Differential Effect of Bursectomy on Antibody Production in a Large and Small Bursa Line of New Hampshire Chickens[1,2] (37622)

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Many investigators have dealt with the possible influence of the size of the bursa of Fabricius on antibody production in chickens. Glick (1) and Jaap (2) first suggested that size of the bursa may play an important role in the efficiency of the immune mechanism. Jaffe and Jaap (3) subsequently found no significant difference in antibody response when a line, selected for over several generations, for large bursa size was compared to other breeds. Sadler and Glick (4) separated White Leghorn families on the basis of bursa size and found higher antibody titers to *Vibrio foetus* in the families possessing the larger bursa size.

The development of a large bursa line (LBL) and small bursa line (SBL) of New Hampshire chickens (5), which differed significantly in bursal size from hatching through five weeks of age, made further studies on the effect of bursa size on antibody production more meaningful. Sato (6) and Rao (7) found no significant differences in agglutinin titers to sheep red blood cells (SRBC) between the SB and LB lines. Rao (7) concluded the selection for a large and small bursa line had not influenced antibody production.

This report deals with the effect of selecting for a large and small bursa size on the early post hatching development of central lymphoid tissue and the subsequent ability of the bird to produce a detectable antibody response.

Materials and Methods. The chickens used in this study were from two lines of birds which differed significantly in the at hatch weight of the bursa of Fabricius: a large bursa line (LBL) and a small bursa line (SBL). These lines were developed from an outbred-closed flock of New Hampshires maintained by Professor L. J. Dreesen of the Mississippi State University Poultry Science Department (5).

All chicks were housed in heated batteries for the first 3 weeks and then transferred to floor pens containing wood shavings. They were fed a basal ration (8) and provided food and water *ad libitum*. No medications were administered. Chicks were randomly assigned to treatments at hatch without regard to sex.

Growth of the bursa of Fabricius and thymus. Chicks were sacrificed from each line (LBL and SBL) at hatch and at 1, 3, 5, 8, 12, 17, 23, and 32 weeks of age. The bursa of Fabricius (BF) and left thymic lobes were removed from each bird, weighed, and recorded along with the total body weight of the bird.

Each weight was converted to mg/g of total body weight and analyzed by means of Duncan's New Multiple Range Test (DNMRT) (9).

Tissue histology. Bursae were fixed in Bouin's Solution, embedded in tissuemat, sectioned at 8 μm thickness, and stained with Hemotoxylin and Eosin.

Effect of bursectomy. Chicks from each line were surgically bursectomized (BSX) at hatch, 1, 3, or 5 weeks of age (10). Chicks BSX at hatch, 1 and 3 weeks of age, were injected iv at 5 weeks with 1 ml of a 7% suspension of washed sheep red blood cells (SRBC) in phosphate buffered saline,

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while chicks BSX at 3 and 5 weeks of age were injected with the 7% SRBC suspension at 7 weeks. These birds were subsequently bled from the brachial wing vein at 3, 7, and 10 days following the SRBC injection.

Within 24 hr following each bleeding, serum samples were heated at 56° for 30 min to destroy complement activity and assayed for sheep agglutinin against a 1.5% washed SRBC suspension utilizing the Microtiter technique (11, 12).

Plaque forming assay. Screening of splenic tissue for antibody producing cells (*i.e.*, plasma cells) was accomplished by an agarless plaque forming assay (13, 14). Means are included with their standard error and probability levels assigned contingent with the amount of variation intrinsic in this assay method.

Results. The bursa of Fabricius of LBL chicks was significantly larger than the bursa of SBL chicks at hatch, 1 and 3 weeks of age. Within both lines the maximum growth of the gland was reached at approximately 3 weeks of age (Table I).

Thymus weights did not differ between the lines. However, a common growth peak for both lines occurred between the 3rd and 5th week.

Histologically, the bursa revealed a dramatic difference in number of differentiating follicles at hatch: the SBL bursal folds being nearly devoid of active follicles while the LBL had the expected dense follicular development (Figs. 1 and 2). Observations on successive ages revealed no differences in follicular development after the 3rd wk post hatching.

TABLE I. Mean Bursa Weights (mg/g) in LBL and SBL Chickens.[a]

Age	LBL	SBL
Hatch	*1.2386^{c} ± 0.1549	0.7439^{cd} ± 0.0546
1 wk	*2.0686^{b} ± 0.2081	1.3183^{b} ± 0.0940
3 wk	*3.5917^{a} ± 0.3336	2.0463^{a} ± 0.2153
5 wk	1.1145^{c} ± 0.0739	0.9162^{c} ± 0.0675
8 wk	0.4545^{e} ± 0.0582	0.6117^{cd} ± 0.0984
12 wk	0.7349^{d} ± 0.1596	0.3721^{de} ± 0.0532
17 wk	0.7173^{d} ± 0.1286	0.4000^{de} ± 0.0834
23 wk	0.4546^{de} ± 0.1033	0.1071^{e} ± 0.0364
32 wk	0.1323^{e} ± 0.0365	0.0797^{e} ± 0.0073

[a] Each mean represents 8 observations and is included with its standard error. Means within lines and among ages (columns) not followed by a common letter superscript (a, b, c, d, or e) are significantly different, $p < 0.05$ (9).

* Asterisks designate significant differences ($p < 0.01$) between lines within age classes (rows).

SBL birds BSX at hatch failed to produce antibody to a primary immunization with SRBC (Table II). The agglutinin titers of LBL birds BSX at hatch or 1 week of age were significantly lower than control titers 3 days after SRBC injections but not 7 days after injection.

A second trial verified the absence of detectable antibody in the SBL BSX at hatch group, one bird in ten producing a peak titer value ($\log_2$) of 2, while in the remainder no detectable titer was found.

LBL and SBL birds BSX at 3 and 5 weeks of age revealed no significant modification in antibody production.

A significant difference in plaque forming cells (PFC) was observed between the SBL BSX at hatch and SBL control groups ($p \leq 0.06$). The number of PFC between LBL controls and SBL controls was not different. The PFC number of LBL BSX at hatch birds was not different from LBL controls, while SBL BSX at hatch and LBL BSX at hatch groups were significantly different ($p \leq 0.15$) in the number of PFC.

Discussion. Our concept of the pattern of avian bursa growth has not remained constant over the past 60 years. Early work demonstrated that bursa regression occurred at the age of sexual maturity (15), while later work has demonstrated the bursa to regress prior to sexual maturity and as early as 5–8 wk of age (1, 5, 16). Unfortunately many authors continue to wrongfully relate that bursa regression occurs at the time of sexual maturity.

In the present study, the bursae of LBL chicks grew significantly faster and were significantly larger than the bursae of SBL chicks to 3 weeks of age. This rapid period of bursal growth, hatch to 3 weeks, followed by regression between 5–8 weeks of age, was common to both lines. The pattern of growth in this report agrees with other data from this laboratory (1, 5, 16, 17). The thymus

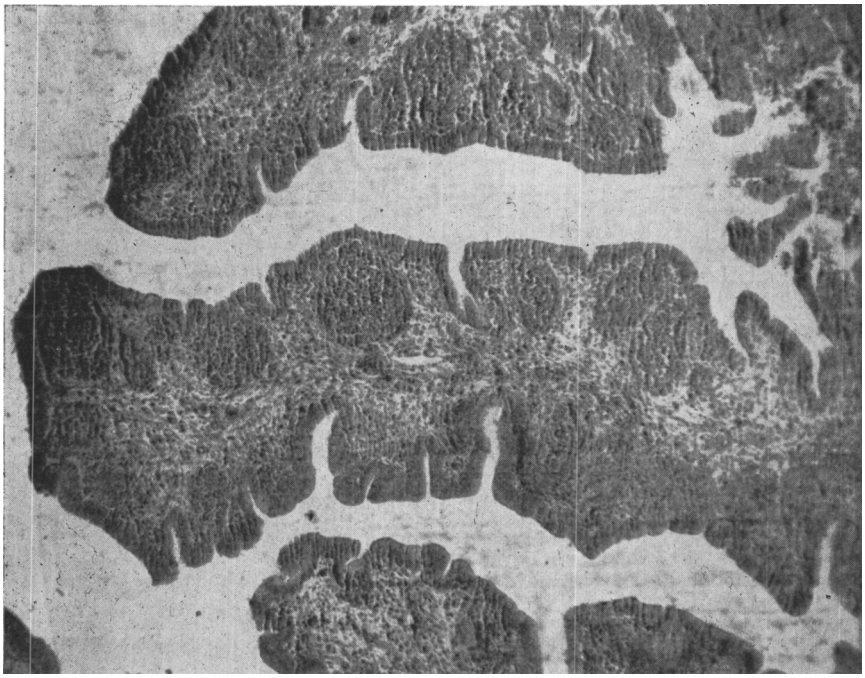

FIG. 1. Bursa of Fabricius from a hatchling SBL chick. Note the relative lack of active follicles within the folds. (×100, H & E).

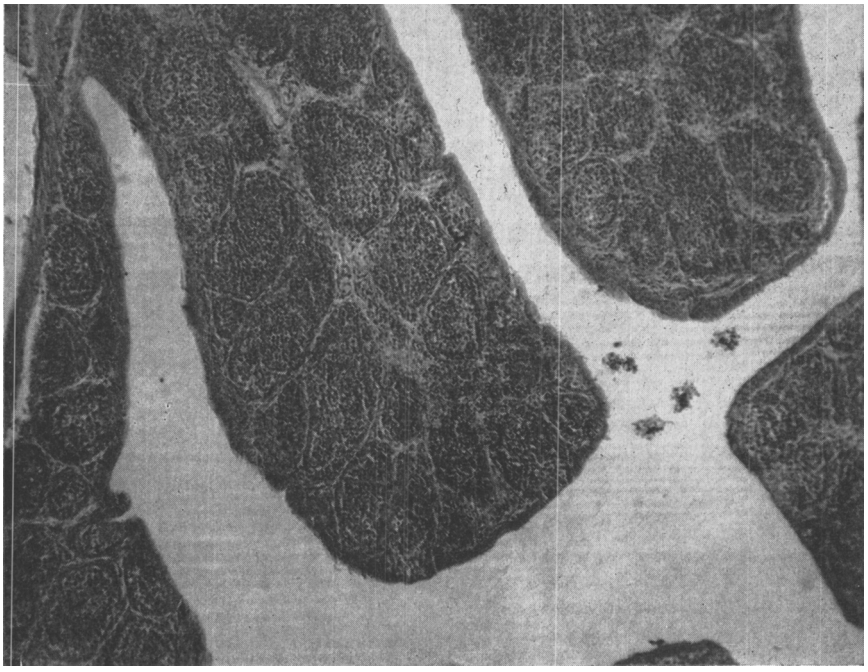

FIG. 2. Bursa of Fabricius from a hatchling LBL chick. Follicular development is extensive (×100, H & E).

TABLE II. The Mean Agglutinin Titer of 5-Week-Old Birds to an iv Injection of a 7% Suspension of SRBC (log_2).[a]

Line	BSX	Days post injection		
		3	7	10
LBL	Hatch	$1.17^{cd} \pm 0.54$	$4.17^{c} \pm 1.54$	$2.17^{b} \pm 0.60$
	1 wk	$2.33^{c} \pm 0.33$	$6.33^{ab} \pm 0.56$	$3.33^{ab} \pm 0.42$
	3 wk	$5.50^{ab} \pm 0.72$	$6.50^{ab} \pm 0.43$	$3.17^{ab} \pm 0.79$
	Control	$6.17^{a} \pm 0.87$	$5.17^{bc} \pm 0.54$	$3.17^{ab} \pm 0.79$
SBL	Hatch	$0.00^{d} \pm 0.00$	$0.00^{d} \pm 0.00$	$0.00^{c} \pm 0.00$
	1 wk	$2.50^{c} \pm 0.43$	$4.83^{bc} \pm 0.95$	$3.83^{ab} \pm 0.54$
	3 wk	$7.00^{a} \pm 0.63$	$7.50^{a} \pm 0.43$	$4.33^{a} \pm 0.61$
	Control	$4.33^{b} \pm 0.56$	$8.00^{a} \pm 0.52$	$4.00^{ab} \pm 0.26$

[a] Each mean represents 6 observations and is included with its standard error. Comparisons were made between lines, among treatments and within bleedings; means not followed by a common superscript are significantly different, $p < 0.05$ (9).

has been found not to differ in weight (5) or cell number (17) between LBL and SBL chicks. A lack of thymus weight differences (mg/g total body weight) have been substantiated by this study. Histologically the bursa of the hatchling SBL bird is nearly devoid of follicular development, while the LBL bursa is well populated with actively differentiating follicles (Figs. 1 and 2). The apparent lack of follicular development in the SBL bursa at hatch suggests that the SBL bursa may be retarded in its embryonic development.

Theoretically the LBL should possess a more active bursa embryonically by virtue of the greater number of cellular units (17) and, thus, at hatching would be immunologically more mature. This maturity would be difficult to measure after several weeks since the SB line would have had sufficient time to develop and seed the minimal number of units (cellular or humoral) necessary to prepare the birds for antibody production. BSX at hatch would prevent further maturation of the immune system by direct bursal control and would be a reflection of bursal activity during embryonic development. Therefore, to compare the immunocompetence of the LB and SB lines, birds were surgically bursectomized at hatch and at sequential ages after hatching. SBL birds bursectomized at hatch showed no detectable agglutinin response to SRBC at 6 weeks of age (of 16 birds, one had an agglutinin titer of 2), while LBL birds BSX at hatch continually produced an agglutinin response similar to control birds. Bursectomizing SBL birds at 1 week of age did not eliminate antibody production but significantly reduced peak titers. On the other hand, agglutinin values for SBL birds bursectomized at 3 and 5 weeks of age were comparable to control titers. This would suggest the SBL bursa had by 1–3 weeks of age sufficiently seeded the peripheral lymphoid tissue so that removal of the bursa no longer interfered with immunocompetence. The earlier maturation, greater number of cells (17), and subsequent earlier release of cells or a humoral substance from the bursa of LBL chicks would account for this lines' earlier immunocompetence. Plaque counts support these conclusions in that the LBL BSX at hatch group did not differ from the LBL control group while the SBL BSX at hatch group was significantly lower in plaque forming cells than the SBL control group ($p \leq 0.06$). The plaque counts also suggest that fewer bursal dependent cells exist in the spleens of SBL birds bursectomized at hatch than the LBL BSX at hatch group ($p \leq 0.15$). The reduced number of bursal follicles in hatchling SBL chicks verifies the concept of retarded immunogenic development.

The SBL of chickens presents itself as a model for further studies on humoral (bursal mediated) immune mechanisms, since its ability to produce detectable antibody to a

primary immunization may be eliminated without the added complications of steroids, cyclophosphamide, or irradiation.

Summary. SBL birds bursectomized at hatch failed to produce hemagglutinin to a primary injection of SRBC. The antibody titer of LBL birds BSX at hatch did not differ significantly from controls. BSX at 1 week reduced the antibody titer in the SBL birds. BSX at 3 and 5 weeks had no effect on hemagglutinin levels. The number of PFC of SBL BSX at hatch chicks was significantly lower than controls and LBL BSX at hatch birds. There were fewer lymphoid follicles at hatch in the bursae from SBL birds than in the bursae from LBL birds. The SBL chickens offer a model for further studies on humoral immune mechanisms, since the ability of SBL chicks to produce detectable antibody to a primary immunization may be eliminated without further treatment with steroids, cyclophosphamide, or irradiation.

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