

EXPERIMENTAL GLOMERULONEPHRITIS

VII. THE ABSENCE OF AN AUTOIMMUNE ANTIKIDNEY RESPONSE IN NEPHROTOXIC SERUM NEPHRITIS*, †

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It is generally agreed that in nephrotic serum nephritis (NTN) there are two immunologic reactions, that of the heterologous nephrotic antibody (NTAb) with glomerular antigens of the host, and later that of host antibody with the glomerular fixed heterologous gamma globulin. In addition, it has been suggested that the host may make an autoantikidney response presumably to renal antigens formed and/or liberated during the above reactions. An autoantikidney response would, of course, provide a suitable explanation for the chronic progression of NTN. Attempts to demonstrate such a response by the transfer of NTN between parabiotic partners or with the transfer of cells have been made but without clear-cut results (1, 2).

Since the implications of an autoantikidney response would be of great importance for both clinical and experimental nephritis, we have tested for its presence by as direct a method as possible. This procedure has involved transplanting normal isologous kidneys into rats in various stages of NTN and following the fate of such kidneys in the presence or absence of the host's nephritic kidneys. Presumably the transplanted normal kidney should be vulnerable to any antikidney response of the host and would, therefore, serve as an indicator. In addition, using isologous rats, we have repeated the studies attempting to transfer NTN by parabiosis. Neither of these experimental approaches has provided any evidence for the existence of an autoantikidney response in this disease. Moreover, these experiments have permitted the identification of a population of NTAbs which dissociates from the tissues of the

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nephritic rat and which fixes to the transplanted kidney or the kidney of the normal parabiont.

Materials and Methods

Experimental Animals.—Three strains of rats were used; Buffalo (Simonsen Laboratories, Gilroy, California), and Lewis (Microbiological Associates, Bethesda, Maryland) both of which are inbred and histocompatible strains and Sprague-Dawley which is an outbred strain.

NTN.—NTN was induced by the intravenous injection of 2 to 10 mg of rabbit nephrotoxic gamma globulin (NTGG) containing 40 to 500 μ g of kidney-fixing antibody (KFAb). The amount of KFAb depended on the particular experiment and was given in 1 to 3 intravenous injections. Rabbit NTGG consisted only of gamma-2 globulin (IG-G) (3). The content of KFAb was determined using I^{131} -labeled NTGG (4). Some experiments also included rats which, besides receiving the injection of NTGG, were also immunized to rabbit gamma globulin (GG) in incomplete Freund's adjuvant in order to produce a more severe nephritis characterized by a continuous immunologic reaction in the glomeruli (5). Rats were immunized with 1 to 2 injections of 5 mg of rabbit GG (obtained from Pentex, Inc., Kankakee, Illinois, Lot 33), usually given at the time of NTGG injection and 1 week later.

Fluorescent Antibody Studies.—These studies employed the technique and antisera described previously (3). Fluoresceinated sheep anti-rabbit GG, rabbit anti-rat GG, and rabbit anti-rat Beta 1C globulin (β 1C) were used. Tissue complement (C') fixation was used to determine tissue lesions capable of fixing C' *in vitro* employing guinea pig serum and fluoresceinated anti-guinea pig β 1C-globulin (3). The criteria for identification of the patterns of glomerular fluorescence were the same as before (3).

Antibody determination.—Rat antibodies to rabbit GG were determined by precipitation in gel using Preer's technique (6). Some parabiotic pairs were also tested for immune elimination of a tracer dose of I^{131} -labeled rabbit GG, *i.e.* rats received 200 μ g of rabbit GG containing 2 to 4 μ c of I^{131} and were followed with whole body counting from 1 to 3 days; urine was also collected during this period and total, trichloroacetic acid precipitable and non-precipitable (non-protein bound I) fractions determined for both members. Because proteinuric rats have an increased rate of elimination of intact rabbit GG, the increased excretion of non-protein bound I in the urine was taken as the most reliable evidence of antibody; nephritic rats, *per se*, do not show increase excretion of non-protein I in the urine.

Urinary Protein.—Proteinuria was determined on 24-hour collections of urine by the sulphosalicylic acid method (3). Proteinuria of over 20 mg per 24 hours was considered abnormal. The urine of the parabionts was collected in special metabolic cages which permitted the separate collection from each member.

Blood Urea Nitrogen (BUN).—BUN was determined by phenate-hypochlorite method using commercially available reagents (Hyland Laboratories, Los Angeles, California). Normal BUN values ranged from 15 to 26 mg per cent.

PROCEDURE

The first experiment consisted of the surgical transplantation of normal isologous kidneys to rats with NTN. Lewis rats weighing 300 to 400 gm were used. NTN was induced by either 1 to 3 intravenous injections of rabbit NTGG totaling from 300 to 500 μ g of KFAb or by immunization to rabbit GG after the administration of 275 μ g of KFAb, as described before. Kidney transplantation was performed 10 to 30 days after the first injection of KFAb and always to rats with proteinurias exceeding 100 mg per 24 hours.

All transplants were performed by Dr. Lee. The normal isologous kidney was transplanted to the right pelvic area of the nephritic recipient; the vascular anastomoses with the vessels

of the donor were made to the lower abdominal aorta and vena cava of the recipient; the donor ureter was implanted directly into the recipient's bladder (7). At the time of the transplant, the recipient's right kidney was removed. The recipients were followed with weekly urinalysis and BUNs for periods of 7 to 35 days, after which the recipient's own left kidney was removed and the transplant was checked for possible complications. The rats then were followed with urinalysis and BUN for 4 to 7 more weeks before sacrifice. The control in this experiment was the transplant of a normal isologous kidney to a normal recipient. The controls were treated in the same way and followed for similar periods of time.

The second experiment consisted of parabiosis of rats with NTN to normal rats. Buffalo, Lewis and Sprague-Dawley rats, weighing 150 to 200 gm were parabiosed in intrastain combinations. Parabiosis was performed by the Bunster and Mayer technique with a common peritoneal cavity (8). Representative rats were tested for cross circulation by either; (a) examining for bluing of the skin and mucous membranes of one partner after intravenous injection of Evans Blue to the other, or (b) determining the degree of transfer of a radioactive-labeled protein after its injection to one member of the pair. Bovine serum albumin was labeled with I^{131} and injected in one member ($100 \mu\text{g}$ with 2 to 3 μc); both partners were bled for estimation of blood radioactivity 2 and 24 hours later. All pairs tested had extensive cross circulation by 4 to 6 days after parabiotic union.

Three groups of rats with NTN were parabiosed to normal rats. Group I consisted of 45 rats which were injected with 200 to 300 μg of KFAb and parabiosed 1 to 21 days later; these rats developed classical NTN with an immediate and continuous proteinuria ranging from 80 to 200 mg per day. Included in this group were: 20 Buffalo rats, 8 of which were parabiosed 1 day after injection and followed from 16 to 57 days, and 12 of which were parabiosed 2 weeks after injection and followed from 7 to 73 days; 12 Lewis rats, 11 of which were parabiosed 1 day after injection and followed for 30 days and 1 of which was parabiosed 15 days after injection and followed for 40 days; 13 Sprague-Dawley rats, 4 of which were parabiosed 2 days after injection and were followed from 14 to 28 days and 9 of which were parabiosed 21 days after injection and were followed for 12 to 60 days. Group II consisted of 5 Lewis rats injected with 250 μg KFAb, immunized to rabbit GG on the same day, parabiosed 8 days later and followed for 22 days. These rats developed an immediate and continuous proteinuria usually ranging from 100 to 300 mg per day. Group III consisted of 8 Buffalo rats injected with 40 μg of KFAb, immunized to rabbit GG on the same day, parabiosed 1 day later and followed for 110 days. These rats developed proteinuria 6 to 12 days after injection ranging from 80 to 120 mg per day. After the pairs were followed for the periods indicated above, they were either sacrificed or separated surgically. After separation, the member with NTN was followed for 4 to 6 weeks with weekly urinalysis; the normal partners were immunized at the time of separation and 1 week later with rabbit GG and then followed with urinalysis for 4 to 6 weeks. Renal biopsy was usually taken from each member at the time of separation.

Included in all the above groups were control rats treated with NTGG the same way but not parabiosed. Several additional controls were included: (a) parabiosis of normal Buffalo and Lewis rats in intrastain combination, this control served to determine if parabiotic union *per se* was able to induce renal abnormalities in one or both parabionts; (b) parabiosis of 2 normal Sprague-Dawley rats or normal Buffalo to Lewis rats; these served to determine the renal changes induced by parabiotic intoxication. Parabiotic intoxication was determined by clinical appraisal (wasting) and by microhematocrit determination of each member; and (c) parabiosis of normal Buffalo or Lewis rats to isologous rats which had several types of renal abnormalities or insufficiency, *i.e.* hypertension (induced by unilateral nephrectomy plus compression of the remaining kidney) (9), aminonucleoside nephrosis (induced by administration of 20 to 30 mg of aminonucleoside of puromycin), or bilaterally nephrectomized rats.

These groups served to determine if renal abnormalities in one partner produced any changes in the normal partner. All controls were followed for 4 to 8 weeks with weekly urinalysis.

RESULTS

Transplantation of Normal Kidneys to Rats with NTN.—The results of twenty successful transplants are summarized in Table I. The experimental animals were followed for periods of 1 to 5 weeks with the left kidney of the nephritic *in situ*; at the end of this period the left nephritic kidney was removed and the rats followed for an additional 4 to 7 weeks. Two of the rats died of complications; 1 developed a volvulus with gastrointestinal obstruction

TABLE I
Transplantation of a Normal Isologous Kidney to Rats with NTN

Induction of NTN	No. of animals	Transplanted kidney with left nephritic kidney (1 to 5 weeks)		Transplanted kidney alone (4 to 7 weeks)	
		No proteinuria	Recovery BUN	No proteinuria	Normal BUN
NTAb alone.....	10	7/10*	2/2	10/10	10/10
NTAb + Rabbit GG immunization..	10	2/10‡	8/8	10/10	8/10§
Total.....	20	9/20	10/10	20/20	18/20

Numerator represents number of rats with particular result; denominator represents number of rats examined.

* 1 additional had significantly reduced proteinuria.

‡ 5 additional had significantly reduced proteinuria.

§ Transplanted kidney in the 2 animals with incomplete return of BUN to normal showed hydronephrosis (BUNs ranged 35 to 40 mg per cent).

8 days after the second surgical intervention, and the 2nd died of acute necrotizing papillitis 26 days after the second intervention. However, the data obtained on these 2 were sufficient to permit their inclusion in the group. The remaining rats did not show any complications and developed normally with no signs of weight loss, edema, etc.

During the first period in which the left nephritic kidney remained in place, 9 of the 20 rats showed a decrease in proteinuria to normal values; of these 9, 7 were in the group which was given NTAbs only, and 2 in the group given NTAbs plus rabbit GG in adjuvant. The decrease in proteinuria occurred in the majority of the rats between 4 and 14 days posttransplantation. Six additional rats showed a marked decrease in proteinuria from average levels of 242 mg/day pretransplantation to 56 mg posttransplantation. Of these, 1 was in the group given NTAbs only and the remaining in the group given NTAbs plus rabbit GG in adjuvant. During the second period, after removal of their

left nephritic kidney, none of the transplanted rats had proteinuria. Total urinary volume did not change significantly between the period in which the left nephritic kidney was left *in situ* and the period in which it was removed and the transplanted kidney was left alone. Urinary volumes varied from 8 to 30 ml per 24 hours. Control nephritic rats which did not receive transplants experienced no decrease in their proteinuria, maintaining levels fluctuating between 140 to 400 mg per 24 hours.

Ten of the nephritic rats had elevated BUN at the time of transplantation and all had a decrease to normal values 1 week later. The BUNs in this group ranged from 30 to 86 mg per cent with an average of 47.1 and they decreased to an average of 21.0. Of these, 2 had a subsequent slight increase in BUN (35 to 40 mg) which apparently was related to hydronephrosis of the transplant caused by periureteral fibrosis. At the time of sacrifice the other 18 rats with transplanted kidneys had completely normal BUNs.

Histologic studies of the transplanted kidneys showed no evidence of proliferative, exudative or membranous glomerulonephritis (Fig. 1). However, 8 of the 20 kidneys had minor glomerular alterations such as slight, focal increase in PAS material in the axial regions and in an occasional peripheral loop. These changes were best seen in 1 transplanted kidney which had been followed for 86 days. These 8 kidneys, when studied by fluorescent antibody technique, showed positive staining for rabbit GG indicating transfer of the rabbit NTA_b. Fifteen of the transplanted kidneys showed minimal postoperative complications such as hydronephrosis, focal pyelonephritis, or papillitis. However, in only 2 of these was hydronephrosis severe enough to compromise the function of the transplant (see Table I).

Electron microscopy of 4 of the transplanted kidneys of group I (37, 50, 62 and 63 days after transplantation), revealed no significant glomerular lesions. The kidneys examined 62 and 63 days after transplantation showed occasional focal areas of basement membrane thickening and of subendothelial deposits but endothelium and epithelial foot processes were normal.

The nephritic kidneys of recipients of normal kidneys were compared with the kidneys of NTN rats which did not receive transplants to see whether transplantation of a normal kidney affected the morphologic development of the nephritis. The rats receiving NTA_b only and no transplant showed the usual changes of NTN—thickening of glomerular capillary basement membranes, glomerular lobulation, adhesions and crescents. The recipients of transplants had, at time of transplantation, changes identical to those in the NTN controls. During the first posttransplantation period, the disease in the remaining nephritic kidney progressed but at a rate slower than that in the non-transplanted NTN controls. At removal, the 2nd nephritic kidney showed less scarring than those of the non-transplanted controls. The control rats receiving both NTA_b and immunization to rabbit GG showed an advanced glomerular scarring with

5 to 10 per cent of glomeruli completely hyalinized. Transplantation of normal kidneys in such nephritic rats altered the morphologic picture in the nephritic kidneys in three ways: the glomerular lesion became more cellular and less scarred, there was diffuse proliferation of fibroblasts in the interstitium and the tubules became dilated and contained hyaline casts.

By fluorescent antibody studies, 13 of 18 transplants, 7 of 8 in rats receiving NTAb only and 6 of 10 in those receiving NTAb plus immunization with rabbit GG, showed positive staining for rabbit GG in the glomerular capillary walls, indicating transfer of the NTAb from tissues of the recipient. Rabbit GG was noted in a faint, membranous pattern involving all capillaries. Staining for rat GG was positive in a weak and usually discontinuous membranous pattern in 12 of the 13 kidneys which were positive for rabbit GG; those negative for rabbit GG were negative for rat GG. Rat β 1C-globulin was positive in a weak and usually discontinuous membranous pattern in 8 of the 12 kidneys which were positive for rat GG; the remaining did not show specific fluorescence for rat β 1C-globulin.

Seven controls were studied in which a normal isologous kidney was transplanted to a normal rat and followed 47 to 176 days. None showed proteinuria or increase in BUN. Pathologically, 2 showed moderate degrees of hydronephrosis because of ureteral compression by fibrous tissue; 2 presented small areas of focal pyelonephritis, 1 had a small area of infarction in the lower pole. Three of the 7 transplanted kidneys exhibited mild glomerular changes, mainly in the form of an increase in PAS-positive material in the axial regions. Fluorescent antibody studies on 2 kidneys disclosed traces of rat GG and β 1C-globulin in an axial distribution.

Parabiosis of Rats with NTN to Normal Rats.—The results of these experiments are summarized in Table II. None of the normal partners of any of the strains used showed clinical evidence of nephrosis or renal insufficiency. No proteinuria was noted in any of the normal partners of the inbred pairs. Only 1 of the 13 normal Sprague-Dawley partners showed a mild proteinuria of 20 mg per 24 hours 17 days after parabiotic union. There was no weight loss or any evidence of parabiotic intoxication in any of the inbred pairs. However, 60 per cent of the Sprague-Dawley pairs showed evidence of mild to moderate parabiotic intoxication.

Proteinuria in the nephritic partners varied depending on the amount of NTGG injected and on whether or not immunization to rabbit GG was induced. Nephritic partners of the pairs in group I showed a substantial decrease in proteinuria during the whole period of parabiotic union. Of the 20 Buffalo rats of group I, 8 had return to normal levels of proteinuria and 6 showed significantly reduced levels; of the 12 Lewis rats of group I, 5 showed normal levels of proteinuria and 3 showed significantly reduced levels; of the 13 Sprague-Dawley nephritics of group I, 2 showed normal levels of proteinuria

while 3 showed significantly reduced levels. Proteinuria in the nephritics with significantly reduced levels ranged from 20 to 65 mg per 24 hours. Daily urine volumes in the nephritic partners varied from 7 to 15 ml while normals ranged from 10 to 45 ml. No decrease in proteinuria was noted in the Lewis nephritics of group II. The 8 Buffalo rats which comprised group III did not become proteinuric during the period of parabiotic union. Control nephritics of all

TABLE II
Intrastrain Parabiosis between Rats with NTN and Normal Rats

Strain	Group	Nephritic partner	Normal partner	
		Proteinuria	Proteinuria	Nephritis
Buffalo.....	I (20)	12/20*	0/20	0/20
Lewis.....	I (12)	7/12‡	0/12	0/12
Sprague-Dawley.....	I (13)	11/13§	1/13	0/13
Lewis.....	II (5)	5/5	0/5	0/5
Buffalo.....	III (8)	0/8	0/8	0/8
Total.....	(58)	35/58	1/58	0/58

Group I, rats given NTA_b (200 to 300 μg of KFA_b) alone and parabiosed 1 to 21 days later.

Group II, rats given NTA_b (250 μg of KFA_b) plus immunization to Rabbit GG and parabiosed 8 days later.

Group III, rats given NTA_b (40 μg of KFA_b) plus immunization to Rabbit GG and parabiosed 1 day later.

Figures in parenthesis indicate number of rats.

Numerator represents number of rats with particular results; denominator represents number of rats examined.

* Six additional rats had significantly reduced proteinuria.

‡ Three additional rats had significantly reduced proteinuria.

§ Three additional rats had significantly reduced proteinuria.

groups, which were not parabiosed, maintained high levels of proteinuria during the period of parabiosis of the experimentals.

Histologic studies at the time of termination of parabiosis showed absence of glomerulonephritis in all of the normal partners examined. There was no increase in glomerular cells and no thickening of the capillary walls (Figs. 2 *a* and 2 *b*). Approximately 10 per cent of the normal partners showed occasional focal increases of PAS-positive material in the axial regions of the glomeruli. There were no apparent differences between these normal partners and the normals of the control groups. The only normal Sprague-Dawley rat in the series which showed proteinuria had severe arteriolar nephrosclerosis. Seven normal partners of group I were examined by electron microscopy and no evidence of glomerular abnormality was obtained.

The nephritic parabionts showed the typical histologic changes of NTN. However, in the majority of nephritic parabionts the degree of histologic abnormalities was somewhat less than in control nephritics given similar treatments and followed for similar periods of time but not subjected to parabiotic union. The control nephritics of group I showed more proliferative activity in the glomeruli (20 to 30 per cent increase in cells); the control nephritics of group II had more scarring of the glomeruli with more crescents and a higher degree of interstitial fibrosis.

Antibody to rabbit GG was tested for in several representative pairs. No precipitating antibody was found in 5 Lewis rats of group I and 5 of group II when tested 30 days after parabiosis. However, evidence of circulating antibody was found by determining the rate of catabolism of a tracer dose of I^{131} rabbit GG. Three Lewis pairs of group II tested, (rats previously immunized to rabbit GG in adjuvant), showed a rapid catabolism of the labeled protein with 59 per cent of the injected I^{131} in the urine in non-protein bound form in the first 24 hours. Three Lewis pairs of group I showed a moderately elevated rate of catabolism of the labeled protein with 34 per cent of the injected I^{131} excreted in non-protein bound form *vs.* 8 per cent in controls. By the same methodology, circulating antibodies were found in 1 of 3 Sprague-Dawley pairs of group I tested 30 days after parabiotic union.

Fluorescent antibody studies were made on most normal partners at the time of separation (Table III). Sixteen of 20 Buffalo rats in group I examined had rabbit GG in the glomeruli, indicating transfer of the NTA_b from the nephritic partner (Fig. 3). Rabbit GG was present in a faint, membranous pattern involving all capillary loops; the fluorescence was fainter but in a distribution identical to that in the nephritic (Fig. 3). As noted in Table III, none of the normal partners of group III showed transfer of NTA_b; the nephritics of this group had received only 40 μ g of KFA_b. Eleven of 11 Lewis rats of group I showed transfer of NTA_b. However, none of 5 of the Lewis rats of group II which had been immunized to rabbit GG showed transfer. Four of the 13 Sprague-Dawley rats showed transfer of NTA_b.

Rat GG and β 1C-globulin were present in the glomeruli of most normal parabionts in a faint, axial pattern identical in distribution to that noted in the normal partners of the control groups. Fifteen rats (8 Buffalo, 5 Lewis, and 2 Sprague-Dawley) of group I also showed rat GG distributed in foci along the glomerular capillary walls but never throughout the whole tuft. Five of these rats, (3 Buffalo and 2 Sprague-Dawley) showed a similar distribution for rat β 1C-globulin. All 15 rats which showed focal positivity for rat GG also had transfer of NTA_b as evidenced by positive reaction for rabbit GG. Tissue C' fixation was negative in rats which did not show transfer of NTA_b (tested were 6 Sprague-Dawley rats of group I, and 2 Lewis rats of group II). It was positive in those showing transfer of NTA_b (tested were 1 Sprague-Dawley rat of group I and 2 Buffalo of group I).

Representative nephritic parabionts of each group and strain were studied by the fluorescent antibody technique. All showed a strong membranous positivity for rat GG and β 1C-globulin and for rabbit GG.

Behavior of nephritic and normal parabionts after separation was followed in 2 ways. In 11 pairs of rats of group I in which the proteinuria of the nephritic partner had been reduced (in 8 to normal levels and in 3 to significantly reduced levels), the members were separated and the nephritic partners followed with weekly urinalysis. Proteinuria returned to elevated figures in all these 11 rats starting from 4 to 14 days after separation. The levels of proteinuria became comparable to the levels of control nephritics of equal duration which

TABLE III
Fluorescent Antibody Studies of Normal Rats in Parabiosis with Rats with NTN

Strain	Group	Rabbit GG	Rat GG	Rat β 1C	Tissue C' fixation
Buffalo	I	16/20	8/20	3/20	2/2
Lewis	I	11/11	5/11	0/11	—
Sprague-Dawley	I	4/13	2/13	2/13	1/7
Lewis	II	0/5	0/5	0/5	—
Buffalo	III	0/4	0/4	0/4	0/2

Group I, rats given NTA_b (200 to 300 μ g of KFAB) alone and parabiosed 1 to 21 days later.

Group II, rats given NTA_b (250 μ g of KFAB) plus immunization to Rabbit GG and parabiosed 8 days later.

Group III, rats given NTA_b (40 μ g of KFAB) plus immunization to Rabbit GG and parabiosed 1 day later.

Rabbit GG was in faint but membranous pattern involving all loops. Rat GG and β 1C were in a faint and discontinuous membranous pattern.

Numerator represents number of rats with particular results; denominator represents number of rats examined.

had not been parabiosed. The histology of the kidney 4 weeks after separation, when compared to biopsies taken at the time of separation, showed definite progression of the glomerulonephritis.

The normal partners of 6 Lewis pairs of group I after separation were immunized to rabbit GG and followed for 4 weeks. Three developed a mild membranous glomerulonephritis with proteinuria ranging from 20 to 40 mg per 24 hours. The normal partners of 5 Sprague-Dawley pairs of group I after separation also were immunized to rabbit GG. Despite the absence of detectable transferred NTA_b, 3 of these rats developed a mild membranous glomerulonephritis and proteinuria of 20 to 80 mg/24 hours within 6 weeks.

No significant abnormalities were noted in the normal partners of any of the isologous intrastrain parabiotic control groups, regardless of the nature of the renal disease of their partners. No proteinuria was observed. Histologic studies

revealed no changes except occasional deposits of PAS-positive material in the mesangial zones of the glomeruli of a few rats. This change had no relation to the time of parabiotic union or the type of primary disease of one member of the pair. Rats with severe aminonucleoside nephrosis after parabiosis to isologous normals showed a decrease in proteinuria to normal levels. This decrease in proteinuria became evident 2 to 3 weeks after parabiotic union and persisted throughout the period of parabiosis.

The non-isologous pairs showed some renal changes presumably related to parabiotic intoxication. All 8 normal Sprague-Dawley pairs showed evidence of moderate parabiotic intoxication. However, no proteinuria was obtained in any of these. Histopathological studies of their kidneys showed congestion and moderate axial thickening of the glomeruli, usually more marked in one member of the pair. Fluorescent antibody studies showed deposition of rat GG and β 1C-globulin in an axial distribution. Four pairs of normal Buffalo rats united with Lewis rats showed severe parabiotic intoxication. All the Buffalo members became polycythemic with hematocrits of 60 to 74 per cent and showed proteinurias ranging from 35 to 90 mg per 24 hours. The Lewis members became runted and anemic with hematocrits of 20 to 40 per cent but none showed proteinuria. Histopathological studies showed that the Buffalo members exhibited congestion of the glomeruli with axial thickening and an increase in PAS-stainable material; the Lewis members exhibited few changes. By fluorescent antibody technique, both members showed moderate amounts of rat GG and β 1C-globulin in an axial distribution.

DISCUSSION

These experiments would seem to exclude convincingly the presence of an effective autoantikidney response in NTN. In neither severe, early, nor advanced NTN was there evidence of an antikidney response capable of significantly affecting, morphologically or functionally, either a transplanted isologous normal kidney or the kidneys of an isologous normal parabiont. In the case of the renal transplants, this was true whether one of the host's nephritic kidneys was present or not. Renal injury induced not only by NTAb but also by aminonucleoside and hypertension failed to induce an autoantikidney response detectable in the kidneys of normal isologous parabionts, thereby, casting considerable doubt on the possibility that glomerular injury, *per se*, leads to autoimmune responses.

These results are in agreement with a number of earlier studies. Protection of one kidney by clamping off its blood supply for the first few minutes after injection of NTAb has been reported (10, 11). This observation, while perhaps complicated by non-specific ischemic renal changes, would certainly be incompatible with an autoantikidney response. Similarly, in newborn animals, NTN can be induced in the functioning juxtamedullary glomeruli but this disease

does not progress to involve the glomeruli which become functional several days later (12, 13). Also, rats made tolerant to rabbit GG will not develop severe progressive nephritis after injection of rabbit NTGG as will normal rats (14). This last experiment, plus the demonstration of enhancement of nephritis in such tolerant subjects by injection of anti-rabbit GG, strongly suggests that the progressive course of NTN is dependent in large part upon the host's response to the heterologous NTAb. Whether the apparent continuing dissociation and reassociation of the heterologous NTAb in the tissues of the host provides a mechanism capable of causing significant renal injury remains to be determined. Finally, on the basis of other experiments in which kidneys with NTN were transplanted to normal isologous recipients, it seems that if severe enough glomerular injury is produced by the initial reaction of NTAb, the glomeruli will be unable to recover even in the absence of continuing injury so that a chronic nephritis will ensue (15).

While these observations directly contradict those of others who have claimed transfer of NTN by parabiosis or cell transfer, they do provide possible explanations for this disagreement. First, since NTAb can dissociate from a nephritic rat and pass to a normal parabiotic partner, a possible mechanism, albeit not autoimmune, for transfer of nephritis exists. While this transfer of NTAb did not cause disease in the present experiments, it is possible that it might in another situation with more NTAb or a better anti-gamma globulin response by the normal parabiont. Second, the fact that proteinuria can be associated with parabiotic intoxication makes this a possible mechanism when the parabiotic partners are not isologous as was the case in earlier studies (1).

Two interesting incidental observations have come out of these experiments. One of these is the response of the nephritic kidney in the presence of normal, functioning renal tissue. Both in the kidney transplant and parabiosis experiments the progression of the nephritic process was apparently slowed and proteinuria, which was the only parameter of renal function studied, improved. A similar observation was made in parabiosis of rats with aminonucleoside nephrosis to normal rats. One apparent reason for this is the normal renal function provided by transplant or parabiont. As soon as this normally functioning renal tissue was removed, the nephritic process again progressed as in control nephritic rats. This apparent sparing of diseased nephrons by normal nephrons may find explanation in the studies of Bricker and Hinman on unilateral renal disease which describe a physiological adaptation that takes place between normal and diseased populations of nephrons (16, 17, 18). It thus becomes apparent that some of the pathological processes seen in the progressive stage of NTN can be partially slowed or ameliorated upon placing the kidney in a more favorable milieu.

The second observation has to do with the transfer of NTAb molecules from the tissues of the originally injected rat to the kidneys of a normal rat in para-

biosis or to a normal transplanted kidney days or even weeks after its injection. This observation was first made by Seegal (see reference 19) in parabiosis experiments and has been quantitated in our laboratory by the use of I^{125} -labeled NTGG. The transfer apparently depends upon the continuous dissociation and reassociation of at least some of the NTAbs with the tissue antigens. On the basis of other studies, the antibody primarily involved in this transfer may have a low avidity for rat tissue antigens and, therefore, dissociate readily (20). It appears from these experiments that the antibody levels of the rat to rabbit NTGG must be relatively low in order to permit the dissociated NTGG molecules to remain in the circulation and to reach the normal parabiont or the transplanted kidney. These NTAbs molecules presumably could be present in the circulation as single molecules or as small antigen-antibody complexes and in either form reach the normal kidney. The amounts of transferred NTAbs are too small to cause significant renal injury by themselves in the conditions of our present experiments. These amounts range from 2 to 10 μ g of KFAbs in the parabiosis experiments and from 30 to 90 μ g in kidney transplants. However, these small amounts of NTAbs in the presence of a persistent anti-rabbit GG response will cause renal injury. This situation, which has been reported previously (5), was noted in Lewis and Sprague-Dawley normal parabionts which, after parabiosis, were separated and immunized to rabbit GG. The Sprague-Dawley rats, despite showing no rabbit NTGG by fluorescent technique, became proteinuric after immunization indicating that they did have small amounts of NTGG in their glomeruli. Normal rats immunized to rabbit GG do not become proteinuric (5).

CONCLUSIONS

Rats with nephrotoxic serum nephritis were studied for the presence of a possible autoimmune response to renal antigens formed and/or liberated during the immunologic reactions taking place in the glomeruli. The experiments consisted of transplantation of a normal isologous kidney to a nephritic rat and parabiosis of a normal rat to a nephritic rat. Neither functional nor morphologic abnormalities were noted in the normal kidneys in either situation. Transfer of the rabbit nephrotoxic antibody to the normal kidneys was noted in both experiments, indicating a continual dissociation and reassociation of nephrotoxic antibody with the tissue antigens of the host. Some of the nephritic rats showed a decrease in proteinuria and a slowing in the progression of the nephritis during the period in which the normal kidney was transplanted or a normal rat was united in parabiosis.

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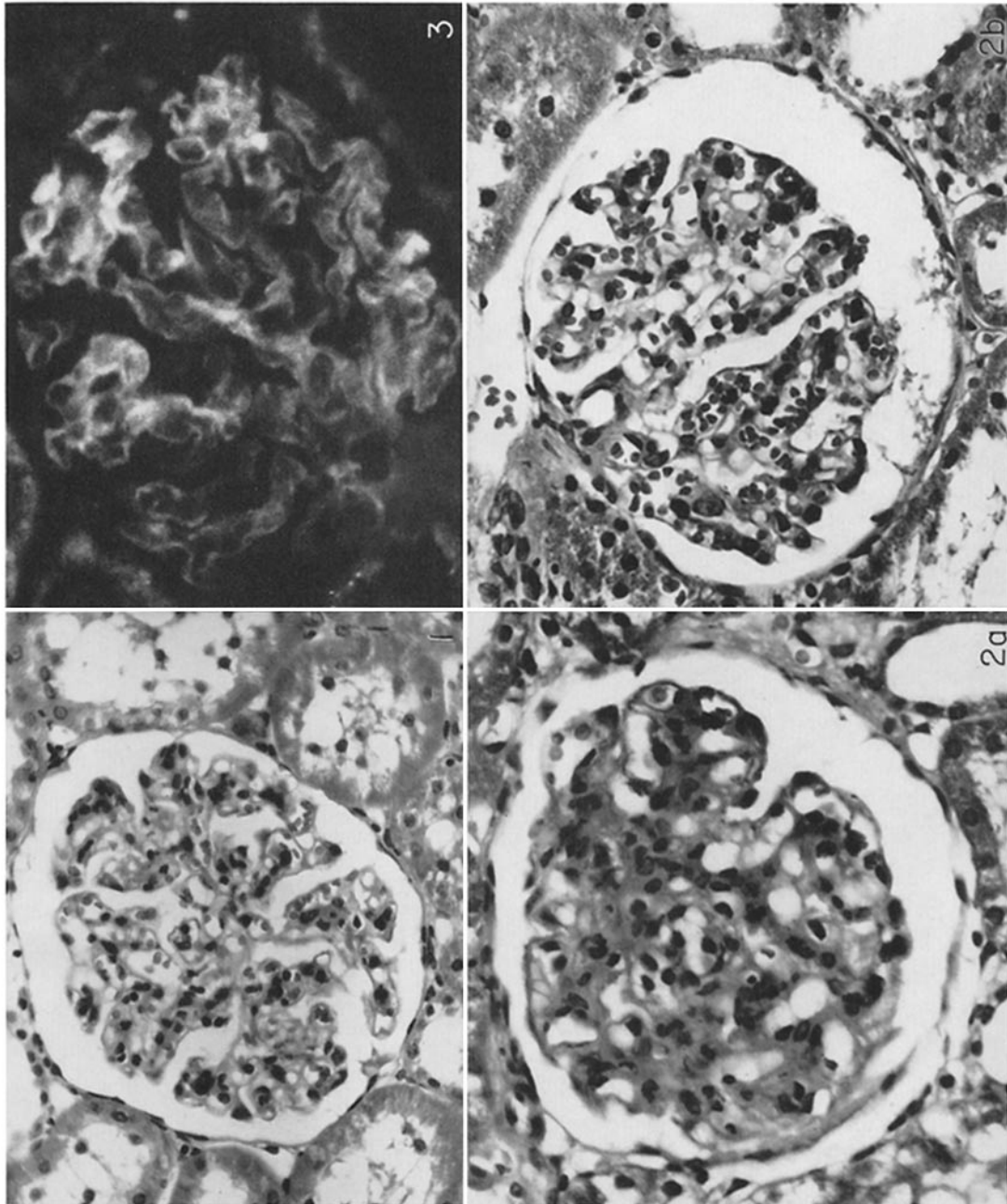
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EXPLANATION OF PLATE 36

FIG. 1. Photomicrograph of representative glomerulus from transplanted kidney. Kidney was transplanted to a NTN rat (NTN induced by NTA_b plus immunization to rabbit GG) and followed for 62 days. Histologically it appears normal. $\times 350$.

FIGS. 2 *a* and 2 *b*. Photomicrographs of representative glomeruli from kidneys of nephritic and normal members of parabiotic pair. Parabiosed were Sprague-Dawley rat with NTN (group I) and normal Sprague-Dawley rat. Parabiosis lasted 42 days. Normal partner did not show proteinuria and had moderate parabiotic intoxication. Nephritic partner showed reduction of proteinuria during parabiosis. Fig. 2 *a* shows a glomerulus from the nephritic partner showing thickened capillary walls, increased number of cells in the mesangia and adhesion to Bowman's capsule. Fig. 2 *b* shows a glomerulus from the normal partner showing moderate dilation and congestion of the capillaries but no evidence of glomerulonephritis. $\times 263$.

FIG. 3. Fluorescence micrograph of glomerulus from kidney of normal member of parabiotic pair stained with fluorescent anti rabbit GG. Fluorescence is in a weak but uniform membranous pattern. Pair consisted of a Sprague-Dawley rat with NTN (group I) and a normal Sprague-Dawley rat. Parabiosis lasted for 20 days. $\times 350$.



(Unanue *et al.*: Experimental glomerulonephritis. VII)