5. Oliver, W. J., J. Lab. Clin. Med., 1963, v62, 449.
6. Euler, U. S. v., in Hormones in Blood, C. H. Gray & A. L. Bacdarach, ed., Academic Press, New York, 1961, p515.
7. Euler, U. S. v., Lishajko, F., Acta Physiol. Scand., 1961, v51, 348.
8. Pisano, J. J., Crout, J. R., Abraham, D., Clin. Chem. Acta, 1962, v7, 285.
9. Brunjes, S., Wybenga, D., Varner, J. J., Jr., Clin. Chem., 1964, v10, 1.
10. Swingle, W. W., DaVanzo, J. P., Glenister, D., Wagle, G., Osborne, M., Rowen, R., Proc. Soc. Exp. Biol. & Med., 1960, v104, 184.

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Effects of Neonatal Thymectomy on Spontaneous Murine Autoimmune Disease.* (32307)

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The possible pathogenetic role of the thymus in autoimmune disease has been the subject of much recent investigation. Tumors and other thymic abnormalities occur in patients with dysgammaglobulinemia; thymic epithelial hyperplasia and/or germinal center formation are described in systemic lupus erythematosus (SLE) and myasthenia gravis (1).

Atypical epithelial hyperplasia and structures resembling germinal centers were described by Holmes and Burnet in the thymus of NZB mice with autoimmune hemolytic anemia and nephritis(2). These authors considered the changes to be representative of the development of "forbidden clones" which initiate autoimmune reactions. As neonatal thymectomy is known to impair the full development of immunologic competence in many animal species, it was logical to attempt to determine the effect of neonatal thymectomy in murine strains with autoimmune disease. On the one hand, Helyer and Howie reported the earlier appearance of auto antibodies and more florid renal disease in neonatally thymectomized animals(3,4); whereas Holmes and Burnet noted that the appearance of Coombs antibodies was delayed 2 to 3 months in NZB mice thymectomized 2 to 6 days after birth(5,6). We report herein on the effect of neonatal thymectomy in two F_1 hybrid strains, NZB-NZW and NZB-NZC.† Nephritis is the major manifestation of the disease in the former, hemolytic anemia in the latter(7).

Material and methods. Thymectomies were done within 24 hours of birth by the technique of Helyer & Howie(3). Two groups served as controls: nonthymectomized and sham thymectomized animals. In the latter the thymus was dissected but left in place. Mice from newborn litters were assigned at random to the 3 groups.

Studies were made on mice which survived to weaning: 24 neonatally thymectomized (Tx) NZB-NZW F_1 mice and 25 controls (11 sham operated; 14 unoperated); 21 Tx NZB-NZC F_1 mice and 17 sham operated and unoperated controls. As there were no differences in the 2 control groups of each strain they are considered together. All mice were inspected and weighed weekly; blood was drawn biweekly for hematocrit, Coombs tests, antinuclear antibodies and LE cells(8). Animals were sacrificed when ill. A complete autopsy was done in 66 mice; completeness of thymectomy was confirmed by gross inspection and histologic examination in all but 2 NZB-NZC mice.

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†A breeding colony of mice of the NZB, NZW & NZC strains was kindly provided by Dr. B. J. Howie, Dunedin, New Zealand.

TABLE I. Percent Survivors in the NZB-NZW F_1 and NZB-NZC F_1 Mice at Various Ages.*

	NZB-NZW			NZB-NZC		
Age (mo)	Neonatal Tx (24)	Experimental control (25)	Colony (164)	Neonatal Tx (21)	Experimental control (17)	Colony (23)
6	79	92	94	81	94	96
9	63	69	71	66	82	96
12	45	46	52	33	53	96

* Calculated according to Merrell and Shulman(9). Number of animals in each group is in parentheses.

Results. Clinical findings. A fatal wasting syndrome was not observed before 4 months of age. Four of 21 Tx NZB-NZC mice and 3 of 24 Tx NZB-NZW mice developed steady weight loss and hunched posture, and died or were sacrificed between 4 and 11 months of age. The weight gain of the remaining Tx mice was similar to that of the controls. The survival rate, calculated according to the method of Merrell and Shulman(9), is summarized in Table I, in which data for the whole colony(8) are presented for comparison. The survival appears to be slightly decreased in both Tx groups.

The hematocrit levels and incidence of positive Coombs tests were similar in Tx and control mice of both strains.

The test for antinuclear antibodies was positive in Tx animals of both strains earlier and more frequently than in their respective controls. At 12 and 16 weeks the test was positive respectively in 14 and 17 of 24 Tx NZB-NZW animals, but only in 3 and 4 of 25 controls. In the NZB-NZC strain the test was positive at 16 weeks in 8 of 21 Tx animals, in none of 16 controls. By 32 weeks, 11 of the Tx animals, but only 1 control, had a positive test for antinuclear antibodies. The serum proteins were studied in 51 animals (Table II). There was no significant difference in the β and γ globulin levels of the experimental and control groups in each strain.

Significant proteinuria was found more frequently in Tx NZB-NZW animals than in controls. The concentration of protein in bladder urine was 100 mg/100 ml or greater in 11 of 16 Tx animals but only in 4 of 14 controls. Significant proteinuria was found in 6 of 15 Tx NZB-NZC mice and in 5 of 12 controls. Significant azotemia was not observed in either strain.

Pathologic findings. The splenic index (spleen weight/body weight $\times$ 10^3) was similar in Tx and control NZB-NZW mice. By contrast, the splenic index of control but not of Tx NZB-NZC mice increased considerably with age, and, over 8 months of age, was significantly higher than that of the Tx animals ($\chi^2_{[1]} = 8.1$, $p<0.01$). A detailed semiquantitative analysis (graded 0 to 4+) of the histologic findings in the spleen was made using masked coded slides (Table III). Despite the difference in spleen size of Tx and control NZB-NZC animals, detailed histologic analysis did not reveal significant morphologic differences save perhaps for the fact that hemosiderosis was less prominent in the spleens of the Tx NZB-NZC animals, and that plasma cells and Russell bodies were perhaps slightly more prominent in the spleens of Tx animals of both strains.

Only a few lymph nodes were examined.

TABLE II. Serum Proteins in Thymectomized and Control NZB-NZW and NZB-NZC F_1 Mice.*

Strain	Thymectomy or control	No. of animals	Total protein	Albumin	Globulins			
					$\alpha 1$	$\alpha 2$	β	γ
NZB-NZW	Tx	13	7.41 ± .75	3.04 ± .58	.78 ± .29	.82 ± .27	1.50 ± .23	1.19 ± .41
"	C	11	6.88 ± 1.29	2.58 ± .66	.97 ± .25	.83 ± .27	1.32 ± .27	1.21 ± .26
NZB-NZC	Tx	14	7.24 ± 1.60	2.89 ± .79	.86 ± .30	.79 ± .32	1.50 ± .47	1.09 ± .47
"	C	13	6.95 ± 1.10	3.15 ± .63	.81 ± .38	.72 ± .35	1.30 ± .42	.94 ± .34

* The results in g/100 ml are expressed as mean ± 1 S.D.

TABLE III. Detailed Semiquantitative Analysis of Histology of Spleen from Neonatally Thymectomized and Control NZB-NZW and NZB-NZC F_1 Mice.*

Histologic feature	NZB-NZW		NZB-NZC	
	Tx (5 mice)	Control (7 mice)	Tx (12 mice)	Control (10 mice)
Presence of Malpighian follicles with germinal centers	2.0	2.3	.8	.5
Pyroninophilic reticulum cells	2.0	1.6	1.0	.9
Plasma cells	3.0	2.3	1.4	.9
Extramedullary hematopoiesis	1.0	1.1	2.4	2.3

* Average scores on a scale 0-4.

No significant differences were noted. In the thymus of the control NZB-NZW mice there was massive replacement of the medulla with pyroninophilic reticular cells and plasma cells, and foci of these cells were also found in the cortex. The findings in the thymus of the control NZB-NZC animals did not differ strikingly from those in strains of mice without autoimmune disease.

A detailed analysis of the renal histology from 41 animals was made, using masked coded slides. Differences between Tx and control animals were slight in both strains. Glomerulitis and glomerulonephritis(8,10) were observed in one each of 11 Tx NZB-NZC mice but in none of 12 controls. Although the number of animals is small, it is likely that neonatal thymectomy slightly accelerated the renal lesions in NZB-NZW mice. Glomerulonephritis was observed in 4 of 11 kidneys from Tx NZB-NZW animals (average age 44 weeks) and in 2 of 8 controls (average age 54 weeks).

Discussion. The effects of neonatal thymectomy in NZB-NZW mice were similar in character but less striking than those reported in this strain by Helyer and Howie (4). In Tx NZB-NZC mice life span was more definitely shortened, anemia was slightly less frequent and splenomegaly did not occur. The appearance of antinuclear antibodies and the occurrence of the disease was accelerated by neonatal thymectomy in both strains, and the effects were more striking in females. The reasons for this enhancement of the disease in neonatal Tx animals are not clear, but the results resemble in many respects those reported in neonatal Tx mice of hybrid strains which do not have autoimmune disease(11); they are also consistent with the view that the thymus may be defective from birth as a regulating mechanism of the development of autoimmune clones.

New evidence favors the hypothesis that the thymus and bursa of Fabricius are central lymphoid tissues with different influences on the ontogenesis of the immune mechanism, certainly in the chicken and probably in the mammal(1). When the present experiments were started thymectomy was thought to suppress the whole immunologic mechanism including plasma cells and antibody production. The new data(1) suggest that the development of cellular immune functions (*e.g.*, delayed hypersensitivity, homograft rejection and graft *versus* host capability) is controlled by the thymus. The bursa appears to influence germinal center and plasma cell formation, immunoglobulin synthesis and specific antibody production. These are characteristic abnormalities in human SLE and murine autoimmune disease, and their occurrence was not prevented by neonatal thymectomy in the present experiments. This suggests an alternate possibility that the as yet incompletely defined mammalian analog of the bursa of Fabricius may play a pathogenetic role in murine autoimmune disease more important than that of the thymus.

Summary. Thymectomies were done on the first day of life in NZB-NZW and NZB-NZC F_1 hybrid mice. Sham thymectomized and nonthymectomized animals served as controls. Survival was somewhat shortened in thymectomized animals. The appearance of antinuclear antibodies, but not of Coombs positive hemolytic anemia was accelerated. There was little effect of thymectomy on the morphology of spleen and lymph nodes. When considered in relation to recent work on the bursa of Fabricius the results suggest that the mammalian analog of the bursa,

rather than the thymus, may play a more important role in the pathogenesis of the murine autoimmune disease.

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1. Good, R. A., Gabrielsen, A. E., Cooper, M. D., Peterson, R. D. A., Ann. N. Y. Acad. Sci., 1966, v129, 130.

2. Burnet, F. M., Holmes, M. C., J. Path. Bact., 1964, v88, 229.

3. Helyer, B. J., Howie, J. B., Lancet, 1963, v2, 1026.

4. Howie, J. B., Helyer, B. J., in The Thymus: Experimental and Clinical Studies, Wolstenholme, G. E. W. & Porter, R., ed., Little, Brown, Boston, 1966, p360.

5. Holmes, M. C., Burnet, F. M., Aust. J. Exp. Biol. Med. Sci., 1964, v42, 589.

6. ———, in The Thymus: Experimental and Clinical Studies, Wolstenholme, G. E. W. & Porter, F., ed. Little, Brown, Boston, 1966, p381.

7. Howie, J. B., Helyer, B. J., Ann. N. Y. Acad. Sci., 1965, v124, 167.

8. Miyasato, F., Manaligod, J., Pollak, V. E., Arch. Path., 1967, v83, 20.

9. Merrell, M., Shulman, L. E., J. Chron. Dis., 1955, v1, 12.

10. Manaligod, J., Pirani, C. L., Miyasato, F., Pollak, V. E., Nephron, 1967, v4, 215.

11. Miller, J. F. A. P., in The Thymus: Experimental and Clinical Studies, Wolstenholme, G. E. W. Porter, R., ed., Little, Brown, Boston, 1966, p153.

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Action of Alkali Metals on Papillary-Cortical Sodium Gradient Of Dog Kidney.* (32308)

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It is generally accepted that a countercurrent transfer mechanism is responsible for the concentrating process in mammalian kidney. Evidence in favor of this view has been reviewed(1). As a further generality, it seems that sodium transfer out of the ascending limb of the loop of Henle is the "single effect" which underlies the countercurrent system. The following observations support this hypothesis: 1) A gradient in sodium concentration is found between papilla and cortex and the gradient is related to the final osmolality of the urine(2,3,4). 2) In micropuncture studies, it has been found that fluid collected from an ascending limb is usually hypo-osmotic to fluid collected from an adjacent descending limb(5). 3) Under free flow conditions, the ascending limb is electronegative by 8-10 mv to the interstitium(6,7).

Several observations, however, are difficult to explain if sodium movement out of the ascending limb is the only mechanism responsible for urinary concentration. 1) Using the Gertz technique, Gottschalk found that a fluid droplet was reabsorbed from the descending limb but not from the ascending limb(8). This result would indicate solution transfer in the opposite direction to that predicted if no *ad hoc* assumptions are invoked. 2) Although Marsh and Solomon did not find collapse of the descending limb droplet under all stop-flow conditions, they were unable to detect the establishment of any significant electrochemical gradients in either ascending or descending limbs under stop-flow conditions utilizing the Gertz technique(7). 3) Thurau and Henne have found volume flow in the ascending limb to be greater than that in the descending limb(9).

If, in fact, sodium transport is the only factor responsible for establishing the corticopapillary concentration gradient, further insight into this problem might be gained from a study of the effects of alkali metals on the concentrating process. In many transport systems alkali metals interact, and more specifically, lithium competes with sodium

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