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Received January 18, 1965. P.S.E.B.M., 1965, v118.

Restoration of Antibody Producing Capacity in Bursectomized Chickens by Bursal Grafts in Millipore® Chambers.* (30070)

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Recent studies have established that the bursa of Fabricius plays a major role in antibody production in birds(1). Neonatally bursectomized chickens fail to respond to antigenic challenge with an increase in number of plasma-cellular elements(2) and exhibit a striking suppression of antibody formation, whereas the delayed responses remain unchanged(3). The immunologic deficiency caused by removal of bursa could be partially restored by treating bursaless birds with a saline extract of bursa(4) or by grafting bursectomized chickens with bursa from newly hatched or 8-week-old chickens(2). These experimental findings suggest that the bursa may exert a hormone-like function in the development of immunological responsiveness in birds. To explore this hypothesis further, a study was made of the effect of bursa grafts in cell-impermeable millipore® chambers on antibody production to human erythrocytes in bursectomized chickens.

White Rockland chickens were surgically bursectomized under ether anesthesia within 24 hours after hatching. At the age of 8 weeks one group of bursectomized chickens had implanted a millipore chamber containing bursa from a 4-day-old chicken. A second group of bursaless birds was implanted with an empty millipore chamber, and a third group of non-operated birds served as controls. The chambers were made by cementing 2 discs of nylon supported millipore filter having a pore size of 0.45 μ (Millipore Filter Co., Bedford, Mass.) to lucite rings (inner diameter 17 mm, height 3 mm). The bursae were evaginated prior to placement in the chamber, which was thereafter filled with

* This work was supported by U.S.P.H.S. grant AI-01289-08. We are indebted to the invaluable assistance of Dr. Katarina Isakovic.

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TABLE I. Antibody Response in Bursectomized Chickens with Implanted Millipore Chambers.

Group	No. of chicks	Material implanted	No. of chickens with hemagglutination titer (log_2) 1	2	3	4	5	6	7	8	9	≥10	Mean antibody titer (log_2)
Bursectomized	10	Empty chamber	3	2	1	3	1	0	0	0	0	0	2.7
"	11	Chamber with bursa	0	0	0	2	1	1	1	3	2	0	6.5
Control (non-operated)	16	None	0	0	0	0	0	0	0	0	1	15	11.0

Hanks' solution, and the chambers were implanted into the right side of the peritoneal cavity (one chamber per chicken). Three days later the chickens of all groups received intravenously 1.5 ml of a washed 10% suspension of human type O red blood cells. Nine days following immunization the chickens were bled and autopsied for inspection of the bursal region. Those birds which showed even very small bursal remnants (5-10 mg) were discarded from the experiment. Every chamber containing bursa was also inspected. The sera were heated at 56°C for 20 minutes and used on the same day for hemagglutinin assay as described previously (2). The identical human O erythrocytes served as antigen for immunization and for assay.

The results listed in Table I demonstrate that bursae from young chickens, when put into millipore chambers and implanted into the peritoneal cavities of bursectomized 8-week-old chickens of the same strain, produced a significant recovery in immunologic competence. Thus, bursectomized chickens implanted with empty chambers were severely deficient in antibody production with average antibody titers (log_2) of only 2.7, none being greater than 6. This could be compared with an average antibody titer of 11.0 for the unoperated control group. The group of bursectomized chickens implanted with a bursa in a millipore chamber, however, produced an average antibody titer of 6.5, with 7 of 11 animals having a titer of 6 or greater. It is of interest that bursectomized chickens which were found at post mortem examination to contain small bursal residues (5-10 mg) were also found to produce fairly large amounts of hemagglutinin.

While these results indicate a significant recovery of antibody forming potential, it is apparent that few grafted animals achieved antibody levels comparable to those of intact animals. Further studies involving the parameters of time and size of graft should enable one to determine the exact degree of restoration possible in this experimental situation.

To obtain preliminary information on the type of antibody restored by bursal grafts, several sera in the grafted and control groups were assayed for mercaptoethanol sensitive antibody(5). A 1:8 dilution of the serum in pH 7.3 phosphate buffer was made 0.1 N in mercaptoethanol and was incubated at 37°C for one hour prior to dilution for hemagglutination assay. Results in Table II indicate that all the antibody in the bursa-grafted group was mercaptoethanol sensitive and hence presumably macrglobulin, while some residual hemagglutinin remained in the control group sera. Further studies involving

TABLE II. Effects of Mercaptoethanol Treatment on Hemagglutinin Titers Produced by Normal and Bursal Grafted Chickens.

Chicken No.	Treatment	Hemagglutination titer (log_2) Pre-mercaptoethanol	Post-mercaptoethanol
2	Bursal graft in chamber	8	0
3	"	9	0
6	"	9	0
8	"	8	0
10	"	8	0
24	Unoperated	12	3
26	"	12	5
27	"	12	5
28	"	12	5
29	"	12	5

more prolonged immunization will be required to determine whether mercaptoethanol-resistant γ-globulin production may also be restored by bursal grafts.

It has been demonstrated(6) that intensely basophilic cells, morphologically very similar to the plasmacellular elements, may occur in the bursa. However, there is no definite evidence that those cells are capable of manufacturing antibodies. Nevertheless, the possibility exists that bursal implants from the millipore chamber may respond to injected antigen and produce antibodies which can easily pass through the chamber pores and enter the circulation. The bursae recovered from millipore chambers 12 days after implantation were of normal color, with easily visible plicae, although markedly involuted.

Histologic examination (haematoxylin-eosin staining) showed various degrees of disruption of cellular architecture. In some cases, the bursal cellular make-up was completely destroyed, so that no lymphoid elements were recognizable. However, in other cases the bursal follicles were well preserved; cortex and medulla were clearly distinguishable. The cells in most of these follicles exhibited the incipient signs of degeneration, and the interfollicular zone was characterized by a proliferation of connective tissue.

One further possibility to be considered is that the millipore chambers used are in fact permeable to cells as has been reported by Capalbo *et al*(7). However, in our hands(8) empty or salmonella vaccine-baited millipore chambers with 0.45 μ filters have been left in the peritoneal cavity for as long as 6 weeks without the appearance of detectable cells in the chamber. Since these later chambers, and the ones used in the bursa experiments, incorporated the newer nylon-backed microweb filters which have a substantially decreased porosity,‡ it is possible that this alone could account for the differences in permeability observed. In addition, species differences might exist in the ability of mononuclear cells to penetrate millipore filters. This possibility is being currently investigated.

The results presented here strongly suggest that the bursa of Fabricius has an endocrine function with respect to the chicken's potential to manufacture circulating antibodies, in addition to a potential cellular function. These results are in concordance with previous findings(4,6), in which the endocrine-like function of bursa was demonstrated, using different experimental approaches.

Summary. Neonatally bursectomized chickens were seriously defective in antibody production to human red cells. When grafted with a bursa in a millipore chamber, significant restoration of antibody formation occurred. The antibody formed in grafted chickens was mercaptoethanol sensitive. An endocrine function for the bursa of Fabricius in respect to production of antibody is strongly indicated by these results.

‡ Millipore Catalog MF.64, p7.

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Received January 5, 1965. P.S.E.B.M., 1965, v118.