "nephrotic" ELP is meant the type of electrophoretic pattern of serum shown in Fig. 1 and Fig. 2 (bottom row).

‡1 SD.

Elution of Kidney-Bound Proteins.—Whole mouse kidneys were minced with scissors and forced through fine mesh tantalum gauze. The resulting slurry was washed in phosphate-buffered saline, pH 7.2, at 4°C until a colorless supernatant was obtained. The washed sediment was incubated with 0.02 M citrate buffer, pH 3.2, for 2 hr at 37°C with constant stirring. 10 volumes of buffer were incubated with each volume of kidney sediment. The sediment was then centrifuged at 2000 *g* for 30 min and the supernatant was dialyzed against phosphate-buffered saline, pH 7.2, for 48 hr at 4°C. The dialysate was concentrated by pervaporation to a final protein concentration of 10 mg/ml.

RESULTS

Clinical Observations.—The syndrome of acute allogeneic disease did not develop in any of the various groups of mice. None of the animals appeared runted, and neither dermatitis nor diarrhea was observed. 3–5 months after inoculation of the parental cells, about one-third of the recipients had a nephrotic syndrome characterized by ascites, proteinuria, hypoalbuminemia, and

lactescent serum (Table I, Figs. 1–3). This was not seen in the older animals. However, some bias favoring a higher incidence of the nephrotic syndrome in younger mice was introduced because animals were usually killed when overt

FIG. 1. Electrophoretic patterns of serum proteins from nephrotic CAF_1 mice with chronic allogeneic disease. A severe reduction in serum albumin with an accompanying increase in an alpha globulin was characteristic. In mouse C36-33, the only detectable protein was an alpha globulin.

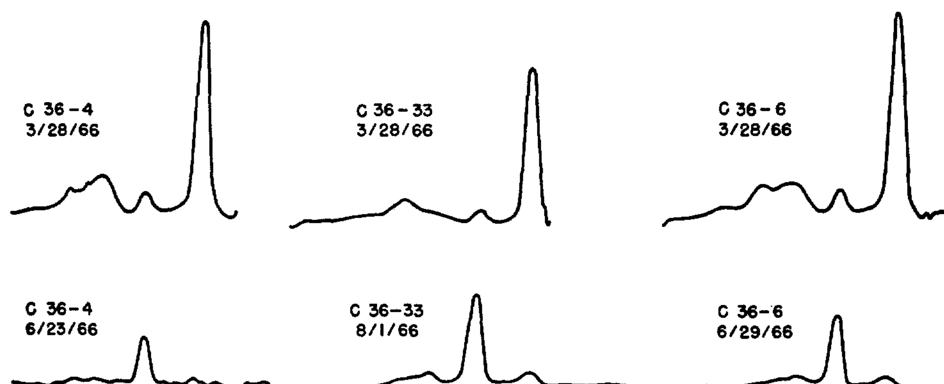

FIG. 2. Serum electrophoretic patterns before (top line) and after (bottom line) the development of glomerulonephritis and the nephrotic syndrome. Note the disappearance of albumin and gamma globulins and the appearance of a homogeneous band in the alpha globulin region.

ascites developed. In a few instances, spontaneous disappearance of ascites was noted.

Azotemia rarely occurred even when the nephrotic syndrome was severe. Of 87 animals tested, only three had blood urea nitrogen levels exceeding two standard deviations of the normal value (27.9 ± 7.6). The nephrotic syndrome occurred in about 10% of mice studied 6–9 months after injection of BALB/c spleen cells; but proteinuria of at least 300 mg/100 ml was present in 40% of these animals (Table I). In still older mice, only 10% had proteinuria exceeding

300 mg/100 ml; nevertheless, histological evidence of glomerulonephritis was present in more than one-half of them (Tables I and II). By contrast with younger mice, these older animals tended to develop hyperglobulinemia (Fig. 4).

Pathology of the Kidney.—The kidneys of 50 6-month old normal CAF_1 mice were examined (Table III). The only abnormalities, found in one-third of the animals, were focal pyelitis and periarterial accumulations of lymphocytes and plasma cells. Glomerular capillary basement membranes were thin and smooth

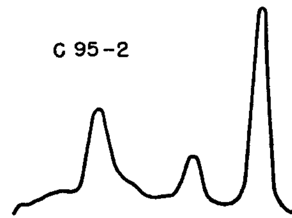

FIG. 3. Representative serum electrophoresis from a CAF_1 mouse with diffuse membranous glomerulonephritis 10 months after receiving 200×10^6 parental spleen cells. Hyperglobulinemia is present.

TABLE II

Incidence of Histological Evidence of Glomerulonephritis

Age	Light microscopy	Fluorescent microscopy
months		
1–2	0/27	0/7
3–5	28/56	21/23
6–9	22/32	22/25
10–12	25/45	20/20

and mesangial staining was scanty. The kidneys of 50 12-month old normal CAF_1 mice were also examined (Table III). They differed from those of the 6-month old animals in that the glomerular capillary membranes were slightly thicker and mesangial staining was present.

No renal abnormalities were found in the groups of mice killed 1–2 months after injection of the parental cells (Table II). The principal lesion that developed subsequently was localized to the glomerulus. In its earliest form, it was characterized by focal thickening of the capillary basement membranes of most glomeruli. As the disease progressed, this thickening became generalized, and with the PAS reaction it appeared serrated (Figs. 5 and 6). Hyalinization and sclerosis of lobules and adhesions to Bowman's capsule accompanied these membrane changes in the advanced lesion (Figs. 7 and 8). In addition, dilation of tubules, accumulations of hyaline droplets in tubular epithelial cells, protein

casts, and focal accumulations of lymphocytes and plasma cells were present in these kidneys. Amyloid could not be detected by the Congo red stain.

Immunofluorescence Studies.—The kidneys of 30 normal CAF_1 mice, ranging in age from 1 to 12 months, were examined for the presence of gamma globulin

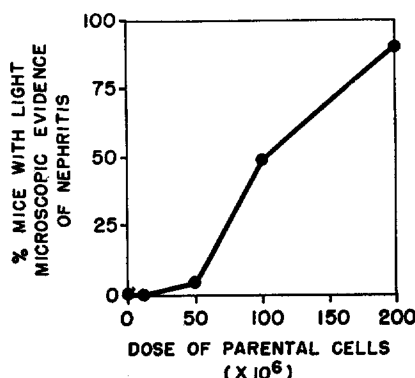

FIG. 4. Correlation between dose of parental spleen cells injected and the incidence of light microscopic evidence of glomerulonephritis in the F_1 recipients.

TABLE III
Normal CAF_1 Mice

Age	Body weight	Proteinuria	Membranous glomerulonephritis		
			Light	Immuno-fluorescent	Electron
months	*g*	*>30 mg/100 ml*			
6	27.3 ± 2.1	0/50	0/50	0/16	0/5
12	32.7 ± 2.2	0/50	0/50	0/14	0/7

Incidence of proteinuria and membranous glomerulonephritis in control mice. Light, immunofluorescent, and electron indicate the type of microscopic examination carried out on these animals. Numerator, number of mice with an abnormality; denominator, number of mice examined.

and β_{1C}-globulin deposits (Table III). Gamma globulin was detected in the mesangium of mice over the age of 3 months, and especially in 1-yr old animals. β_{1C}-globulin deposits were not found in the glomeruli of any of the control mice. Gamma globulin deposits were not found along capillary basement membranes of the normal mice, and their glomeruli could always be differentiated from those of the experimental animals.

In the experimental animals, deposits of gamma globulin were found in glomeruli, and in advanced lesions they could be demonstrated in tubular casts, as droplets in the epithelial cells of some proximal convoluted tubules and occa-

sionally in Bowman's capsule and in the basement membrane of some periglomerular tubules.

The earliest recognizable abnormality was deposition of gamma globulin along glomerular capillary basement membranes in an irregular, finely beaded pattern. As they enlarged, the deposits projected from the extraluminal aspect of the membrane (Figs. 9 and 10). Similar immunoglobulin deposits were sometimes observed free in the glomerular capillary wall in a speckled pattern. Rarely, the entire wall of a single capillary was uniformly stained, imparting a "wire loop" appearance. Sclerotic glomerular lobules appeared as dense, uneven, fluorescent masses. Although variation in staining was observed between glomeruli in a given kidney and between different lobules of a single glomerulus, the irregular deposits along capillary basement membranes were always present. The variability just mentioned was due to differences in the size of the deposits and in the amount of additional staining in the mesangium and capillary walls.

Fluorescent staining for β_{1C}-globulin generally conformed to the capillary pattern described above, with patchy fluorescence visible along capillary basement membranes, within capillary walls and in the mesangium.

Several representative lesions were also stained with a fluorescein-conjugated antiserum against the lymphocytic choriomeningitis (LCM) virus (supplied by Dr. John Hotchin). LCM antigens could not be detected in these preparations by this method.

Electron Microscopy.—The kidneys of 12 normal CAF_1 mice, ranging in age from 2 to 12 months, were examined by electron microscopy (Table III). No abnormalities were noted and the glomeruli and tubules appeared comparable to previous descriptions (13) (Fig. 11). In the 1-yr old mice there were discrete projections of the epithelial surface of the glomerular basement membrane, which was otherwise thin and smooth (Fig. 12).

24 experimental animals, killed at the following times after injection of parental spleen cells, were studied: 1 month (two mice); 2 months (four mice); 3 months (four mice); 4 months (eight mice); 6 months (two mice); and 12 months (four mice). No abnormalities were found in the mice examined 1 and 2 months after receiving BALB/c spleen cells. In the remaining animals the lesion, which principally involved the glomeruli, was characteristic and, once established, uniform.

The earliest recognizable abnormality was swelling and fusion of the foot processes of the visceral epithelial cells (Fig. 13). Later, the foot processes were replaced by sheets of cytoplasm, which were closely applied to the glomerular basement membrane (Fig. 14). In such kidneys, the glomerular basement membrane appeared thickened in all glomeruli studied. This thickening was patchy in some capillary loops and diffuse in others. It was also variable, ranging from mild in one area to marked in another. Numerous round or irregularly shaped projections from the epithelial side were a constant feature in the thickened

segments of the basement membrane (Fig. 14). Dense, osmophilic intramembranous and subepithelial deposits were also observed. These deposits appeared finely granular and homogeneous. It was difficult to distinguish them from the lamina densa in some areas, and the over-all effect was that of a greatly thickened basement membrane (Fig. 15). In other areas, round or oval, homogeneous deposits were seen within large, hemispheric projections from the epithelial side of the basement membrane (Fig. 15). These smooth projections were covered by the swollen, fused foot processes of the epithelial cells (Fig. 15). Still other deposits, irregular in shape, projected from the thickened lamina densa and were contiguous with similar material in the foot processes of the visceral epithelial cell (Fig. 16). Subendothelial deposits were not seen. Amyloid was not present in any of the kidneys examined.

The visceral epithelial cells were hypertrophied and contained a profuse rough endoplasmic reticulum, multiple Golgi apparatus, numerous vacuoles, abundant cytosomes and multivesicular bodies (Figs. 14–16). Microvilli were frequently observed, often in areas where disrupted epithelial cells were discharging their contents into Bowman's space. Fibrils were abundant but microtubules were not conspicuous. Amorphous aggregates and protein droplets were not seen in the cytoplasm of the visceral epithelial cells. There was marked swelling and vacuolization of endothelial cells of some glomeruli in three mice. The lumens of these glomeruli were markedly reduced and fibrinoid deposits were seen. In all other animals examined, changes in the endothelial cells were minimal, consisting of slight swelling of the cytoplasm, some increase in the rough endoplasmic reticulum, and a moderate enlargement and distortion of their fenestrations (Figs. 13 and 15). The lumens of the capillaries were not narrowed, and plugging by polymorphonuclears or erythrocytes was not observed (Figs. 14–16, 18).

A constant and early feature of the lesion was the presence of a basement membrane–like substance containing osmophilic deposits in the mesangial matrix (Fig. 17–18). However, this material was not present in the cytoplasm of the mesangial cells, which were only moderately increased in number and slightly hyperplastic (Fig. 18). The infiltration of the mesangial matrix was at times massive and distorted the architecture of some glomeruli.

Elution of Kidney-Bound Proteins.—Acid eluates prepared from kidneys of mice with severe proteinuria contained a single precipitin band when reacted with rabbit antisera to mouse serum protein. This single band was identified as mouse IgG by analysis in gel diffusion. The eluates were also analyzed by indirect immunofluorescent methods. Sections of normal mouse kidney were incubated with the eluate, washed, and then stained with fluorescein-conjugated rabbit anti-mouse globulin. Binding of the eluate to normal renal tissue could not be detected by this method.

Incidence and Correlation of Renal Lesions.—The latent period between the

injection of at least 100×10^6 BALB/c spleen cells and the development of glomerulonephritis was about 3 months (Tables I and II). Following this incubation period, 50% of all the mice had glomerulonephritis recognizable by light microscopy. In the 6–9 months period of observation this was 68%; 1 yr after treatment with foreign lymphoid cells 55% of the mice had nephritis detectable by light microscopy (Table II). These results probably do not represent the true incidence of glomerular disease, since immunoglobulin deposits were found in the kidneys of virtually all mice when examined at least 3 months after they were given BALB/c spleen cells (Table II).

Fig. 4 shows that the minimal nephritogenic (as judged by light microscopy) dose of parental spleen cells was 100×10^6. When 200×10^6 cells were given, almost all recipients had glomerulonephritis.

16 mice received 150×10^6 BALB/c spleen cells that were killed by alternate freezing (dry ice–acetone bath) and thawing (37° water bath). None of these animals developed proteinuria, and in no instance was there histological evidence of glomerulonephritis. The kidneys of three of these animals were examined by immunofluorescence and none contained gamma globulin deposits in the capillary basement membranes.

DISCUSSION

It is important at the outset to distinguish acute allogeneic disease from its chronic form, which is the subject of this report. The "runting" syndrome of wasting, diarrhea, and dermatitis does not occur in chronic allogeneic disease. Symptoms appear relatively late and the mortality rate is low. Furthermore, in contrast to the lymphoid atrophy typical of the acute form (1), lymphoid hyperplasia is a hallmark of chronic allogeneic disease (14). The reason for the distinctive differences between acute and chronic allogeneic disease is unknown; in any case, there is convincing evidence that an immunological reaction of the donor cells against host antigens initiates both conditions (15, 16).

Chronic allogeneic disease provides a unique opportunity to examine the consequences of acquiring cells that are immunologically reactive against one's own antigens. In this sense, it may be useful as a model of the type of autoimmunization that could occur if lymphoid cells developed anti-"self" reactivity. In the model, autoreactive cells are represented by the graft. In actuality, they could result from a mutation, or from the action of an infectious agent or a chemical. An intriguing aspect of this model is that, like some types of spontaneous human (17) and murine (18) autoimmune disorders, lymphoid neoplasms may follow immunological disease (19).

Previous examples of chronic allogeneic disease have revealed several syndromes. (C57Bl/6 $\times$ DBA/2)F_1 mice given C57Bl/6 spleen cells developed immunohemolytic anemia (5). Rats manifested a variety of lesions: chronic dermatitis resembling scleroderma (20), polyarthritis with chronic synovitis,

myocarditis, and cardiac valvulitis (21). The present experiments are the first to demonstrate glomerulonephritis in chronic allogeneic disease.

Nephritis is known to occur spontaneously in some strains of mice. In old C57Bl mice, for example, amyloid is frequently deposited in the glomeruli (22). Necrotizing papillitis is found in association with renal amyloidosis in other mice (23). Neither of these two disorders was found in the animals we studied. Intercapillary glomerulosclerosis occurs in several strains, including CAF_1 hybrids (24). This condition is characterized by thickening of the axial portion of the glomerular lobules and deposition of gamma and β_{1C}-globulins in these areas (25). Electron-dense material has been observed in the same locations and advanced lesions may have thickened glomerular basement membranes (26). Normally, intercapillary glomerulosclerosis appears in old mice; its development is greatly accelerated by whole body X-irradiation (24). None of the 50 normal 6-month old CAF_1 mice we studied had this lesion. 50 normal 1-yr old CAF_1 mice were also examined, and light microscopic lesions of early intercapillary glomerulosclerosis were found in most of them. Faint, but definite deposits of gamma globulin were present in the mesangium of 12/12 of these animals. Electron microscopy of the kidneys was carried out in 12 normal CAF_1 mice. Five of them were 6 months of age or younger and seven were 12 months old. In no instance were changes indicative of membranous glomerulonephritis observed. However, slight thickening of the mesangial zone and discrete projections of an otherwise thin and smooth glomerular basement membrane were commonly found. Electron-dense deposits were never seen in these projections of the membrane, and the foot processes, including those covering the projections of the basement membrane, were always thin. In all cases, these mild abnormalities, which were found only in 1-yr old mice, could be readily distinguished from the lesions in the experimental animals.

The evidence that the nephritis we observed was evoked by the allogeneic cells is: (*a*) it did not occur in untreated mice; (*b*) it failed to develop when killed cells were given; (*c*) its incidence was proportional to the number of living cells administered. Results of Experiment (*b*) also indicate that an antigen in the donor inoculum was not involved in the pathogenesis of the nephritis. Additional work, to be presented in connection with other experiments, has shown that the injection of CAF_1 spleen cells into either BALB/c or CAF_1 mice is ineffective in producing nephritis.

From recent studies it has become clear that two different immunological processes are capable of producing glomerulonephritis (27). One mechanism requires the specific interaction of antibody with basement membranes located in the wall of the glomerular capillaries. Experimentally, antiglomerular basement membrane antibodies can be induced by immunization with heterologous (28), homologous (29) or autologous (30) basement membranes. The lesion provoked by these antibodies is a mixed proliferative and membranous glomerulo-

nephritis that has as its primary electron microscopic change an irregular thickening of the luminal aspect of basement membranes accompanied by focal subendothelial deposits of electron-dense material (31). The immunofluorescent staining pattern in this lesion is a smooth, uniform, linear fluorescence overlying all glomerular capillary basement membranes (32).

The second pathogenetic mechanism in glomerulonephritis involves neither antigens native to the glomerulus nor anti-kidney antibodies. Instead, the renal lesion is provoked by immune complex depositions in the walls of glomerular capillaries. The pathogenesis of this lesion was first suspected in human serum sickness (33) and later it was elucidated in considerable detail in studies of acute and chronic experimental glomerulonephritis of rabbits (34). The chronic glomerular lesion produced by soluble antigen-antibody complexes is morphologically distinct from that produced by anti-kidney antibodies. By light microscopy, its chief characteristic is irregularly thickened basement membranes (35). Electron microscopic studies consistently show thickening of the basement membrane, fusion of epithelial foot processes and discrete subepithelial deposits of electron-dense material (36). The fluorescent staining pattern in this case is characterized by irregular, beaded deposits of host gamma globulin and complement along capillary basement membranes (37). These deposits project from the extraluminal aspect of the membrane and they correspond geographically to the electron-dense deposits visible by electron microscopy.

On the basis of currently available criteria, the renal lesion that developed in CAF_1 recipients of BALB/c lymphoid cells was an immune complex-induced glomerulonephritis. The light microscopic picture was one of membranous glomerulonephritis, the fluorescent staining pattern was characteristically beaded and irregular, and the thickening of the basement membrane seen by electron microscopy was always accompanied by subepithelial deposits. Acid eluates obtained from diseased kidneys stained no part of normal mouse kidneys when examined by the indirect fluorescent technique. Had antibody to native renal antigens been responsible for the nephritis, positive fluorescent-staining reactions should have been found when the eluate was tested against normal kidney tissue.

The immune complex mechanism of glomerular damage is highly significant, for no limitation is placed on the source of the antigen. Theoretically, any substance that can form a soluble complex with its homologous antibody could provoke glomerulonephritis. It has been shown that even autologous renal tubules (38) and thyroglobulin (39) contain antigens suitable for generating nephritogenic immune complexes. Recently, DNA has been identified, along with anti-DNA antibodies and complement, in the nephritis of human lupus (40) and in NZB mice (41). The results of other studies indicate that streptococcal antigen and host antibody are on the glomeruli of patients with acute

poststreptococcal nephritis (42, 43). The renal disease that develops in mice chronically infected with the lymphocytic choriomeningitis (LCM) virus has all the features of an immune complex–induced disease. Acid eluates from their affected kidneys contain antibodies to the LCM virus and viral antigen is present in the lesion, thus definitively establishing the pathogenesis of the nephritis (44). We sought LCM antigen in the nephritis of chronic allogeneic disease with no success. Nevertheless, the demonstration of glomerulonephritis as a sequela of chronic LCM infection suggests the possibility that other infectious agents can, through immune complex deposition, lead to the development of lesions in organs for which they have no tropism. Of particular interest in this regard is the development of glomerulonephritis in mice injected with mouse leukemia viruses (45, 46).[1]

The results of our studies suggest that autoreactive immunologically competent cells—as represented by the parental lymphoid graft in the chronic allogeneic disease model—could generate nephritogenic immune complexes from any number of autoantigens. In addition, normally tolerated, indigenous agents might also serve as antigenic stimuli for a new population of immunologically competent cells that lacks tolerance of them. Further studies of chronic allogeneic disease may elucidate how immunological disorders could arise following responses of abnormal, intolerant lymphoid cells to normal antigens.

SUMMARY

Glomerulonephritis, often accompanied by the nephrotic syndrome, developed in CAF_1 mice following the administration of spleen cells from normal BALB/c mice. The renal lesion was membranous glomerulonephritis. When studied with fluorescein-conjugated antisera to either mouse gamma globulin or β_{1C}-globulin, the glomeruli contained beaded and irregular deposits of these immunoproteins. The ultrastructure of the lesion was characterized by thickening of the glomerular basement membranes and the presence of electron-dense subepithelial deposits. Acid eluates of the diseased kidneys contained gamma globulin that failed to bind to sections of normal kidneys. These findings conform to the type of nephritis provoked by immune complexes. They indicate that this type of immune injury can be based on the reaction of intolerant immunocytes to normal antigens.

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BIBLIOGRAPHY

1. Van Bekkum, D. W., and M. J. de Vries. 1967. Radiation Chimaeras. Academic Press, New York.

[1] Eluates prepared from kidneys of CAF_1 mice given BALB/c spleen cells were examined by Dr. Wallace Rowe for the presence of mouse leukemia virus antigens and antibodies, with negative results.

2. Billingham, R. E., and L. Brent. 1959. Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. *Phil. Trans. Roy. Soc. London, Ser. B.* **242**:439.
3. Mathé, G., J. Bernard, M. J. de Vries, L. Schwarzenberg, M. J. Larrieu, C. M. Lalanne, A. Dutreix, J. L. Amiel, and J. Surmont. 1960. Nouveaux essais de greffe de moelle osseuse homologue après irradiation totale chez des enfants atteints de leucémie aiguë en rémission. Le problème du syndrome secondaire chez l'homme. *Rev. Hematol.* **15**:115.
4. Armstrong, M. Y. K., J. André-Schwartz, and R. S. Schwartz. 1968. Immunological homeostasis and leukemia: an experimental model in the mouse. *In* Perspectives in Leukemia. W. Dameshek, editor. Grune and Stratton, New York. 133.
5. Oliner, H., R. Schwartz, and W. Dameshek. 1961. Studies in experimental autoimmune disorders. I. Clinical and laboratory features of autoimmunization (runt disease) in the mouse. *Blood.* **17**:20.
6. Lerner, R. A., R. J. Glassock, and F. J. Dixon. 1967. The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J. Exptl. Med.* **126**:989.
7. Mardiney, M. R., and H. J. Müller-Eberhard. 1965. Mouse β_{1C} globulin: Production of antisera and characterization in the complement reaction. *J. Immunol.* **94**:877.
8. Clark, H. F., and C. C. Shepard. 1963. A dialysis technique for preparing fluorescent antibody. *Virology.* **20**:642.
9. Leduc, E., V. Marinozzi, and W. Bernhard. 1963. The use of water-soluble glycol methacrylate in ultrastructural cytochemistry. *J. Roy. Microscop. Soc.* **81**:119.
10. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.
11. Richardson, K. C., L. Jarrett, and E. H. Fink. 1960. Embedding in epoxy-resin for ultra thin sectioning in electron microscopy. *Stain Technol.* **35**:313.
12. Venable, J. H., and R. A. Coggeshall. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell. Biol.* **25**:407.
13. Spargo, B. H. 1966. Structure of the kidney. *In* The Kidney. F. K. Mostof, and D. E. Smith, editors. Williams and Wilkins, Baltimore. p. 17.
14. Armstrong, M. Y. K., R. S. Schwartz, and L. Beldotti, 1967. Neoplastic sequelae of allogeneic disease. III. Histological events following transplantation of allogeneic spleen cells. *Transplantation.* **6**:1380.
15. Simonsen, M. 1962. Graft versus host reactions. Their natural history, and applicability as tools of research. *Progr. Allergy.* **6**: 349.
16. Schwartz, R. S. 1965. The activation of experimental and clinical immunologic diseases by x-irradiation and alkylating agents. *Ann. N. Y. Acad. Sci.* **123**:64.
17. Schwartz, R. S., and N. Costea. 1966. Autoimmune hemolytic anemia: clinical correlations and biological implications. *Seminars Hematol.* **3**:2.
18. East, J., and M. A. B. de Sousa. 1966. The thymus, autoimmunity and malignancy in New Zealand Black mice. *Natl. Cancer Inst. Monograph.* **22**:605.
19. Schwartz, R. S., and L. Beldotti. 1965. Malignant lymphomas following allogenic disease: transition from an immunological to a neoplastic disorder. *Science.* **149**:1511.

20. Stastny, P., V. A. Stembridge, and M. Ziff. 1963. Homologous disease in the adult rat, a model for autoimmune disease. I. General features and cutaneous lesions. *J. Exptl. Med.* **118:**635.
21. Stastny, P., V. A. Stembridge, T. Vischer, and M. Ziff. 1965. Homologous disease in the adult rat, a model for autoimmune disease. II. Findings in the joints, heart and other tissues. *J. Exptl. Med.* **122:**681.
22. Dunn, T. B. 1954. The importance of differences in morphology in inbred strains. *J. Natl. Cancer Inst.* **15:**573.
23. West, W. T., and E. D. Murphy. 1965. Sequence of deposition of amyloid in strain A mice and relationship to renal disease. *J. Natl. Cancer Inst.* **35:**167.
24. Guttman, P. H., and H. I. Kohn. 1960. Progressive intercapillary glomerulosclerosis in the mouse, rat and chinese hamster, associated with ageing and x-ray exposure. *Am. J. Pathol.* **37:**293.
25. Guttman, P. H., K. D. Wuepper, and H. H. Fudenberg. 1967. On the presence of Gamma G and β_{1C} globulins in renal glomeruli of aging and neonatally x-irradiated mice. *Vox Sanguinis.* **12:**329.
26. Guttman, P. H. 1966. Ultrastructural studies of delayed changes in the kidney of the mouse irradiated at birth. *Radiation Res.* **27:**652.
27. Unanue, E. R., and F. J. Dixon. 1967. Experimental glomerulonephritis: immunological events and pathogenetic mechanisms. *Advan. Immunol.* **6:**1.
28. Steblay, R. W. 1963. Glomerulonephritis induced in sheep by injection of heterologous glomerular basement membrane and Freund's complete adjuvant. *J. Exptl. Med.* **116:**253.
29. Heymann, W., D. B. Hackel, S. W. Harwood, S. G. F. Wilson, and J. L. P. Hunter. 1959. Production of nephrotic syndrome in rats by Freund's adjuvant and rat kidney suspensions. *Proc. Soc. Exptl. Biol. Med.* **100:**660.
30. Unanue, E. R., F. J. Dixon, and J. D. Feldman. 1967. Experimental allergic glomerulonephritis induced in the rabbit with homologous renal antigens. *J. Exptl. Med.* **125:**163.
31. Bencosme, S. A., and B. J. Bergman. 1962. The ultrastructure of human and experimental glomerular lesions. *Intern. Rev. Exptl. Pathol.* **1:**139.
32. Steblay, R. W. 1963. Glomerulonephritis induced in monkeys by injections of heterologous glomerular basement membrane and Freund's adjuvant. *Nature.* **197:**1173.
33. Von Pirquet, C. E. 1911. Allergy. *Arch. Internal Med.* **7:**259.
34. Dixon, F. J., J. D. Feldman, and J. J. Vazquez. 1961. Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. *J. Exptl. Med.* **113:**889.
35. Ehrich, W. E., J. Seifter, and C. Forman. 1949. Experimental serum disease. A pathogenetic study. *J. Exptl. Med.* **89:**23.
36. Andres, G. A., B. C. Seegal, K. C. Hsu, M. S. Rothenberg, and M. L. Chapeau. 1963. Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody. *J. Exptl. Med.* **117:**691.
37. Dixon, F. J., T. S. Edgington, and P. J. Lambert. 1968. Nonglomerular antigen-antibody complex nephritis. Fifth International Immunopathology Symposium. P. Miescher and P. Grabar, editors. Schwabe and Company, Basel. 17.

38. Edgington, T. S., R. J. Glassock, J. I. Watson, and F. J. Dixon. 1968. Autologous immune complex nephritis induced with renal tubular antigen. *J. Exptl. Med.* **127**:573.

39. Weigle, W. O., and G. J. High. 1968. The behavior of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. *J. Immunol.* **98**:1105.

40. Koffler, D., P. H. Schur, and H. G. Kunkel. 1967. Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. Exptl. Med.* **126**:607.

41. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exptl. Med.* **127**:507.

42. Michael, A. F., K. N. Drummond, R. A. Good, and R. L. Vernier. 1966. Acute post streptococcal glomerulonephritis: Immune deposit disease. *J. Clin. Invest.* **45**:237.

43. Markowitz, A. S., and C. F. Lange. 1964. Streptococcal related glomerulonephritis I. Isolation, immunochemistry, and comparative chemistry of soluble fractions from type 12 nephritogenic streptococci and human glomeruli. *J. Immunol.* **92**:565.

44. Oldstone, M. B. A., and F. J. Dixon. 1967. Lymphocytic choriomeningitis. Production of antibody by "tolerant" infected mice. *Science.* **158**:1193.

45. Recher, L., T. Tanaka, J. A. Sykes, T. Yumoto, G. Seman, L. Young, and L. Dmochowski. 1966. Further studies on the biological relationship of murine leukemia viruses and on kidney lesions of mice with leukemia induced by these viruses. *Natl. Cancer Inst. Monograph.* **22**:459.

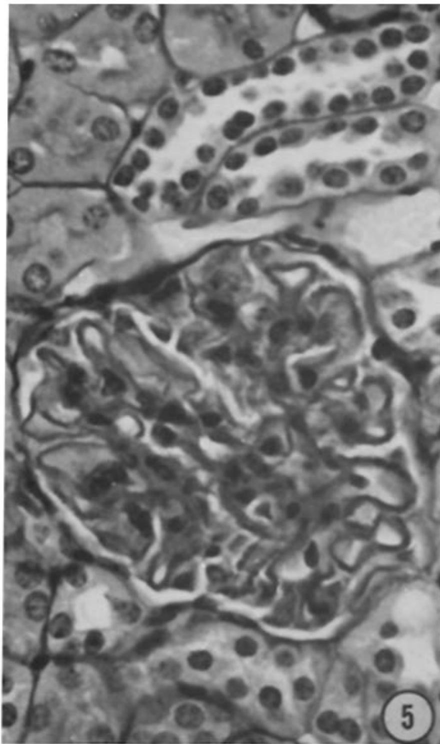
46. Gude, W. D., V. K. Jenkins, A. C. Upton, and R. L. Tyndall. 1964. Glomerulosclerosis in mice with myeloid leukemia. *Proc. Soc. Exptl. Biol. Med.* **115**:691.

FIG. 5. Diffuse membranous glomerulonephritis, which appeared 4 months after parental spleen cell injection. PAS, × 520.

FIG. 6. Diffuse membranous glomerulonephritis with confluent PAS-positive deposits on the extraluminal aspect of capillary basement membranes, which impart a serrated appearance. The luminal edge of the basement membranes is smooth and uniform in appearance. PAS, × 1170.

FIG. 7. Diffuse membranous glomerulonephritis with intraluminal and mesangial deposition of PAS-positive material, adhesions to Bowman's capsule and periglomerular lymphoid infiltration. PAS, × 520.

FIG. 8. Advanced, sclerosing membranous glomerulonephritis. PAS, × 520.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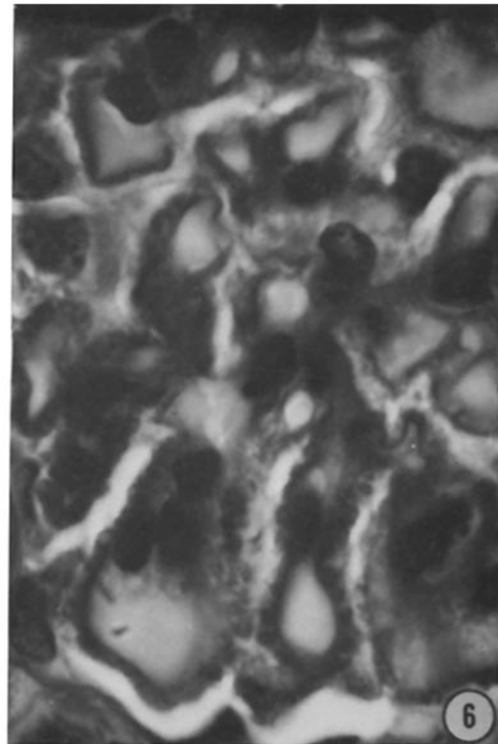
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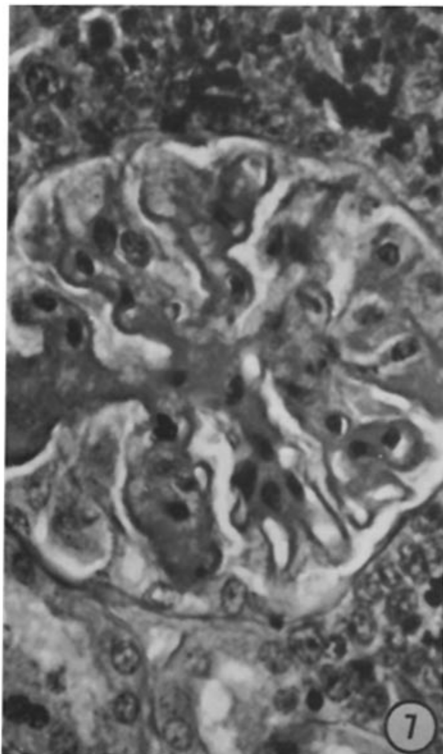
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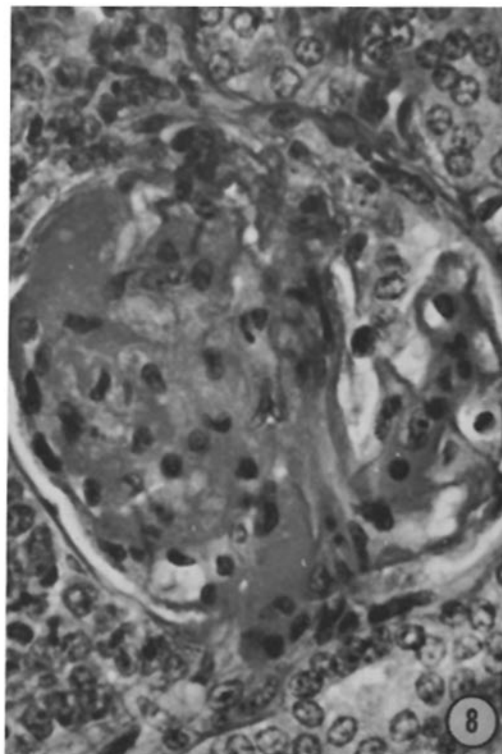
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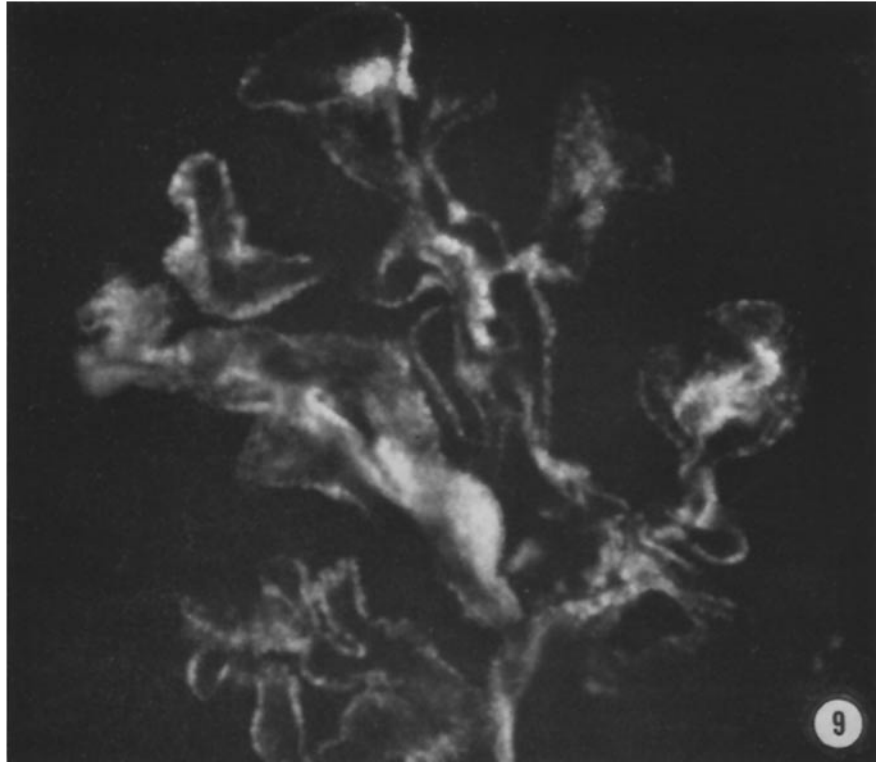
FIG. 9. Glomerulus from CAF_1 mouse 4 months after injection of 200×10^6 parental spleen cells, stained with fluorescein-conjugated rabbit anti-mouse gamma globulin. Irregular, finely beaded deposits of mouse gamma globulin outline the glomerular capillary basement membranes. Diffuse staining for gamma globulin is also visible in the mesangium of the glomerulus. $\times$ 520.

FIG. 10. Oil immersion photomicrograph of gamma globulin deposits on and along the glomerular capillary basement membrane. $\times$ 1170.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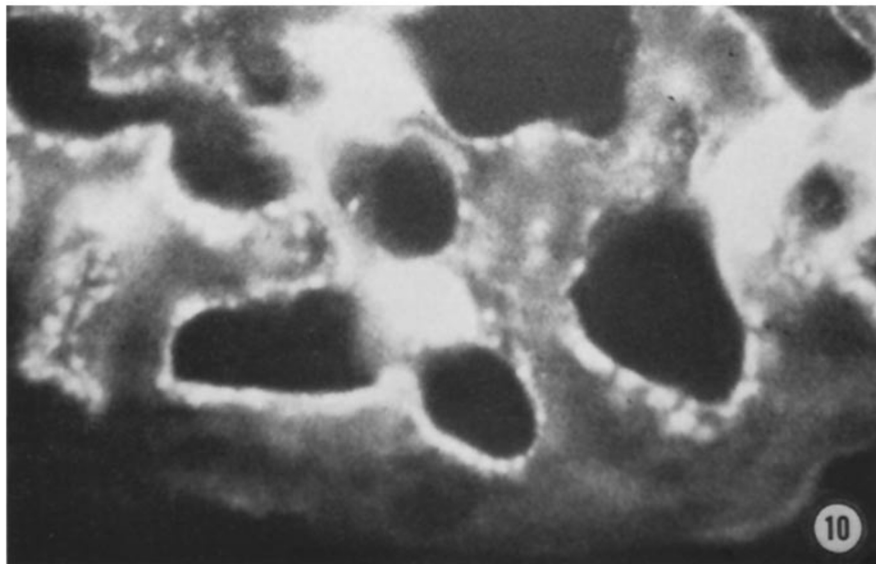
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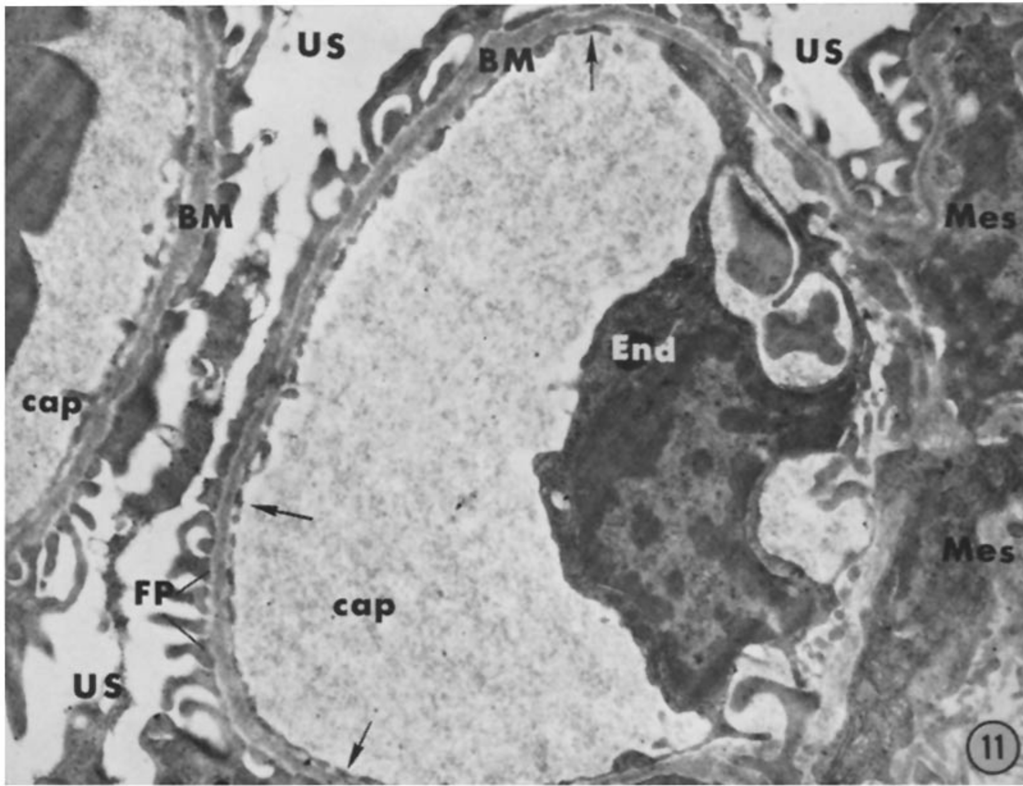
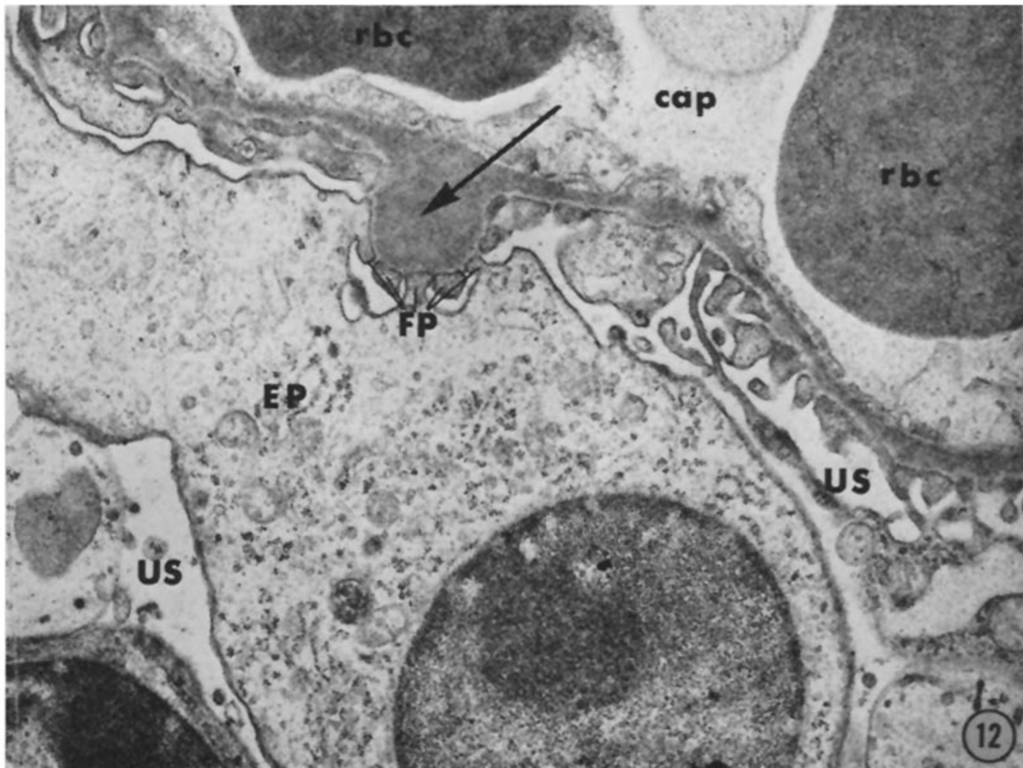
FIG. 11. Glomerular capillary of a normal 1-yr old mouse. The glomerular basement membrane (BM) is thin and covered by conspicuous foot processes (FP). The fenestrated cytoplasm (arrows) of an endothelial cell (End) lines the glomerular capillary (cap). Urinary space (US) and mesangial cells (Mes) are visible. × 10,000.

FIG. 12. Glomerular capillary of a normal 1-yr old mouse. A protrusion of the basement membrane is shown (arrow). Its homogeneous texture is similar to that of the rest of the basement membrane. Normal foot processes (FP) of a visceral epithelial cell (EP) surround this protrusion. Red blood cells (rbc) are seen in the lumen of the glomerular capillary (cap). × 10,000.


US
BM
US
BM
Mes
End
cap
FP
cap
Mes
US
11


rbc
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rbc
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EP
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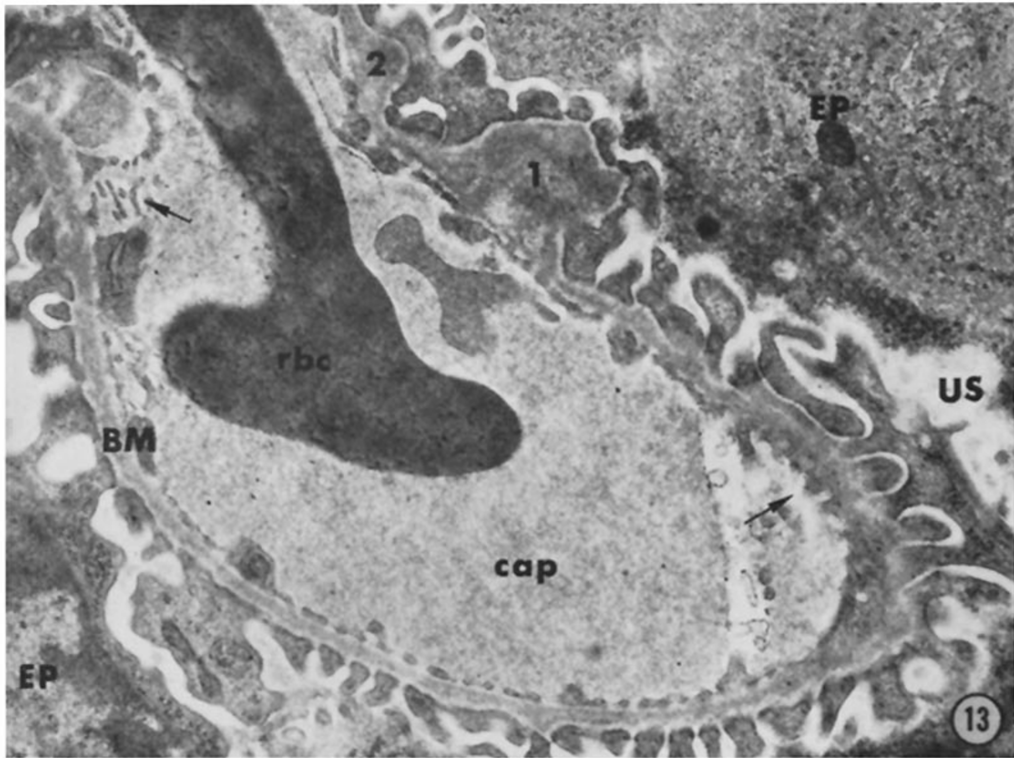
US
12

FIG. 13. Glomerular capillary, early lesion. The glomerular basement membrane (BM) is still thin, but exhibits irregularly thickened areas (1–2). Foot processes appear normal around one protrusion (1) but fused around the next (2). There is focal distortion of the endothelial fenestration (arrows). × 10,000.

FIG. 14. Glomerular capillary, typical lesion. Swollen epithelial cell (EP) exhibits a large vacuole (v). Its coalescent foot processes cover an irregularly thickened basement membrane (BM). Electron-dense deposits (arrows) are visible within the basement membrane and on its epithelial side. × 10,000.


2
EP
1
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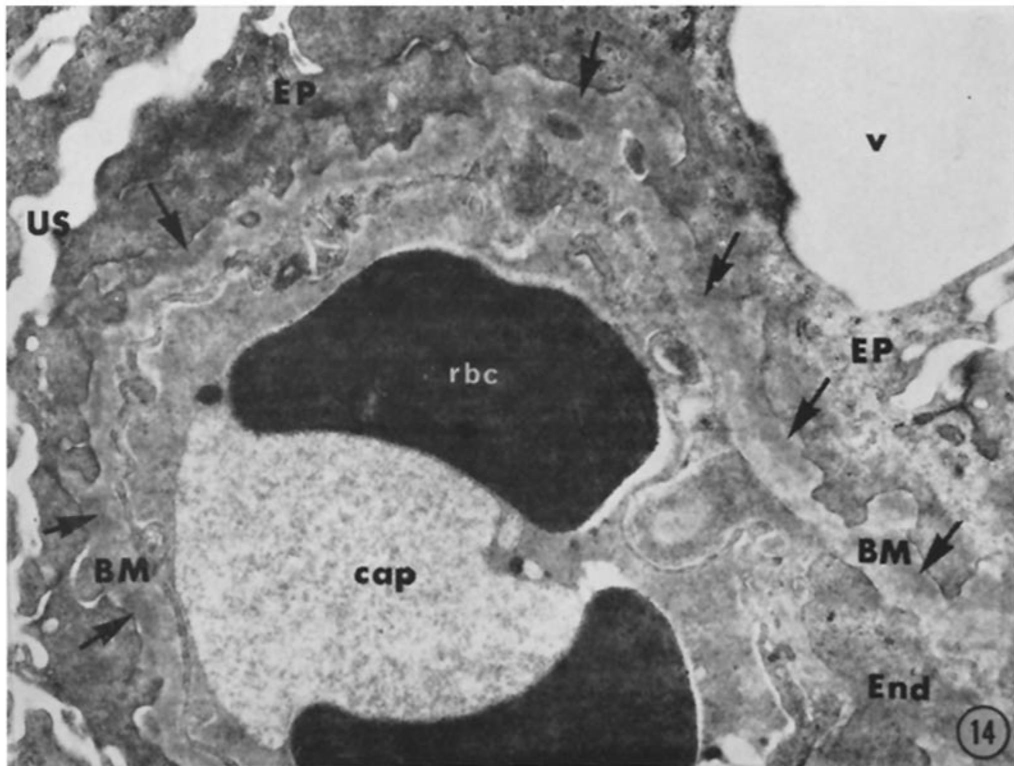
EP
13


EP
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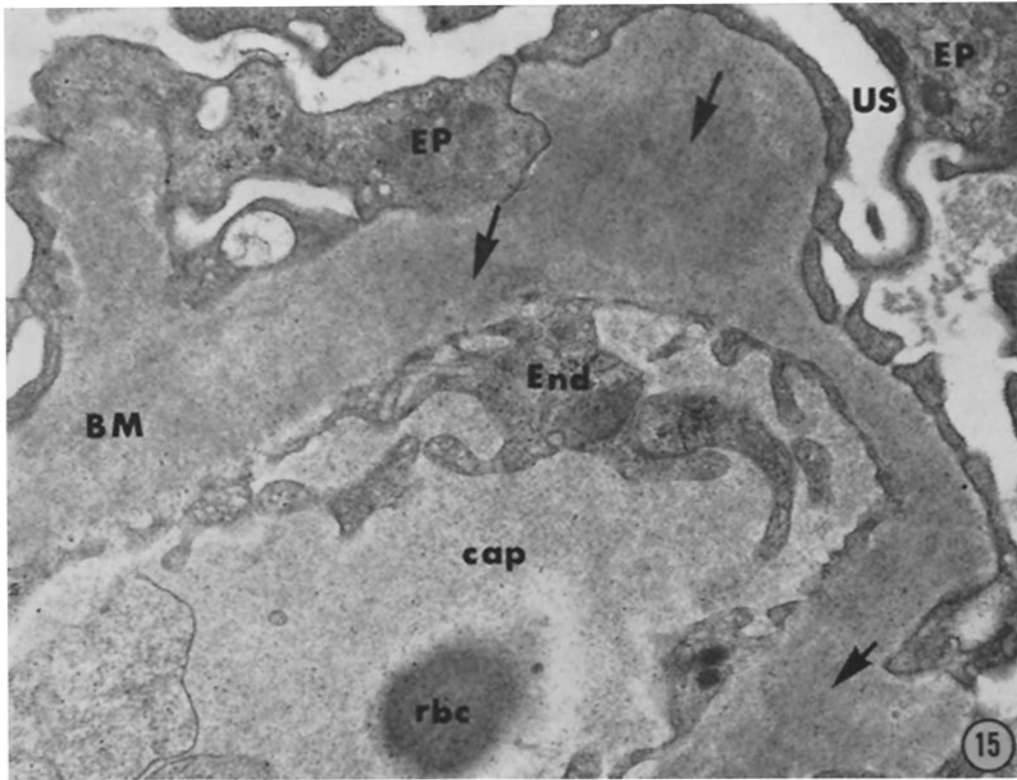
EP
BM
cap
BM
End
14

FIG. 15. Intramembranous deposits are visible (arrows) in the protrusion of the basement membrane (BM) and also within areas where lamina densa and lamina rara are no longer recognizable. Continuous cytoplasmic sheets from visceral epithelial cells (EP) have replaced the foot processes. Endothelial (End) fenestration is slightly increased. $\times$ 24,000.

FIG. 16. Subepithelial deposits (arrows) projecting from the irregularly thickened membrane about the fused foot processes. The visceral epithelial cells (EP) are rich in fibrils (f) and in lysosomes (ly). Numerous small lipid globules (L) are visible in the lumen of the capillary (cap). $\times$ 24,000.


EP
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EP
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rbc
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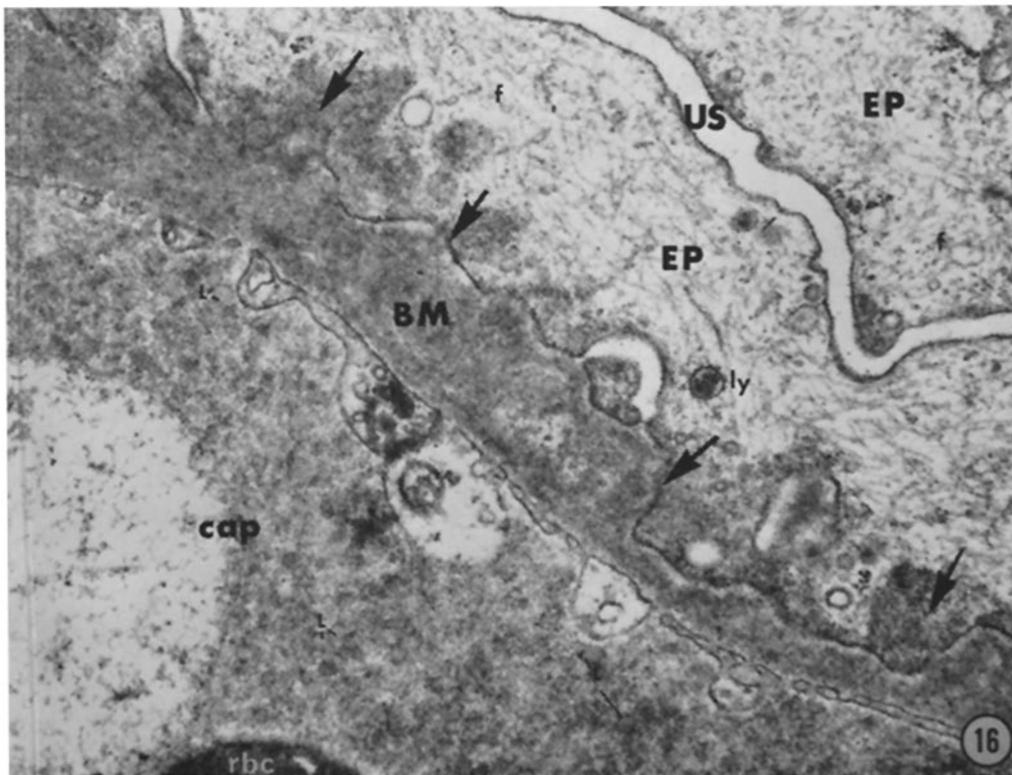
f
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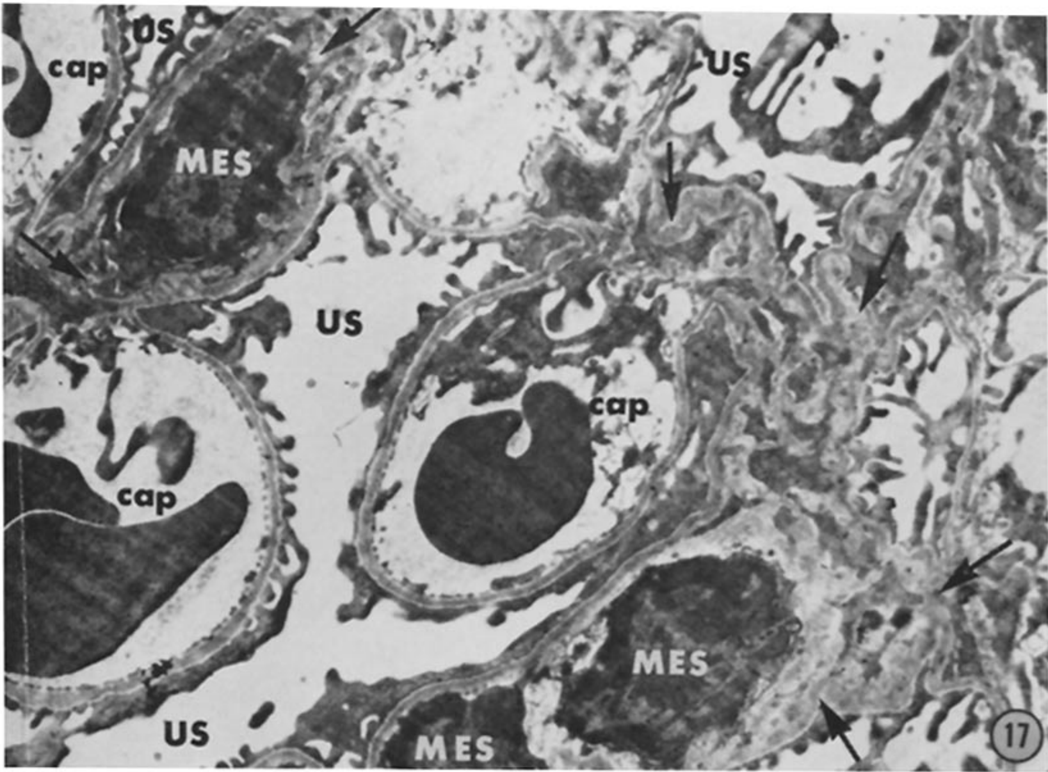
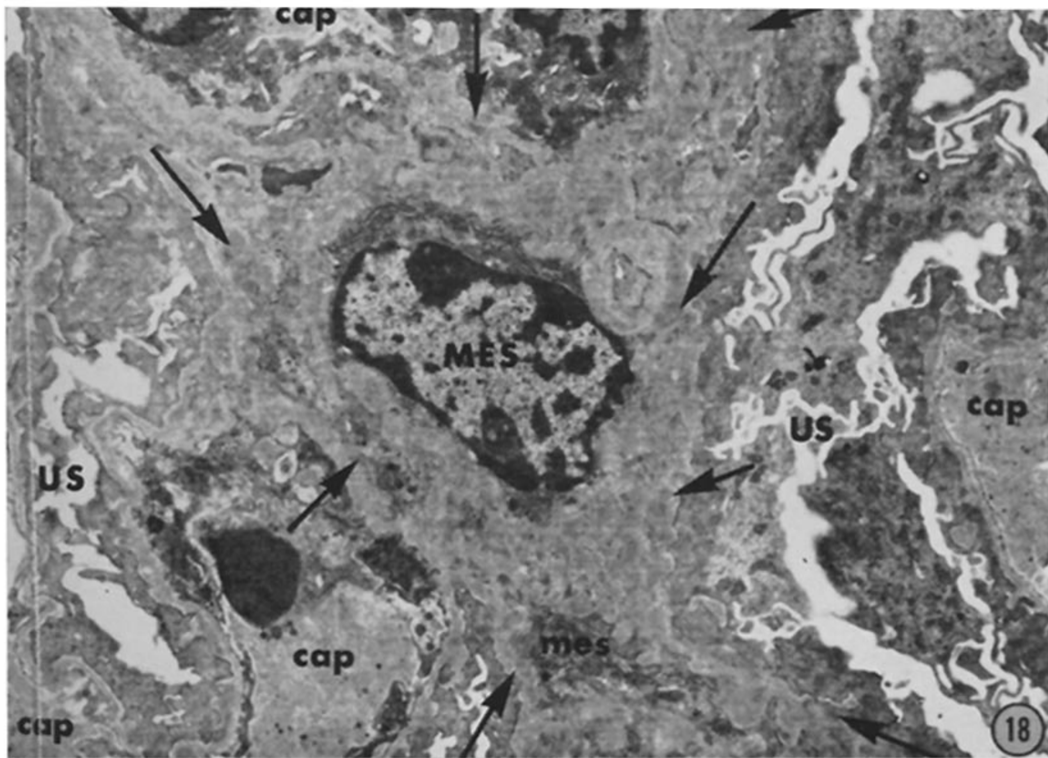
16

FIG. 17. Mesangium of a normal 12-month old mouse. Mesangial cells (MES) surrounded by a clear-cut mesangial matrix are (arrows) shown. Glomerular capillaries (cap) and urinary space (US) are present. × 6000.

FIG. 18. Mesangium, typical lesion. Two mesangial cells (MES) with cytoplasm rich in ribosomes and rough endoplasmic reticulum are shown. In contrast with the intact mesangial cells, the mesangial matrix is heavily infiltrated by a membrane-like substance in which electron-dense deposits are visible (arrows). Several glomerular capillaries (cap) and urinary space (US) are also seen. × 6000.



cap
US
MES
US
US
cap
cap
MES
US
MES
17



cap
MES
cap
US
US
cap
mes
cap
18