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Role of the Appendix in Development of Immunologic Capacity.* (29003)

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Numerous experiments have shown that neonatal thymectomy in the mouse, rat, and hamster markedly reduces the immunologic capacity of the maturing animal(1-7). In rabbits, however, neonatal thymectomy has resulted in some depression of antibody production, but the differences between the antibody levels of the neonatally thymectomized animals and control groups have varied in magnitude and in statistical significance(8-10). Neither delayed hypersensitivity nor transplantation immunity has been discernibly affected by neonatal thymectomy in the rabbit, in contrast to the findings in other species(10,11).

In recent months evidence from a number of laboratories has suggested that immunologic development of the chicken is significantly affected by 2 lymphoid organs, the thymus and the bursa of Fabricius(12-16). Archer *et al*(17) have hypothesized that the rabbit appendix may be a homologue of the bursa of Fabricius of the chicken, based on derivation of both organs from epithelium of the gut, the similarity of their lymphoid development by a budding of follicles from epithelium, their striking morphologic resemblance at maturity, and the apparent status of the appendix as a thymus-independent organ. Thus, the limited effect of neonatal thymectomy on the lymphoid tissues and the immunologic response of the maturing rabbit might be a reflection of compensatory activity of the appendix.

The present studies provide the first experimental test of the hypothesis that the thymus and appendix function synergistically in the development of immunologic capacity.

Materials and methods. Litters of newborn New Zealand white rabbits were separated into groups for thymectomy-appendectomy, thymectomy-splenectomy, thymectomy, appendectomy, or sham-operation. The procedures were performed within 24 hours after birth. No litter was used if the doe had a gestation period of longer than 32 days. An effort was made to randomize the procedures within the litters, so that the number of each litter in each experimental group averaged about the same initially (thymectomy-splenectomy was not included as a procedure in all the litters, and this group is under-represented). The technique of thymectomy has been described(10). The animals were returned to their mothers in the animal quarters of the local rabbitry, and were maintained for 3 weeks on twice weekly doses of 0.2 ml penicillin (Crysticillin, Squibb, 300,000 units per ml). Thereafter, they were given a combination of tetracycline hydrochloride (Polyotic, American Cyanamid) and neomycin sulfate + methscopolamine bromide (Biosol-M, Upjohn) in their drinking

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TABLE I. Antibody Response of Neonatally Thymectomized and Appendectomized Animals to Bovine Gamma Globulin.*

Appendectomized and thymectomized		Thymectomized		Appendectomized		Sham-operated		Thymectomized and splenectomized	
Animal No.	Titer	Animal No.	Titer	Animal No.	Titer	Animal No.	Titer	Anmial No.	Titer
702	160	712	1280	704	640	707	2560	741	320
703	320	719	1280	705	640	708	640	742	1280
709	160	724	5120	718	1280	710	2560	743	2560
713	80	701	640	722	5120	711	2560	744	1280
715	40	706	2560	728	1280	716	5120		
720	80	729	640	730	640	717	5120		
723	320	731	320	734	640	721	5120		
745	80	732	640	736	2560	725	10240		
746	80	733	640	737	640	727	5120		
747	160	739	640			735	2560		
						740	1280		
Geometric mean	121	Geometric mean	971	Geometric mean	1096	Geometric mean	3090	Geometric mean	1076

* Antibody levels were determined by the bis-diazotized benzidine technique of Gordon *et al*(21). Titers are expressed as the reciprocal of the greatest serum dilution demonstrating a positive hemagglutination reaction.

Antibody titers of the sham-operated animals were significantly higher than the appendectomized-thymectomized ($p < .001$), thymectomized ($p = .003$), and appendectomized ($p = .008$) groups; because of the small number of surviving animals the thymectomized-splenectomized group was not included in statistical analyses. Antibody production in the appendectomized - thymectomized rabbits was significantly lower than that of animals subjected to a single procedure: thymectomy or appendectomy ($p < .001$ in both instances). The small difference between the thymectomized and appendectomized group was not significant. The comparisons were made by the "t" test.

water. When self-sufficient, the animals were fed a standard laboratory diet of Purina rabbit chow and offered water *ad libitum*.

At 7 to 9 weeks of age, each animal received an intravenous injection of 10 mg of bovine gamma globulin powder (BGG) (Nutritional Biochemicals) dissolved in 0.5 ml of 0.9% saline. They were bled 9 days later by heart puncture, and each serum assayed for anti-BGG antibody by the bis-diazotized benzidine (BDB) hemagglutination technique of Gordon *et al*(18). Differential white cell counts were done at time of bleeding or shortly thereafter. A number of experimental rabbits were killed between 7 and 11 weeks of age, and the spleen, popliteal lymph node, and sacculus rotundus (a mass of lymphoid tissue at the ileocecal junction) fixed in 10% formol, sectioned and stained with hemotoxylin and eosin.

Results. Table I summarizes the antibody data on the 5 groups of animals. The extreme depression of antibody levels in the thymectomized-appendectomized animals is evident; all of the titers are below the lowest antibody level recorded in the sham-operated group. The titers of the rabbits thymectomized alone or appendectomized alone are clearly intermediate, as are those of the small group of animals thymectomized and splenectomized. This last group was included as a control on the effects of removal of total mass of lymphoid tissue, the spleen approximating the volume of the appendix.

Table II records the differential counts of these same animals. It will be seen that the reduced lymphocyte levels of the appendectomized-thymectomized group are maintained through at least 10 weeks, as compared to the controls. Examination of the lymph nodes of thymectomized-appendectomized animals as late as 9 weeks after birth showed a general failure of lymphoid follicle development and a marked reduction in number of small lymphocytes (Fig. 1), in contrast to the mature lymphoid structure of the nodes in sham-operated rabbits at 9 weeks (Fig. 2). Similarly, in the spleens of the appendectomized-thymectomized animals, studied at 9-11 weeks of age, immature forms of lymphoid follicles are present in which reticular cells

TABLE II. Differential Counts of Neonatally Thymectomized and Appendectomized Rabbits at 9 to 11 Weeks of Age.

Appendectomized & thymectomized				Thymectomized				Appendectomized				Thymectomized & splenectomized				Sham-operated			
Animal No.	N	L	B	Animal No.	N	L	B	Animal No.	N	L	B	Animal No.	N	L	B	Animal No.	N	L	B
702	—	—	—	712	44	54	2	704	33	65	2	741	55	43	2	707	20	74	6
703	52	41	7	719	33	64	3	705	32	56	7	742	44	52	4	708	16	82	2
709	58	36	6	724	56	41	3	718	24	66	10	743	53	45	2	710	32	63	5
713	50	47	3	701	34	41	25	722	19	77	4	744	35	60	5	711	41	58	1
715	53	45	2	706	25	64	11	728	16	84	0					716	27	71	2
720	68	32	0	729	56	42	2	730	47	46	7					717	36	59	5
723	56	36	8	731	36	57	7	734	44	56	0					721	37	55	8
745	51	46	3	732	48	52	0	736	29	69	0					725	41	55	4
746	50	48	2	733	40	56	4	737	39	54	7					727	25	69	6
747	60	40	0	734	27	71	2									735	47	49	3
																740	25	72	3
Mean	55	41	4		40	54	6		32	64	4		47	50	3		31	65	4

N = Neutrophils. L = Lymphocytes. B = Basophils.

predominate and small lymphocytes are almost entirely lacking.

Discussion. The findings provide an initial confirmation of the hypothesis that removal of the appendix and thymus in the newborn rabbit would depress antibody production more than thymectomy alone. Indeed, they suggest that neonatal appendectomy alone has as much effect on antibody-producing capacity as neonatal thymectomy. Although the thymectomy-splenectomy group was small and cannot be evaluated statistically, the antibody levels in those animals were similar to those of thymectomized animals and were higher than those of the appendectomy-thymectomy group. Study of additional animals in this category are needed, but the present findings suggest that the demonstrated effect of thymectomy-appendectomy did not reflect merely the removal of another substantial block of lymphoid tissue since the thymectomy-splenectomy group had substantially higher antibody levels.

The antibody data are supported by the differential counts, showing prolonged depression of the peripheral lymphocyte counts in neonatally thymectomized-appendectomized animals. Extensive studies of spleens and lymph nodes of these animals have been performed through 11 weeks of age, and show that the immunologic defect and relative depletion of lymphocytes in the peripheral

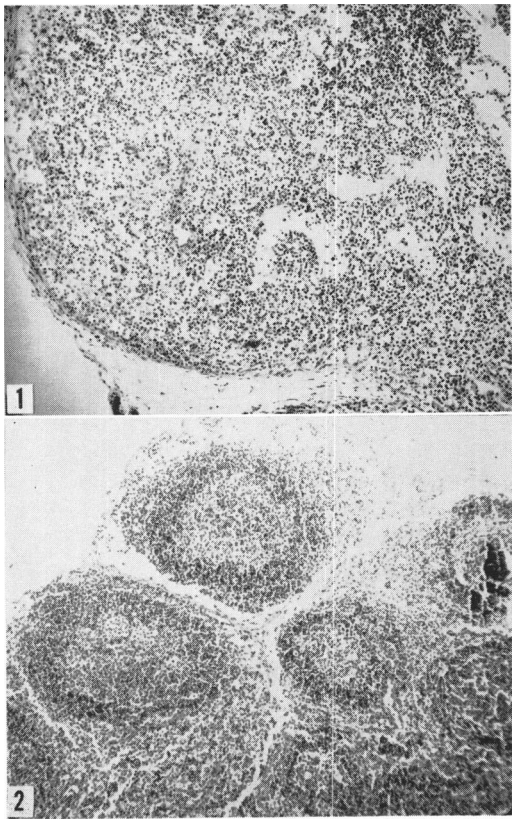

FIG. 1. Peripheral lymph node of a 9-wk-old rabbit appendectomized-thymectomized in the neonatal period. The node shows lack of lymphoid organization and depletion of lymphocytes; × 60.

FIG. 2. Peripheral lymph node of a 9-wk-old sham-operated rabbit showing well developed lymphoid follicles; × 60.

blood are paralleled by a failure of lymphoid development in the nodes and spleen.

It is not yet known how closely the neonatally appendectomized-thymectomized rabbit approximates the immunologic status of neonatally thymectomized mice, rats, and hamsters. Studies of the secondary response to bovine gamma globulin, the antibody response to other antigens, delayed hypersensitivity, and the response to skin homografts are in progress.

We believe, on the basis of these studies and the fast-accumulating data on the effects of the removal of the mammalian thymus and avian bursa of Fabricius, that it is most incisive to think in terms of central as opposed to peripheral lymphoid tissues. Among the central lymphoid tissues essential to full development of immunologic function—function perhaps exercised largely by the lymph nodes and spleen—are the thymus of the mouse, hamster, rabbit, rat and chicken; the bursa of Fabricius of the chicken; and the appendix of the rabbit. It may be that other lymphoid tissues which originate in close proximity to the epithelium of the gut, such as tonsils and even Peyer's patches, function in similar fashion.

Summary. Rabbits thymectomized and appendectomized at birth, and challenged with bovine gamma globulin at 7-9 weeks of age, produce much lower titers of antibody than sham-operated animals. The titers of this group were also significantly lower than those of rabbits subjected to thymectomy alone, appendectomy alone, or combined thymectomy and splenectomy. The suppression of antibody-producing capacity in the thymectomized-appendectomized animals was paralleled by lack of lymphoid tissue development and by low levels of circulating lymphocytes through the period of study.

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