PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 171, 306–314 (1982)

Transplantation of Cultured Bursal Epithelium: A Histologic Study[1] (41516)

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Abstract. Pieces of bursa, taken from one-day-old line 6, subline 1 chicks, were cultured *in vitro* for 13 days. During culture, the fragments gradually lost follicular organization and immunoglobulin (Ig)-positive lymphocytes. The basement membrane associated epithelium proliferated and was later seen diffusely layered under intact plical or specific follicular layers. Cultured bursal epithelium (CBE) was transplanted into the wing web of 14-day-old surgically bursectomized syngeneic recipients. The CBE began to enlarge and become vascularized. Microscopic examination of the transplants 22 days after implantation revealed repopulation of lymphocytes which reorganized into follicle-like structures containing Ig-positive cells. Thus, transplantation of CBE into the wing web of bursectomized chickens may be a good model for simulating the naturally occurring events in avian B-cell development.

The Bursa of Fabricius, a central lymphoid organ, is essential for the development of humoral immunity in the chicken. Following hormonal or surgical ablation, there is a noticeable deficiency in plasma cells, an atrophy or absence of splenic germinal centers, and a reduction in either serum immunoglobulin (Ig) levels or antibody production or both (1–3). These findings resemble those seen in certain types of immune deficiency diseases in humans, e.g., the Bruton type of agammaglobulinemia. Work in this laboratory has shown that immune function in certain patients can be restored following transplantation with cultured thymic epithelium (4). Therefore, we became interested in using cultured bursal epithelium as a possible means of restoring immune function in bursectomized chickens. This preliminary report outlines the conditions used for culturing bursa and describes the histology of the cultured implant before and after transplantation in the wing web.

Briefly, we have shown that cultured fragments of bursa, after lymphocytes have migrated from the tissue or deteriorated *in situ*, will gradually become repopulated with lymphocytes which reorganize into follicle-like structures containing Ig-positive cells.

Materials and Methods. *Chickens.* Five one-day-old line 6, subline 1 chicks (Regional Poultry Research Lab., E. Lansing, Mich.), homozygous (B^2B^2) for the major histocompatibility complex were surgically bursectomized as previously described (5).

Cultured bursal epithelium (CBE). The surgically removed bursae were pooled and minced into pieces approximately 1 mm^3 in volume. The pieces were washed once in phosphate-buffered saline and established as organ cultures on stainless steel wire grids in small plastic tissue culture dishes. RPMI 1640 supplemented with 15% heat-inactivated fetal calf serum, sodium bicarbonate, L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin (Flow Labs. McLean, Va.), 50 μg/ml gentamycin (Schering, Kenilworth, N.J.), and 5 μg/ml fungizone (GIBCO, Grand Island, N.Y.) was added

[1] Supported by Grant HD-07778 from the National Institutes of Health.

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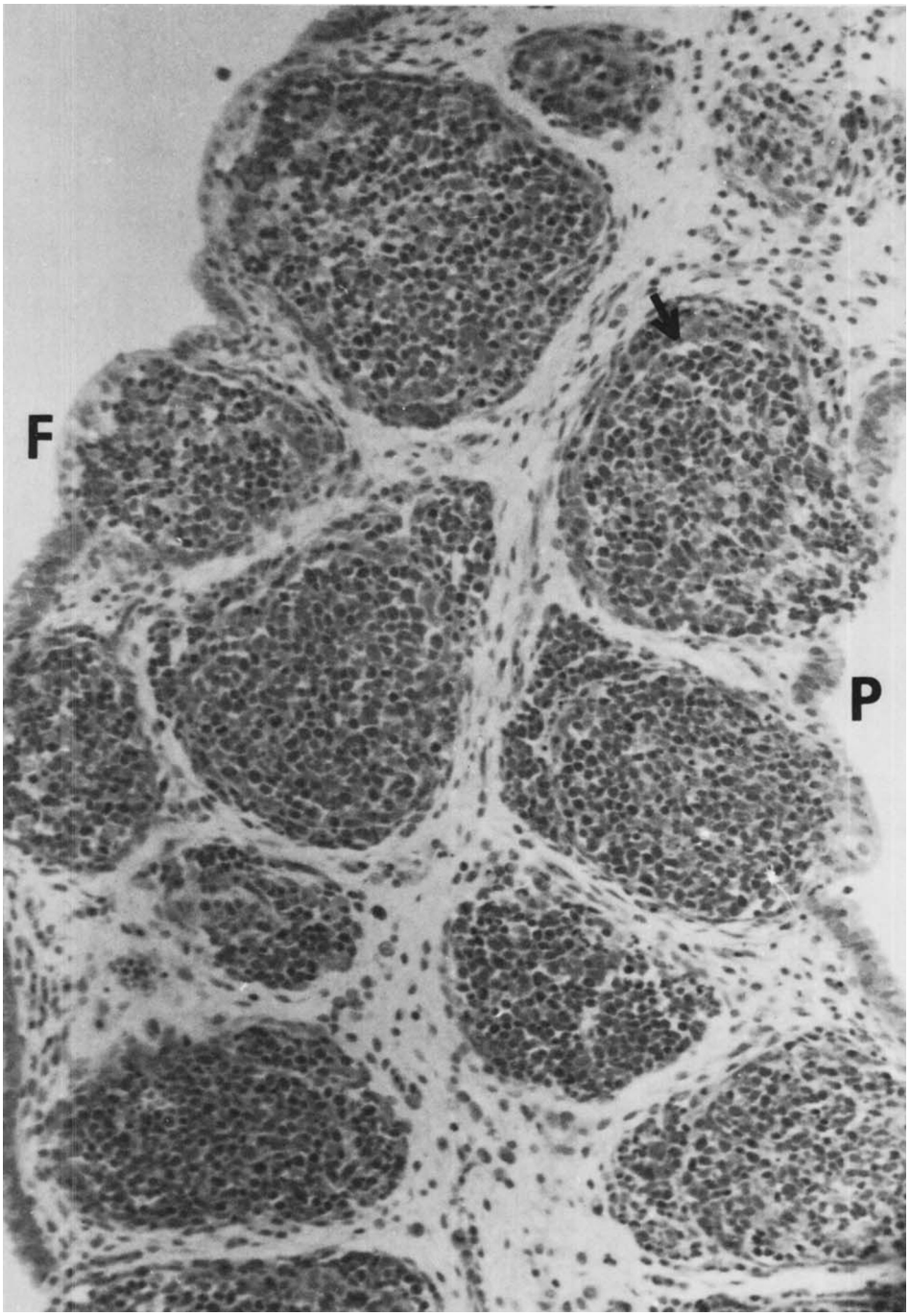


FIG. 1. Bursal fragment after 1 day in culture. Follicular arrangement preserved. Specific follicular (F) and plical (P) epithelium as well as follicular basement membrane (arrow) are clearly seen (×100).

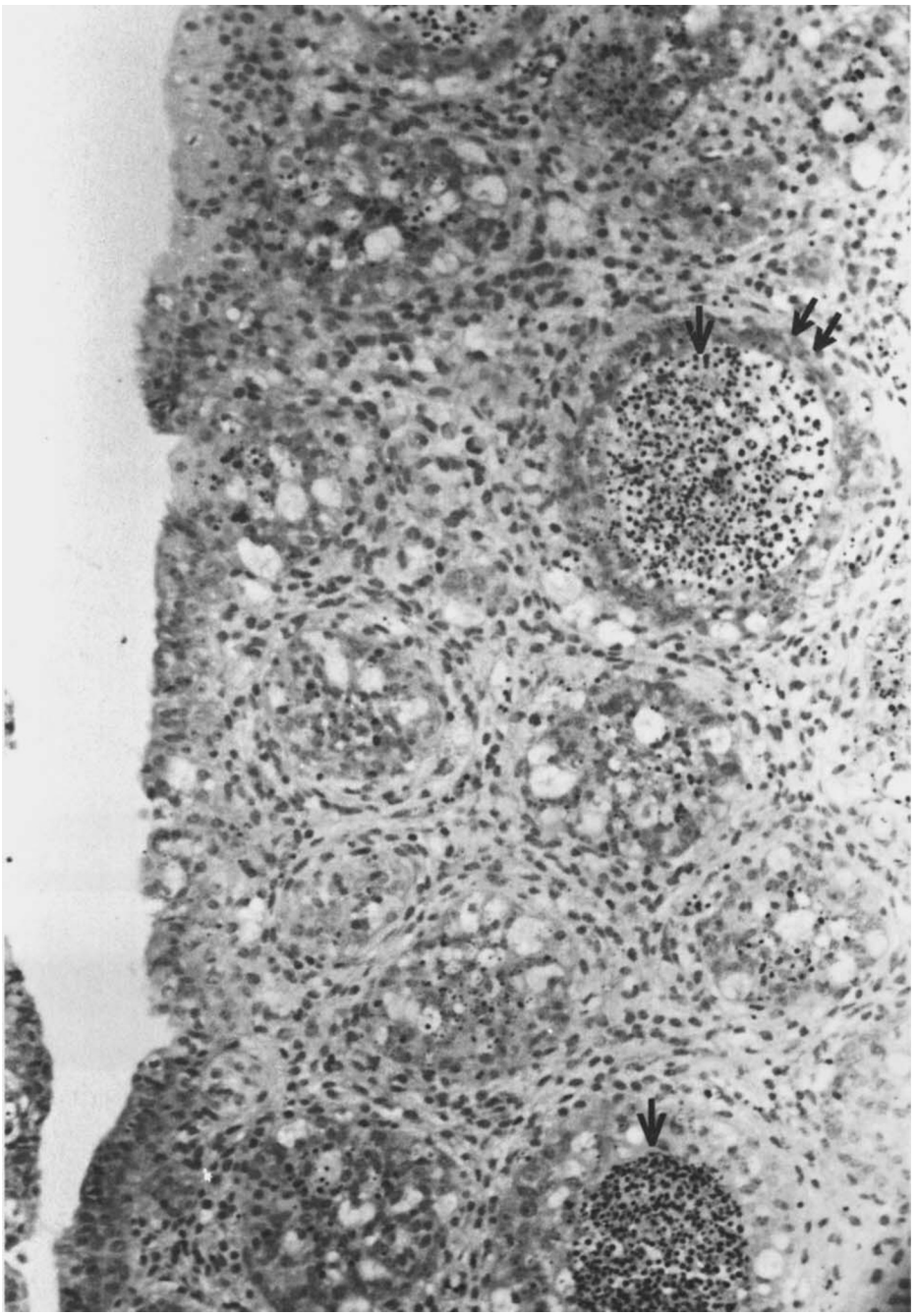


FIG. 2. Bursal fragment after 3 days of culture. Lymphocyte destruction is the prominent feature (single arrows). Basement membrane-associated epithelium is preserved and beginning to proliferate (double arrows) (×100).

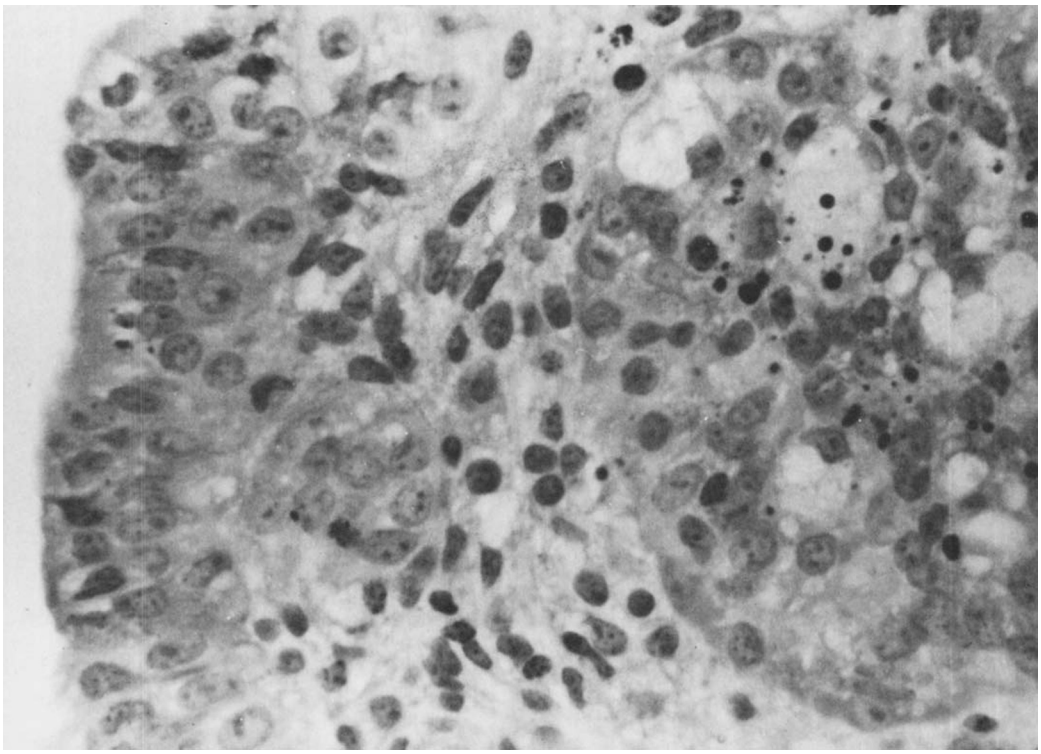


FIG. 3. High-power view of Fig. 2. Nuclear fragments of lymphocytes are seen in the follicle along with epithelial proliferation (×312).

until the grids were submerged and the fluid just touched the bursa pieces. The medium was changed after the first 3 days of culture and again on Day 8. The tissue was incubated in a humidified atmosphere of 5% CO_2 in air at 37° for 13 days.

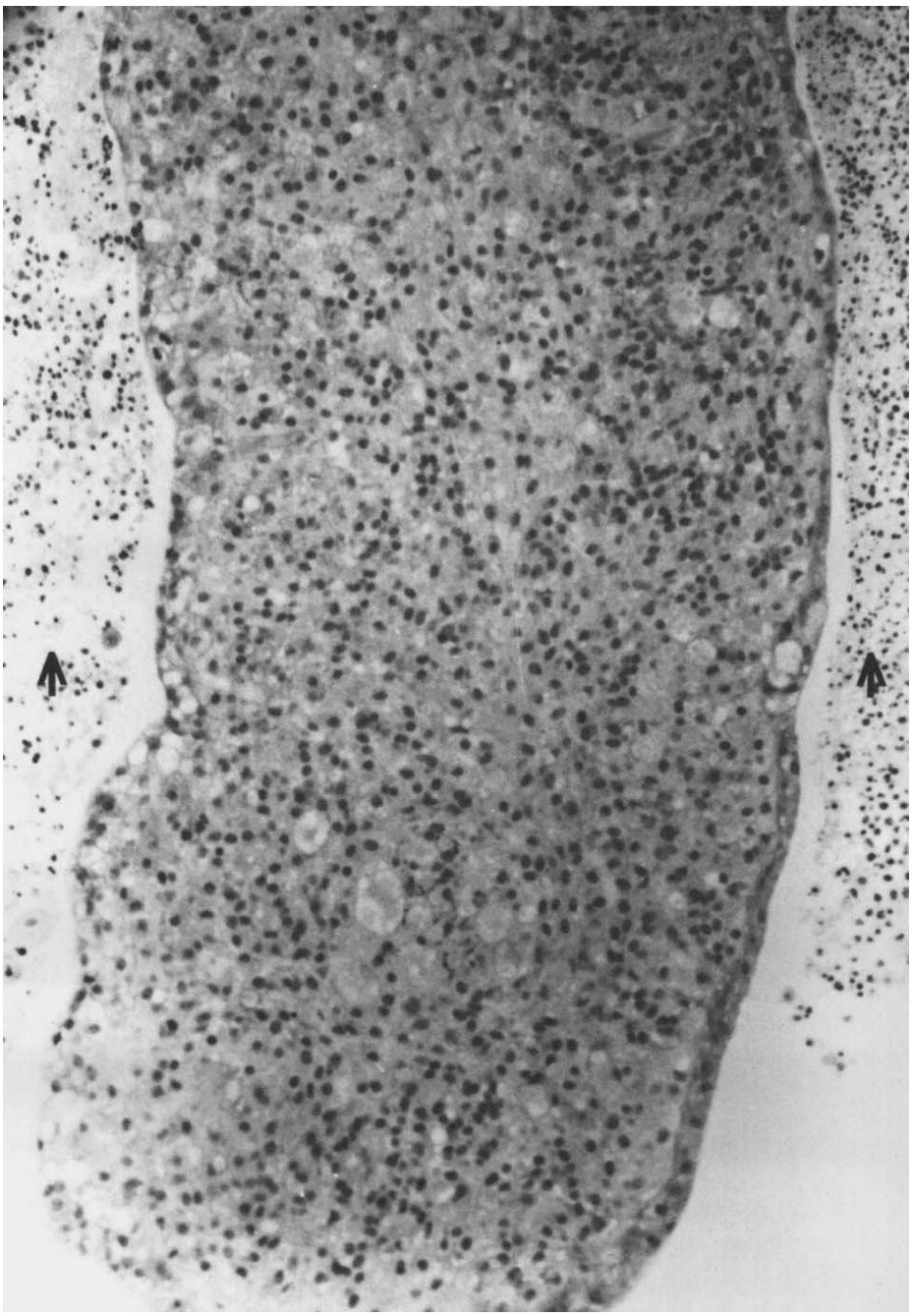
Representative samples of bursal tissue were removed on Days 1, 3, 6, 10, and 13 of culture and frozen or fixed in 10% buffered formalin. Fixed samples were embedded in JB-4 medium (Polysciences, Inc., Warrington, Pa.), sectioned, and stained with hematoxylin and eosin (H&E). Duplicate tissues were stained with an appropriate dilution of rabbit anti-chicken IgG (prepared in our laboratory) followed with FITC-labeled staphylococcal Protein A (Pharmacia Chemical Co., Piscataway, N.Y.). Immunofluorescence was observed using a Zeiss Universal fluorescent microscope with epi-illumination.

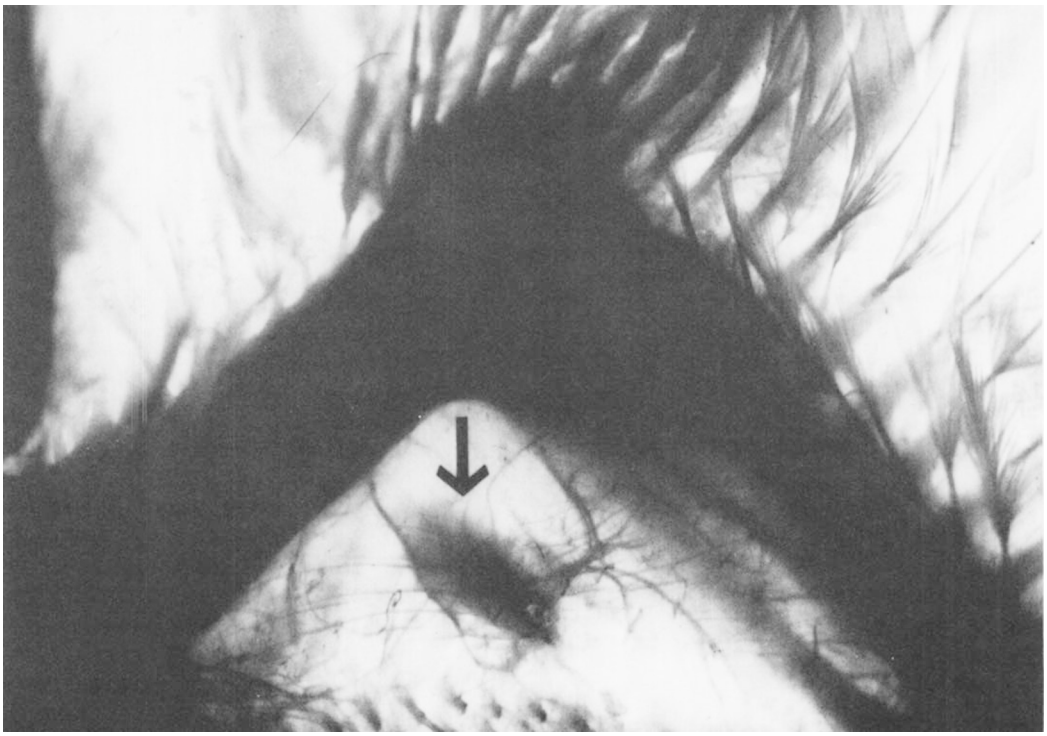
Transplantation. The tissue fragments were removed from culture on Day 13 and pooled in a sterile petri dish containing 5 ml of nonsupplemented RPMI 1640. Meanwhile, the 14-day-old bursectomized chicks were anesthetized with 10–20 μl of methoxyfluorane anesthetic (Pitman-Moore, Inc., Washington Crossing, N.J.), instilled intranasally. A small incision was made in the wing web and 10 pieces of CBE were placed in between the two skin layers forming a pocket. The incision was then sealed with cyanoacrylate adhesive.

Twenty-two days following transplantation, the implants were removed from the wing web and either immersed in liquid nitrogen for frozen sectioning or placed in cold 10% buffered formalin for H&E sections.

The frozen tissue sections were stained with fluorescein-conjugated rabbit anti-chicken γ, μ, and α and examined for the presence of Ig-positive cells.

Results. After 3 days of culture, follicu-





FIGS. 4, 5. Bursal fragment after 10 days culture. Follicular orientation is lost and basement membrane-associated epithelial cells are diffusely layered under plical and specific follicular epithelial layers. Fragment is alymphoid. Emptying of necrotic follicular elements into plical spaces is seen (arrow). (Fig. 4, ×100; Fig. 5, ×312).

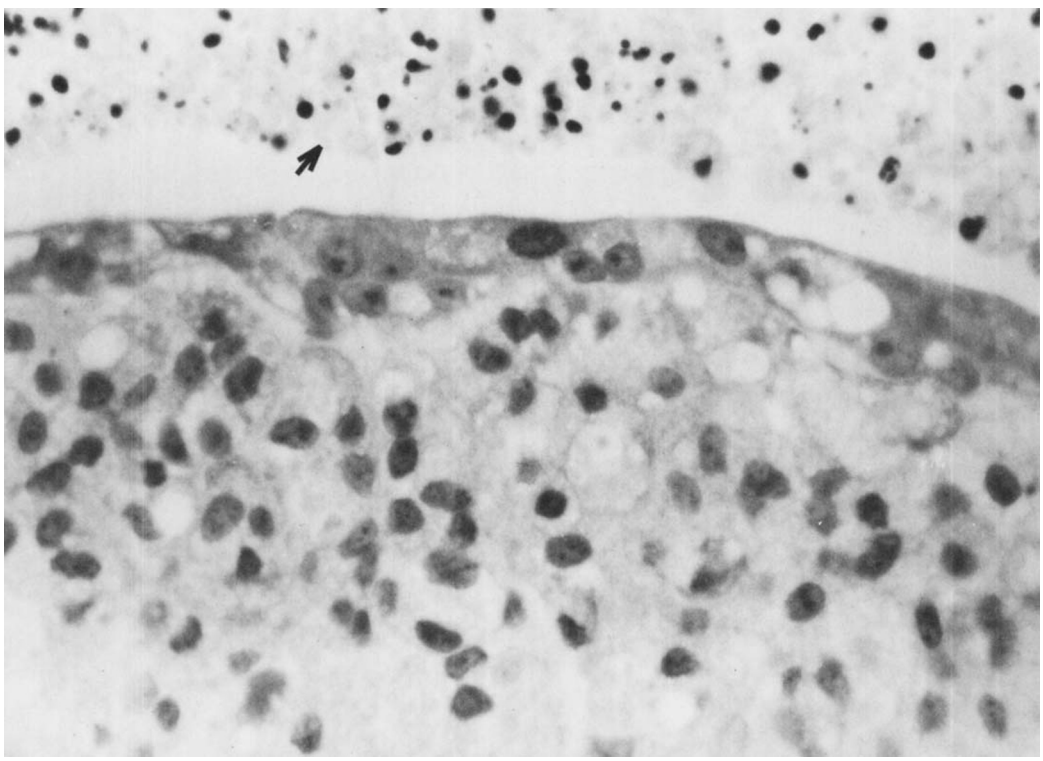


FIG. 6. Fragments of cultured bursal epithelium transplanted 6 days earlier in 14-day-old bursectomized chicks (arrow). Increased vascularity of tissue around transplant is seen.

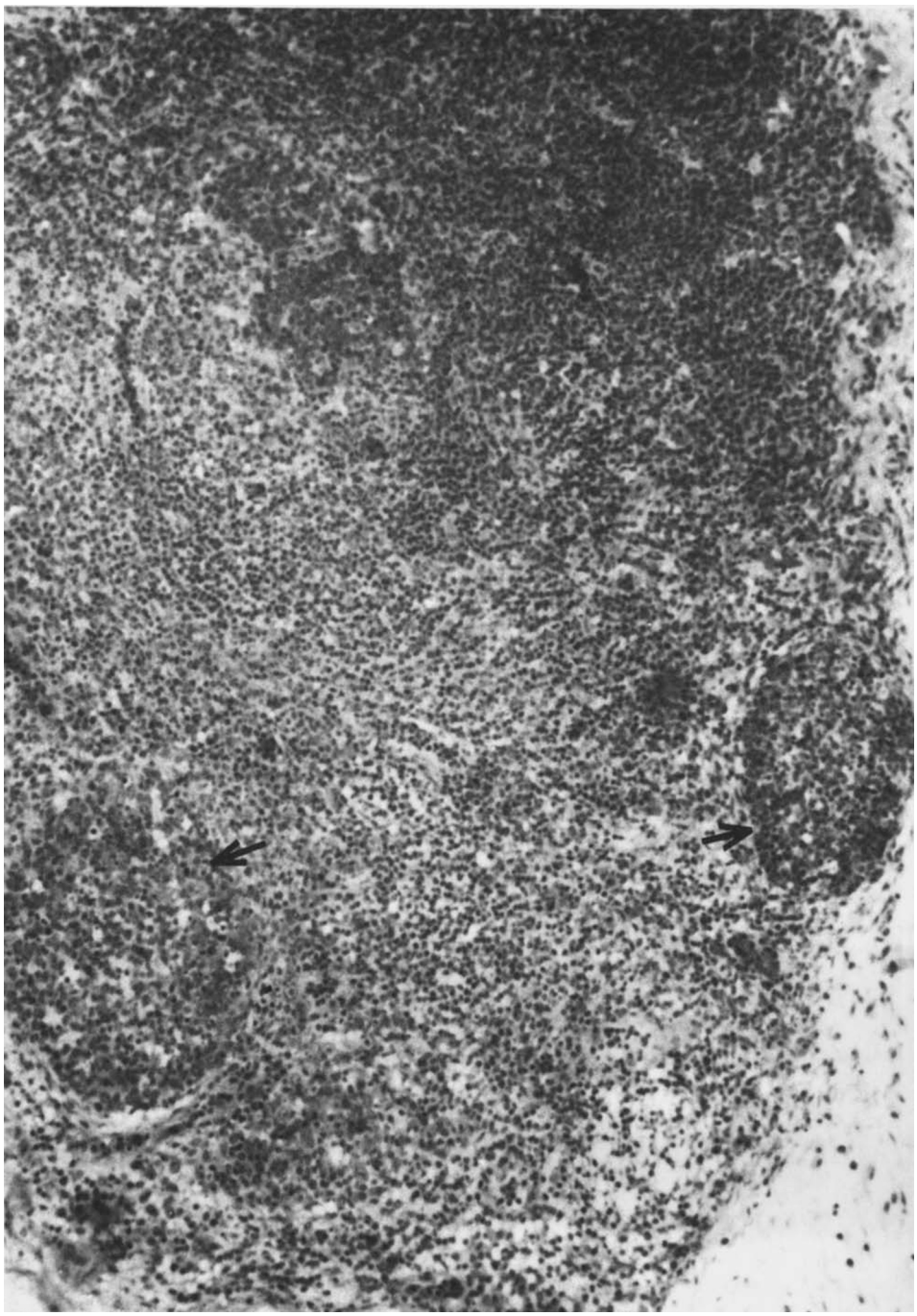


FIG. 7. Sections of cultured bursal fragments implanted 22 days earlier into wing web of 14-day-old chick. Good lymphocyte repopulation with organization into well developed follicle-like structures (arrows) are seen (×63).

lar organization was somewhat preserved, but nearly complete lymphocyte dissolution had occurred. Marked karyorrhexis was apparent, usually in a follicular center, while the basement membrane-associated epithelium remained healthy and appeared to proliferate (Figs. 1, 2, 3). Indirect immunofluorescence indicating the presence of Ig-positive lymphocytes was seen only during the first 36 hr of culture. By Day 10, follicular organization was lost and its epithelium was seen diffusely layered under intact plical or specific follicular layers. Evidence of emptying of the follicular contents into plical spaces as described by Eerola (6) was seen (Figs. 4, 5). Widespread necrosis usually occurred rapidly after Day 14.

On Day 13, the day of transplantation, the fragments were almost totally devoid of lymphocytes. There were no Ig-positive cells seen in the fragments. The bursal epithelium remained intact and appeared viable with no signs of necrosis.

Three to 6 days post-transplantation, the wing web implants in the 14-day-old chicks began to enlarge and become vascularized (Fig. 6). After 22 days, microscopic examination of the transplanted bursal fragments revealed extensive lymphocyte repopulation. There was evidence that follicular organization with corticomedullary demarcation was also reappearing in the implants (Fig. 7). Little necrosis of lymphocytes or parenchymal tissues was observed. In addition, no histological evidence of graft rejection was observed. Previous studies of line 6, subline 1 chickens showed sufficient histocompatibility such that rejection was not anticipated. Stone (7) showed complete acceptance of skin grafts between 42 chickens of this strain. Immunofluorescence revealed collections of Ig-staining cells of all three major classes (Fig. 8).

Discussion. It has been suggested that optimal development of the B-cell repertoire within the bursa is dependent upon close association with the gut environment and its associated antigens (8). Results of our study and others (9) have shown that follicular organization of lymphocytes and appearance of Ig-positive cells within a bursal epithelium can occur at a site other than the gut. In this study, the wing web proved to be an ideal site for both the initial implantation of CBE and also for subsequent visualization of the graft.

The chicks used were homozygous (B^2B^2) for the major histocompatibility complex. Hence, rejection would not be expected and skin grafts have been completely accepted (7). Histologic examination of the transplant did not reveal a cellular infiltrate characteristic of rejection. Furthermore, some of the lymphocytes which were present were organized into structures resembling bursal follicles. Therefore, it is unlikely that they were part of an inflammatory reaction.

The question of whether the lymphocytes in the graft were of donor or host origin was not directly addressed in this study. Previous work in our laboratory with thymus epithelium has shown that after 16–22 days of culture the tissue is nearly devoid of donor lymphocytes. Those few that remain are dead (10). In this study, histologic and immunofluorescent examination of the bursal epithelium after 13 days in culture revealed essential depletion of lymphocytes. The reappearance of Ig-positive cells after transplantation suggests that they were of host origin.

The results of this study further extend the results reported by Eerola (6). Eerola's finding that atrophy of bursal follicles and the repopulation of cultured fragments following transplantation occur in the reverse order of that seen in ontogenesis was also noted by us. In Eerola's study, however, transplantation onto the chorioallantoic membrane was performed. We employed a wing web site. Establishment of vascular channels and, if necessary, appropriate types of endothelium were necessary for repopulation of our transplants. We have further shown that with time the transplant regains both follicular organization and Ig-positive cells. These morphologic characteristics which were lost in culture may return due to an intrinsic capabililty of the epithelium which is expressed after *in vivo* transplantation or may be the result of secondary contact with lymphocytes. Transplantation of CBE into the wing web of bur-

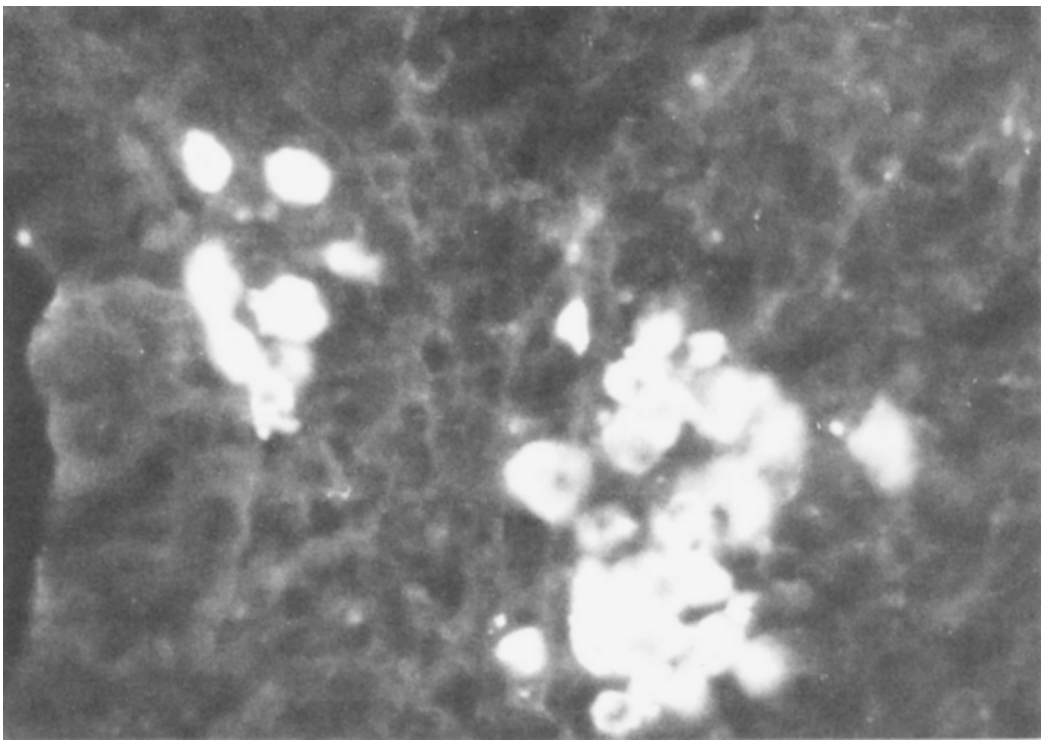


FIG. 8. Immunofluorescence (with anti-μ) of transplant (×308).

sectomized chickens thus may simulate naturally occurring events in B-cell development. Further studies to determine whether the lymphocytes are capable of restoring immune function are in progress.

We are indebted to Dr. M. D. Cooper of the University of Alabama–Birmingham for the gift of FITC-labeled rabbit anti-μ, γ, and α antisera.

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Received June 14, 1982. P.S.E.B.M. 1982, Vol. 171.