

Relationship of the Bursa of Fabricius to Immunologic Responsiveness and Homograft Immunity in the Chicken.* (27423)

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Previous reports have indicated that in chickens younger than 5 weeks surgical removal of the bursa of Fabricius, a lymphoid organ located dorsally on the cloaca, interferes with the development of immunity(1-3). Complete inhibition of bursal development and reduction in thymus and spleen weight can be obtained by administration of 19-nortestosterone on the 5th day of embryonation (4). This report concerns experiments on the antibody-producing ability of bursectomized chickens and on homograft reactivity in chicks hatching from 19-nortestosterone-treated eggs.

Methods. In the first series of experiments, newly-hatched chicks were bursectomized through an incision above the dorsal lip of the cloacal vent and controls were sham-operated with the bursa left intact. Normal chicks were also included as controls. Eight-week-old survivors from these groups were immunized with 3 antigens simultaneously: bovine serum albumin (BSA), 40 mg/kg; killed *Brucella abortus* antigen, 10^9 bacterial cells/kg; and T_2 coliphage, 10^{10} virus/kg. Antigen was administered intravenously. Serum antibody in each group was measured by Farr's ammonium sulfate precipitation technique for radioiodinated BSA(5), the bacterial agglutination test for *Brucella abortus*(6), and the neutralization test for T_2 coliphage (7).

In the second series of experiments, chicks hatching from eggs injected on the 5th day of embryonation with 0.63 mg of 19-nortestosterone in 0.1 ml of corn oil(4), control chicks hatching from eggs injected with 0.1

ml of corn oil only, and normal chicks hatching from untreated eggs were given intraperitoneal injections of adult homologous spleen cells(6) on the first and 12th day after hatching. The 1-day-old chicks received 10^8 cells, and the 12-day-old chicks 2×10^9 cells. The relative degree of depression of homograft immunity was measured by Simonsen's graft-versus-host assay, GVHR(8). This consisted of the ratio of spleen weight to body weight of recipients sacrificed 6 days after cell transfer.

Results. Surgically bursectomized chicks demonstrated deficient antibody-forming capacity to all 3 forms of antigenic stimulation (Tables I and II, and Fig. 1). Note that complete inhibition of antibody-forming ability was not obtained in surgically bursectomized chicks. The response of normal and sham-operated controls was similar.

The most dramatic effects were obtained in the second series of experiments. Chicks hatching from 19-nortestosterone-treated eggs showed a profound reduction in homograft

TABLE I. Antibody Response to 40 mg/kg BSA 7 Days after Immunization in 8-Wk-Old Chickens.

Gamma I ¹³¹ BSA-N bound per ml of undiluted serum (technic of Farr)†		
Normal	Bursectomized*	
4.55	2.07	.08
3.05	1.15	.08
2.06	.41	.08
1.82	.20	.08
1.82	.11	.08
.58	.08	
.41	.08	
.08	.08	
.08	.08	
.08	.08	
Mean‡	1.45	.32
t = 2.41 p = .025		

* Bursectomized 24 hr after hatching.

† ABC — 33 @ .025 gamma I¹³¹ BSA-N/ml(5).

‡ The means were calculated using .08 as the arbitrary lower limit of antigen bound in this experiment.

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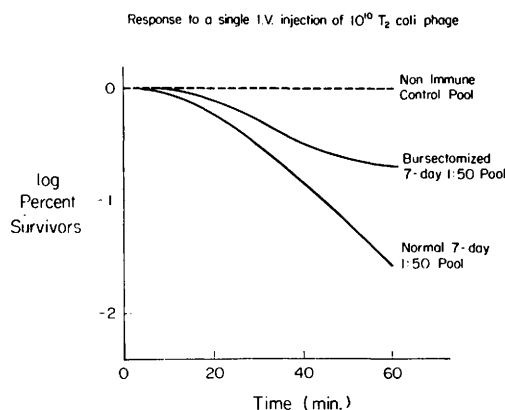


FIG. 1. Phage neutralization by chicken antisera. (Serum obtained 7 days after inj. of antigen.)

TABLE II. Geometric Mean Titers in Serum from 8-Wk-Old Chickens Given 10^8 Killed *B. abortus* per kg.†

Normal—787	Bursectomized*—126
$t = 2.07$	$p = .05$

* Bursectomized at 24 hr after hatching.

† Serum obtained 7 days after inj. of antigen.

immunity, indicated by a greater incidence of the GVHR after injection of adult homologous spleen cells. They also allowed greater proliferation of the adult donor cells, shown in Table III by the ratio of the chimera

GVHR to that of the control. Normal 1-day-old recipients showed only splenomegaly following transfer of adult homologous cells. 19-Nortestosterone-treated recipients showed not only splenomegaly but in addition had white nodules over the liver border and throughout the peritoneal cavity, and subcutaneous effusions of lymph over the abdominal wall, indicating greater proliferation of the grafted cells (Fig. 2).

The effects of 19-nortestosterone treatment were even more pronounced in 12-day-old recipients; 5 of 6 19-nortestosterone-treated recipients showed splenomegaly, nodular growth of donor cells over the peritoneal cavity, subcutaneous effusions of lymph over the abdominal wall, and weight loss characteristic of the runting syndrome. By contrast, control chicks injected with homologous spleen cells on the 12th day after hatching showed only slight splenomegaly as evidence of the graft-versus-host reaction. The 19-nortestosterone-treated chicks not receiving cells had spleens significantly smaller than normal.

Discussion. In the first series of experiments previous findings(1-3) are confirmed. Bursectomy clearly and significantly reduced the subsequent response of chickens to antigenic stimuli.

TABLE III. Graft-versus-Host Reaction in Normal and 19-Nortestosterone-Treated Chicks.

Data on 1-day-old recipients sacrificed 6 days after transfer	No. of cells received	Mean body wt (g)	Mean spleen wt (mg)	GVHR index*	GVHR index	
					Chimera	Incidence of GVHR
					Control	
Normal controls	None	48.3	59.6	1.23	1.85	—
" chimeras	10^8	46.3	105.5	2.27		12/19
19-nortestosterone-treated controls	None	35.8	19.6	.54	2.54	—
19-nortestosterone-treated chimeras	10^8	36.8	48.4	1.35		21/25
Data on 12-day-old recipients sacrificed 6 days after transfer						
Normal controls	None	129.3	210.7	1.62	1.34	—
" chimeras	2×10^9	120.3	264.3	2.19		2/20
19-nortestosterone-treated controls	None	112.4	144.3	1.28	2.12	—
19-nortestosterone-treated chimeras	2×10^9	94.7	258.6	2.73		5/6

* Spleen wt in mg / Body wt in g.

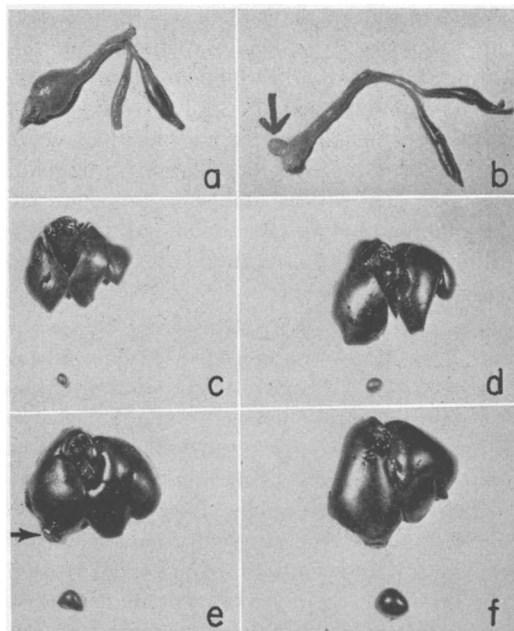


FIG. 2. Organs from 19-nortestosterone-treated chicks (*a*, *c*, and *e*) and normal controls (*b*, *d*, and *f*) at 6 days of age. *a*. Cloaca and trident appendix from a 19-nortestosterone-treated chick. Note ballooning of cloaca and absence of bursa of Fabricius. *b*. Normal cloaca and trident appendix; bursa of Fabricius indicated by arrow. *c*. Liver and spleen from 19-nortestosterone-treated chick. Note small size. *d*. Liver and spleen from normal chick. *e*. Enlarged liver and spleen from 19-nortestosterone-treated recipient of 10^8 adult homologous spleen cells. Note implants of donor cells over the border of liver indicated by the arrow. *f*. Enlarged liver and spleen from normal recipient of 10^8 adult homologous spleen cells.

Homograft immunity is present in normal chicks at hatching(9,10) and is known to develop earlier in ontogenesis than the ability to form circulating antibody(11). Chicks hatching from 19-nortestosterone-treated eggs were unable to form circulating antibody in the studies of Mueller *et al.*(2), and the present studies demonstrate the inadequacy of their response to the more vigorous challenge of transferred spleen cells as well. The 19-nortestosterone-treated chicks have meagerly developed spleens and thymuses, and their mortality rate is high, but those surviving for 12 days or more were unable to reject the transferred cells and were subject to the runt-ing syndrome.

The bursa of Fabricius develops as an out-pouching of the gut endoderm on the 5th day of incubation. Ackerman and Knouff(12) have demonstrated lymphopoiesis in the de-

veloping bursa and have postulated that the lymphocytes develop directly from the lining epithelium of the bursa. The present evidence is consistent with this hypothesis, although it does not rule out the possibility of the bursa producing humoral substances important in control and development of the lymphoreticular system. Presumably 19-nortestosterone, by inhibiting bursal development, suppresses an *anlage* essential to the normal development of the lymphatic system in the chicken and renders it immunologically incompetent. Experiments are in progress to define the relationship of the bursa to the ontogenesis of the lymphoid cells and the immune response.

Summary. Chicks bursectomized at hatching showed reduced antibody production to BSA, *Brucella abortus* antigen, and T₂ coliphage, compared to controls. Chicks hatching from 19-nortestosterone-treated eggs, lacking the bursa of Fabricius, also showed a deficient homograft response to injections of adult homologous spleen cells manifested by splenomegaly, histologic changes characteristic of the graft-versus-host reaction, and signs of the runtting syndrome.

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