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Nephrotoxic Serum Nephritis in Thymectomized Rats.* (28857)

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Injection of serum from rabbits immunized with homogenates of rat kidney (NTS) promptly induces a nephritis in the rat. Apparent fixation of rabbit γ-globulin as well as rat complement within glomerular capillary walls may be demonstrated by immunofluoresence. Following the acute injury, progression to a chronic nephritis not unlike that observed in humans may be noted. Development of the latter has been presumed to result from either a reaction between host's antibodies formed in lieu of the heterologous antibody fixed in the kidney(1) or damaged renal tissue induced by the initial injury(2). In an attempt to gain some insight into the pathogenesis of the chronic nephritis resulting from injections of NTS, particularly that attributed to the role of host antibody production in this process, it was considered worthwhile to observe the clinical, biochemical, morphologic and immunohistochemical features of NTS nephritis in rats thymectomized at birth. This procedure has been observed to suppress the development of autoallergic encephalomyelitis and tuberculin sensitivity, delay the rejection of skin homografts and inhibit the production of antibodies of the precipitating and hemagglutinating type(3).

Materials and methods. One ml of NTS was intravenously injected into 26 two-month-old male and female Long-Evans rats which had been thymectomized at birth and weighed 150-180 g. Twenty intact rats of similar strain, age and weight received a comparable dose of the same NTS. Eight controls received 1 ml of normal rabbit serum (NRS).

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All animals were housed in individual metabolism cages and received a standard ration and water *ad lib*. Daily urinary protein was estimated as described previously(4).

NTS utilized to produce nephritis was prepared in rabbits by intraperitoneal injections of homogenates of kidneys from Long-Evans rats as described previously(4). All sera obtained were pooled and heated at 57°C for one-half hour prior to use.

Surviving rats (Table I) were sacrificed by aortic puncture at 1 and 5 weeks following injection of NTS. Serum BUN, cholesterol, total lipid and total protein were estimated as described previously(4). The thymic region was carefully explored in all thymectomized rats and any possible thymic remnants as well as portions of kidney were fixed in Zenker's fluid. Other portions of kidney were quickly frozen on dry ice and stored at −20°C. Thymus, spleen and adrenals were blotted and weighed and fixed in Zenker's fluid. All tissues were dehydrated in the usual manner. Sections were stained with hematoxylin and eosin and the periodic acid-Schiff technic. Frozen sections of kidney were cut with a cyrostat at −20°C and stained with sheep anti-rabbit gamma-globulin (anti-RGG) and rabbit anti-rat gamma globulin (anti-rGG) conjugated with fluorescein isothiocyanate. These were prepared as described previously(5) as well as purchased commercially.† Purity of all conjugates was assessed prior to use by immunoelectrophoresis and immunologic controls consisted of treatment of sections with respective unconjugated gamma globulins prior to application of conjugated preparations. Fluorescence microscopy was performed with an OSRAM HGO 200 mercury lamp and Zeiss exciter and barrier filters.

Results. Six intact (30%) and 7 (27%) thymectomized rats receiving NTS died during the first week of the experiment. Serum BUN was markedly elevated in these animals to 50-60 mg%. Although histologic appearance of the kidneys from these animals was comparable to that observed in rats sacrificed at one week, they were not included in the final analysis of data. Similarly 4 surviving thymectomized members were also excluded since small fragments of thymus verified by histologic study were identified at autopsy. These animals were also excluded from final analysis. Onset and degree of daily urinary protein (Fig. 1), serum BUN, cholesterol, lipid and total protein were comparable in both thymectomized and intact rats receiving NTS (Table I). Thymectomized rats exhibited slight loss of body weight one week following injection of NTS. Those sacrificed at 5 weeks exhibited weight gain which was less than that noted in intact members. Peritoneal cavities were moist in all NTS-treated animals. Splenic weight was significantly less in all thymectomized rats as compared to intact members ($P<.01$). No difference in adrenal weights was evident and thymic weight was comparable in controls and intact rats receiving NTS. No difference in the above parameters of NTS nephritis were noted in male or female rats of all groups.

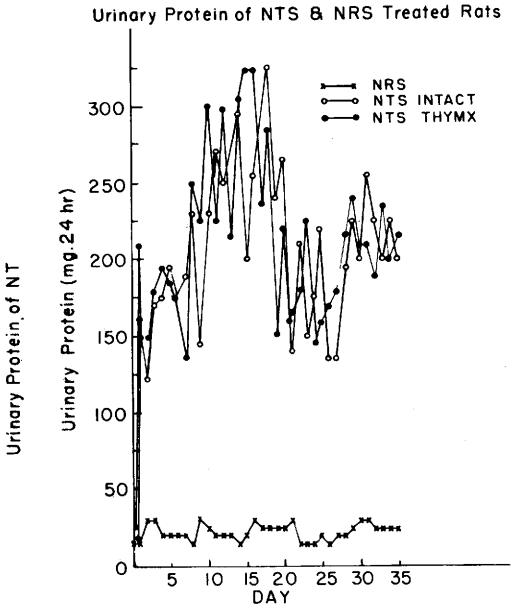

FIG. 1. Daily urinary protein of NTS- and NRS-treated intact and thymectomized rats.

Histological examination of kidneys from thymectomized and intact rats treated with NTS sacrificed at one week disclosed a mixed membranous and proliferative glomerulonephritis. This was characterized by thickening,

† Antibodies, Inc., Davis, Calif.

TABLE I. Body Weight, Biochemical and Organ Weight Changes in Rats Receiving NTS and NRS.

	# *	Change body wt (g)	BUN † (mg %)	Cholesterol (mg %)	Total lipid (mg %)	Total protein † (g %)	Thymus (mg)	Spleen (mg)	Adrenals (mg)
I Intact + NTS									
1 wk	6	+24	24	141 ± 15 ‡	215 ± 30	5.0	205 ± 80	692 ±186	46 ±11
5 "	8	+80	28	152 ± 23	273 ± 35	4.9	243 ± 71	632 ± 94	47 ±10
II Thymx + NTS									
1 wk	5	−11	29	185 ± 38	250 ± 42	4.8	—	332 ± 80	43 ± 5
5 "	8	+45	25	164 ± 21	314 ± 30	5.1	—	318 ± 65	48 ± 9
III Intact + NRS									
1 wk	6	+12	25	87 ± 17	145 ± 24	6.4	179 ± 81	628 ± 84	50 ± 6
5 "	6	+25	24	90 ± 15	160 ± 32	6.2	198 ± 65	549 ±110	53 ± 8

* Surviving or without demonstrable thymus in Group II. † Pooled samples. ‡ Standard deviation.

splitting and irregularity of glomerular basement membranes (Fig. 2 A & B). Glomerular endothelial and epithelial cells appeared increased in number and occasional synechiae of glomerular tufts to Bowman's capsule as well as intracapillary thrombi and a rare crescent were evident. Hyaline casts were noted in lumens of distal tubules and epithelium of proximal convolutions contained variable numbers of hyaline droplets. These glomerular and tubular changes were also apparent in intact and thymectomized rats sacrificed at 5 weeks. In addition, glomeruli of both exhibited focal and occasionally diffuse glomerulosclerosis (Fig. 3 A & B). Synechiae were also more commonly encountered in rats sacrificed at this time. Kidneys from rats receiving an injection of NRS appeared unaltered. Spleens from thymectomized members revealed marked diminution of size and apparent number of lymphoid follicles. (Fig. 4 A & B). Intersinusoidal splenic tissue also contained less lymphocytes in thymectomized than in intact rats. Thymic and splenic structure of intact rats receiving NTS was comparable to that observed in rats receiving an injection of NRS.

Examination of kidneys from both thymectomized and intact NTS-treated rats revealed similar distribution and intensity of specific fluorescence of rabbit gamma globulin within glomerular basement membranes at 1 and 5 weeks. No rabbit gamma globulin could be detected by this technic at these periods in rats receiving NRS (Fig. 5 A, B, C). Although rat gamma globulin could be detected in glomeruli of both intact and thymectomized, NTS-treated rats, its distribution did not appear significantly different from that observed in rats treated with normal rabbit serum and the intensity of the fluorescence was only slightly greater in the former (Fig. 6). On the other hand, tubular casts in all NTS-treated rats exhibited bright fluorescence when treated with anti-rG reaffirming the potency of the conjugate employed.

Discussion. Results indicate that thymectomy performed at birth fails to alter the clinical, biochemical, morphological and immunohistochemical characteristics of the glomerulonephritis induced in rats by administration of NTS. Early deaths following administration of NTS in both thymectomized and intact rats have been attributed to acute renal failure. A similar experience has been noted previously in rats receiving NTS. Although anemia following NTS injection may also be responsible for early death the lot of NTS utilized has not been noted to produce such a hematologic effect. Histologic evidence indicating chronic renal disease in both thymectomized and intact members militates against the presumption that this phase of NTS nephritis results from the effects of host's antibody to the rabbit gamma globulin fixed to kidney tissue or damaged renal substance at least during the time interval studied. In this regard it is of interest that suppression of circulating antibody to heterologous protein as well as the failure to develop such an autoimmune phenomenon as autoallergic encephalomyelitis has been demonstrated in rats thymectomized at birth(3).

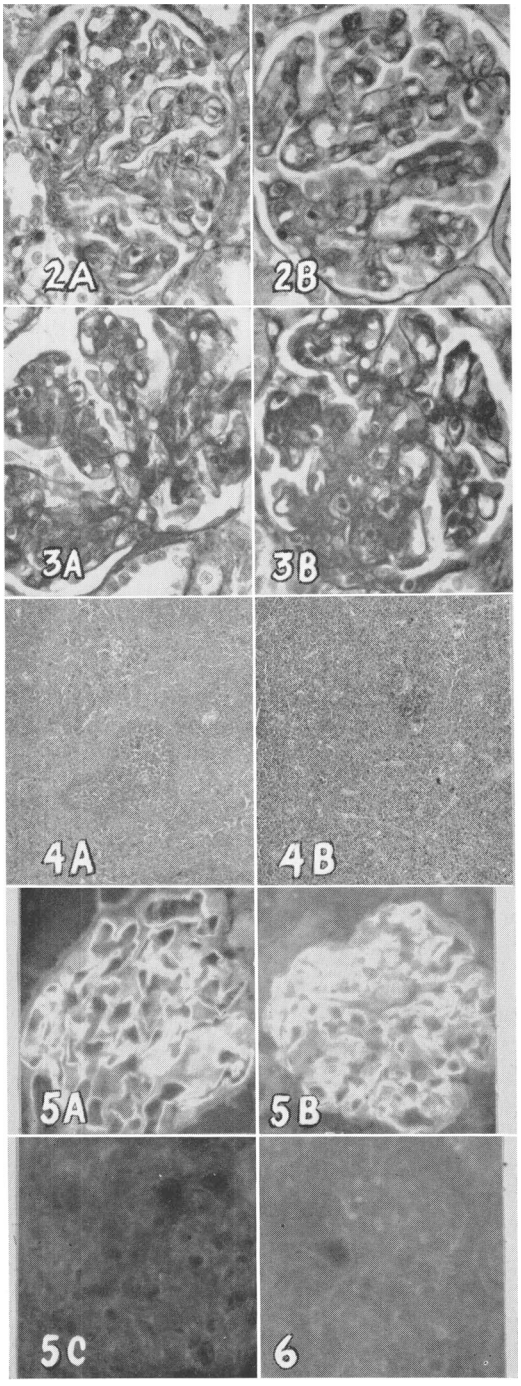

FIG. 2. Proliferative and membranous nephritis in intact (A) and thymectomized (B) rats 1 week after NTS (PAS × 250).

FIG. 3. Focal glomerulosclerosis in intact (A) and thymectomized (B) rats 5 weeks after NTS (× 250).

FIG. 4. Follicular structure of spleen of intact, NTS-treated rat appears normal (A). Spleen follicles are markedly decreased in thymectomized member (B). (× 20)

FIG. 5. Fluorescence of glomerular basement membrane indicative of rabbit γ globulin in intact (A) and thymectomized (B) NTS-treated rats at 5 weeks and its absence in rat receiving NRS (C). (× 200)

FIG. 6. Faint fluorescence of glomerular basement membrane indicative of rat γ globulin in thymectomized NTS-treated rat. Distribution is comparable to that noted in controls. (× 200)

Although host's gamma globulin which could represent antibody was detected in glomeruli of rats receiving NTS, as noted previously by others(1), its similarity in distribution to that noted in controls suggests that it may represent disposition of circulating native protein. The more intense reaction noted in nephritic animals as compared to controls is interpreted as a reflection of greater concentration of this protein in the diseased kidney. Osmophilic deposits, other than antigen-antibody complexes, which most likely represent protein, are not infrequently observed by electron microscopy in glomeruli from rats with experimental renal disease characterized by proteinuria. Serologic information if available might have provided a more definite answer in this regard. Dixon *et al*(6) also failed to discern evidence that foreign protein attached to glomerular basement membrane immunologically participated in development of the chronic glomerulonephritis observed following multiple injections of heterologous proteins. However, more recently Hammer and Dixon(7) have observed that rats made tolerant to RGG at birth failed to develop a secondary phase or chronic form of NTS nephritis. Glomerular localization of host antibody detected by the fluorescent antibody technic and possibly complexes of the host antibody, complement and antigen by electron microscopy(8) were noted in non-tolerant but not in tolerant rats receiving NTS. The distribution of fluorescence appears different from that noted with heterologous protein. Whether this is significant concerning pathogenesis of the lesions in the chronic phase is unclear. In any event, these investigators did not observe evidence of antikidney antibody being formed to account for the chronic disease. The discrepancy of findings concerning the significance of the demon-

strable rGG may be due in part to the shorter period of observation of NTS nephritis reported herein.

Summary. Clinical, biochemical, morphological and immunohistochemical characteristics of renal disease induced in rats with injections of anti-rat kidney rabbit serum (NTS) were similar in adult rats which were thymectomized at birth and in intact members of comparable age. The development of chronic glomerular lesions in thymectomized, NTS-treated rats indicates that progression of the disease is not necessarily dependent upon those immunologic functions related to thymic function, at least during the time interval studied.

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Effect of Butter Fat and Body Weight on Experimental Atherosclerosis in Rabbits.* (28858)

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The importance of diet as a prime factor in the etiology of atherosclerosis in man has been a subject of discussion and investigation for many years. Emphasis has been placed upon the cholesterol content and type of fat in the diet, although the role of body weight has also received some attention.

The literature on the correlation between dietary fat, serum lipids and atherosclerosis has been extensively reviewed by Wigand (17), Katz and Stamler(9) and others. Clinically, it has been demonstrated that significant and sustained reduction in serum cholesterol can be produced by placing the subjects on diets containing large amounts of vegetable fat(1,2,3,10,11,15). This effect has been uniformly attributed to the polyunsaturated character of vegetable oil. In experimental animals, there are conflicting reports concerning the effect of unsaturated and saturated fats. In some studies it has been shown that cholesterol-free diets high in saturated fats produce both hypercholesterolemia and experimental atheromatosis(17). In others, hyperlipemia is said to occur without atheromatosis(8), and in still others neither occurs(16).

There is also controversy as to the influence of body weight on atherosclerosis. For example, in man it has been demonstrated that advanced atherosclerosis was twice as common in the obese than in poorly nourished subjects(18). In rabbits it has been reported that the severity of atherosclerosis is directly proportional to high initial weight and rapid weight gain(4,5). However, the opposite effect has also been described(7).

In view of these controversies, we have attempted to evaluate the effect of butter fat and body weight on serum lipids and experimental atherogenesis in rabbits by feeding them cholesterol, butter and *ad libitum* stock diets in various amounts and combinations.

Material and methods. Two separate experiments were performed, using New Zealand white rabbits of known pedigree. The

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