# Influence of Bursa Implantation Upon Lymphocytic Nodules and Plasma Cells in Spleens of Bursectomized Chickens.* (31382)

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Considerable evidence has accumulated to substantiate that the bursa of Fabricius is essential for the capacity to produce circulating antibodies in birds. The formation and development of the bursa of Fabricius can be completely prevented by *in ovo* administration of testosterone(1-3). Birds hormonally bursectomized *in ovo* or surgically bursectomized at hatching are incapable of forming antibodies to most antigens(1-6). The bursa, although not involved directly in the production of antibody(7), has been shown to restore significantly the antibody producing capacity of bursectomized birds following implantation of bursal tissue enclosed within cell impermeable diffusion chambers(5,6,8). This finding suggests that the bursa acts to confer immunological reactivity upon the bird, at least in part, by means of a humoral (non-cellular) agent. The recent finding that the bursa of Fabricius is responsible for the so-called "bursa-dependent" system in the spleen of the chicken, consisting of lymphocytic nodules and plasma cells(9), has promped the present study to examine the role of the humoral substance from the bursa in the development of these entities.

*Materials and methods. Bursectomy.* Fertile eggs from the regional random-bred White Leghorn population were divided into 2 groups: (A) one group of eggs (experimental) was bursectomized by a 0.1 ml (2.5 mg) injection of testosterone propionate in sesame oil (Schering) on the fifth day of incubation as described previously(6); (B) a second group of eggs, not treated with testosterone, served as a source of normal bursae for subsequent implantation as well as controls, *i.e.*, animals with intact bursae and having normal immunological reactivity.

*Implantation of bursa and immunization.* The bursae used for implantation were obtained from chicks 7 days after hatching. A piece of bursa was implanted either subcutaneously or intraperitoneally into one group of 7-day-old bursectomized chicks. Another group of 7-day-old, testosterone-bursectomized chicks was surgically implanted subcutaneously or intraperitoneally with bursa enclosed within millipore diffusion chambers constructed of two 25 mm plastic cellulose filters of 0.45 $\mu$ porosity (Millipore Filter Corp., Bedford, Mass.). In comparative studies, filters of a smaller porosity, 0.1 $\mu$, were used. As a control, hormonally bursectomized chicks were implanted with empty diffusion chambers.

On the ninth day after hatching, chicks from control and experimental groups were given a 1.0 ml intramuscular injection of killed *Salmonella typhimurium*, standardized at $3 \times 10^9$ cells/ml. Four weeks later, a second injection of *S. typhimurium* was given.

Spleens from control intact chickens, hormonally bursectomized chickens implanted with bursa alone or bursa-filled diffusion chambers and hormonally bursectomized chickens implanted with empty diffusion chambers were studied histologically at the end of the experimental procedure (6 weeks). Spleens were fixed in formal-sublimate-acetic acid, embedded in paraffin, sectioned at 5 $\mu$ and stained with a 0.1% aqueous toluidine blue. Standard bacterial agglutination procedures were performed on sera obtained from birds in all groups.

*Results. Control chicken* (Fig. 1). The spleen of the chicken can be conveniently subdivided into 3 regions: (1) white pulp, consisting of compact accumulations of predominantly small lymphocytes which surround small arteries and arterioles; (2) red pulp, consisting of loose lymphocytic tissue in large part occupied by oval or spindle shaped thickenings of arteriole branches usually surrounded by small lymphocytes; (3) lymphocytic nodules, tightly grouped lymphocytic

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TABLE I. Summary of Antibody Production to *S. typhimurium* and Histological Analysis of Spleen.

| Group | Histological analysis of spleen: No. animals | Mean No. follicles/ long. section | Mean No. plasma cells/ O.I. field | Antibody production: Antibody titer range | Mean titer |
|---|---|---|---|---|---|
| Non-bursectomized control | 10 | 15.6 | 6.4 | 1:256–1:512 | 1:330 |
| Bursectomized with empty diffusion chamber implant | 10 | 0 | 0 | 0 | 0 |
| Bursectomized with bursa-filled diffusion chamber implant | 10 | 5.5 | .45 | 1:64 –1:256 | 1:105 |
| Bursectomized with bursa (alone) implant | 10 | 5.4 | .6 | 1:64 –1:128 | 1:115 |

cells consisting of "blast" cells, large and medium lymphocytes and reticular cells completely encapsulated by a layer of fibrous connective tissue. Lymphocytic nodules were abundant throughout the sections studied (15.4 nodules/longitudinal section of spleen studied).

Plasma cells were not located within the lymphocytic nodule but were present in the red pulp. The concentration of plasma cells was relatively high, 6.4 cells/oil immersion field. A high titer of antibody to *S. typhimurium* (range 1:256 to 1:512, mean 1:330) was obtained from the non-bursectomized control birds.

*Bursectomized chicken* (Fig. 2). No lymphocytic nodules were found in the spleens of the bursectomized birds implanted with empty diffusion chambers. There were dense, non-nodular aggregations of small and medium lymphocytes corresponding to the white pulp of the spleen of the control chicken; however, the cells were more aggregated than in the control spleen. No fibrous membrane capsule was found around the lymphocytic aggregations. Plasma cells were absent in the spleens of bursectomized chickens.

Demonstrable antibody was not detected in the serum of the bursectomized chicken implanted with empty diffusion chambers.

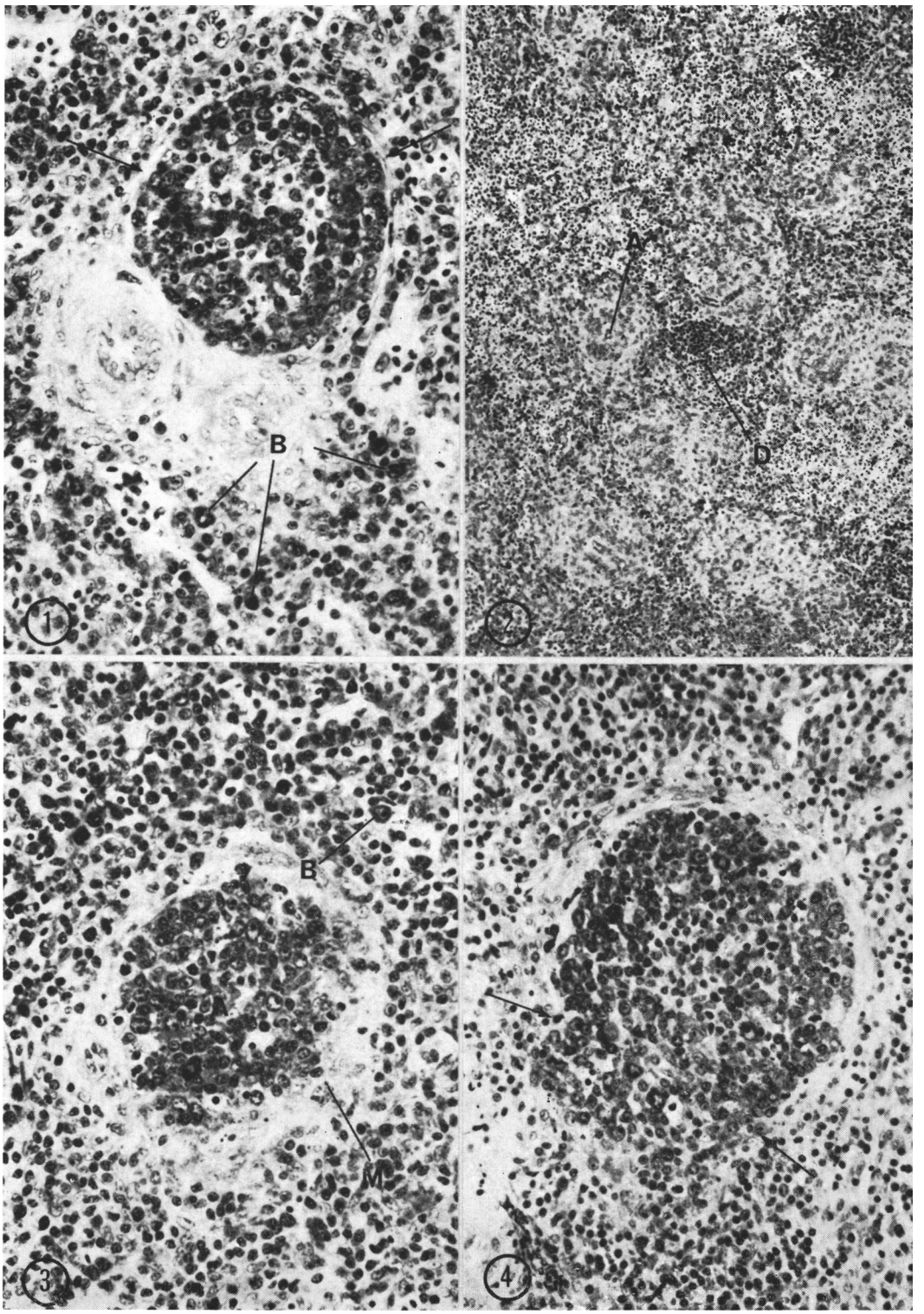
*Bursectomized chicken implanted with bursa alone or bursa-filled diffusion chambers* (Fig. 3 and 4). Lymphocytic nodules were present in the spleens of bursectomized birds implanted with bursa alone and bursectomized birds implanted with bursa-filled diffusion chambers. The shape of the nodule was less uniform than in the spleen of the control chicken. The number of nodules per spleen section was fewer in the bursectomized-implanted birds than in the control (bursa alone 5.3 nodules/longitudinal sections; bursa-filled diffusion chamber 5.6 nodules/longitudinal section). Plasma cells were observed only rarely in the red pulp areas of the bursectomized birds implanted with bursa alone (0.6/oil immersion field) and bursa-filled diffusion chambers (0.45/oil immersion field).

Reconstitution of antibody production (range 1:64 to 1:256, mean 1:105) occurred in the bursectomized birds bearing bursa-filled diffusion chambers as well as in the animals implanted with bursa alone (range 1:64 to 1:128, mean 1:115). No demonstrable differences were detected in studies which employed diffusion chambers constructed of 0.45 or 0.1 $\mu$ porosity filters or in which implants were placed subcutaneously or intraperitoneally.

These results are summarized in Table I.

*Discussion.* Lymphocytic nodules absent in spleens of bursectomized chickens were present following implantation of either bursa alone or a bursa-filled diffusion chamber. Plasma cells, however, were absent in the spleens of both bursectomized birds and bursectomized birds following implantation of bursa.

Evidence that the mechanism of development of the lymphocytic nodules was by


B
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M
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FIG. 1. Lymphocytic nodule with surrounding membrane (arrows) adjacent to 2 arteries with large basophilic cells (B) in the red pulp from spleen of a control chicken. Toluidine blue. × 333.

FIG. 2. Spleen from bursectomized chicken with diffuse lymphocytic area (D) and adjacent artery (A). Toluidine blue. × 86.

FIG. 3. Single lymphocytic nodule from spleen of a bursectomized chicken implanted with bursa alone. Note relative thickness of membrane (M) and sparsity of large basophilic cells (B) in the red pulp. Toluidine blue. × 333.

FIG. 4. Single lymphocytic nodule from spleen of a bursectomized chicken implanted with a bursa-filled diffusion chamber. The surrounding membrane is not complete (arrows). Toluidine blue. × 224.

means of a non-cellular, humoral substance was strongly suggested by the finding that bursa enclosed within cell-impermeable diffusion chambers was capable of forming the lymphocytic nodules.

The bursectomized birds implanted with bursa were found to be capable of producing antibody, although plasma cells were not present in the red pulp. Two alternatives exist for the production of antibody in the bursectomized-implanted chickens in the absence of plasma cells: (1) production of antibody by lymphocytic tissue in other regions of the body, *viz.*, lower cecum and the subcutaneous region of the neck; and (2) production of antibody by lymphocytic nodules. The former is unlikely, since *in ovo* hormonal bursectomy apparently eliminates these areas (10,11).

The lymphocytic nodules of the chicken spleen bear a striking resemblance to the germinal centers of the mammalian lymph nodes and spleen. The reaction to infection of the lymphocytic nodules of the chicken spleen appears to be similar to that of the germinal centers in mammals under the same condition, *viz.*, increased size and number of nodules and an increased mitotic activity and cellular proliferation(12). Antibody has been localized in the germinal centers of the mammalian lymph nodes and spleen(13,14) as well as the lymphocytic nodules of the spleen of the chicken(14).

The mechanism of plasma cell formation would appear not to reside with the bursa humoral factor alone as indicated by the failure of bursa implantation to stimulate plasma cells in the spleens of bursaless chicks. In similar studies(15,16), it was found that implantation of bursa alone (without diffusion chamber) apparently increased the number of plasma cells in the spleens of surgically bursectomized chickens. Lymphocytes from the implanted bursa of Fabricius were reported viable for 8 to 10 days after implantation. In this present study, utilizing hormonal bursectomy, lymphocytes from the bursa implanted without diffusion chamber were found to be viable for only 7 days. Is it possible that immunologically competent lymphocytes migrate from the bursa to the spleen, there to transform into plasma cells under the stimulus of antigen? Hormonal bursectomy results in total elimination of the bursa of Fabricius before the formation of lymphocytes and thus eliminates any possibility for dissemination of lymphocytic cells from the bursa. Although the cellular origin of the plasma cells remains unresolved, it is one theory that lymphocytes may transform into plasma cells in areas where increased concentrations of plasma proteins generally occur, *e.g.*, lymph nodes, spleen and other highly vascular organs(17-19).

Recent evidence of reconstitution of lymphocytic nodules and plasma cells in bursectomized-irradiated birds by means of bursa cell suspensions(20) together with a morphological similarity(21) suggests that the lymphocytic nodules and plasma cells could represent a direct cell line from the bursa lymphoid cell. The presence of antibody to *S. typhimurium* in the absence of plasma cells in the chicken spleen indicates that the antibody production, at least in part, would seem to reside within the lymphocytic nodules. The mechanism of lymphocytic nodule development is by means of the bursa humor factor.

*Summary.* Implantation of donor bursa in hormonally bursectomized chickens restored the immunological reactivity by means of a humoral substance capable of passing through cell-impermeable diffusion chambers. Lymphocytic nodules, shown to develop under the influence of the humoral agent from the

bursa, are felt, in part, to be the site of antibody production. Plasma cell development does not appear to be by means of the humoral substance alone.

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## Changes in the Pituitary of Mice on Diets Supplemented with Egg Yolk, with Extracts of Eggs, or with Cholesterol.* (31383)

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It was demonstrated previously that mice of the T.M. strain maintained on the Rockland rat diet supplemented with egg yolk, with alcohol extract of egg yolk, ether extract of whole egg, or with cholesterol and lard develop, in addition to other malignant tumors, a high incidence of mammary cancer. In controls (on the Rockland rat diet only) mammary cancer occurred rarely and none of the mice on the diet supplemented with hard boiled egg white developed breast adenocarcinoma(1,2). Moreover, in mice of the same strain mainstained on the Rockland rat diet supplemented with cholesterol alone, the incidence of mammary cancer was only 8.6% and there was no lymphosarcoma, while the incidence of lung cancer was 76%. In the females on the diet supplemented with cholesterol, the ovaries, which were often enlarged, had almost no follicles and no corpora lutea, while the germinal epithelium, considerably thickened, formed folds penetrating inside the gonads(3).

Cholesterol apparently stimulates growth, as indicated by the high mitotic rate in the liver, the proliferation of the germinal epithelium of the ovary and the high incidence of lung cancer, while its effect upon ovarian function is inhibitory. The hypofunction of the ovaries, which manifests itself in changes in the estrous cycle (see *Discussion*) is probably the cause of the low incidence of mam-

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