Lymphoepithelial Glands in the Intestines and Cloaca of the Australian Echidna (*Tachyglossus aculeatus*)

Recent studies have led to the proposal that the lymphoid stem cell population differentiates along 2 distinct lines. Lymphoid cells differentiating in the thymus are responsible for the expression of cellular immune mechanisms such as those involved in allograft rejection, and are believed to populate 'thymic-dependent areas' in peripheral lymphoid tissues as found in the spleen and lymph nodes[1,2]. On the other hand, lymphoid cells differentiating in the bursa of Fabricius in chickens, or in possible bursal homologues in mammals, are believed to develop the potential for expressing humoral immunity, and their subsequent population of special 'bursal-dependent' areas in peripheral lymphoid tissues is considered to give rise to cells capable of specific antibody production[3-5].

The search in mammals for a homologue of the bursa of Fabricius has led to the suggestion that subepithelial lymphoid aggregations of the alimentary canal, for example in the rabbit[5-8], represent sites where lymphoid cells undergo a differentiation which is appropriate for the expression of humoral immunity. DIENER and EALEY[9] followed one obvious line of research in studying the immune system of monotremes, the primitive mammals represented by the echidna (*Tachyglossus aculeatus*) and the platypus (*Ornithorhynchus paradoxus*), but concluded that there was no reason to suggest any homology between the appendix of the echidna and the bursa of Fabricius.

During the course of further studies on the alimentary canal of adult echidnas of undetermined age, lymphoid aggregations with a highly unusual appearance were seen in the submucous layer of the ileum, large intestine and cloaca.

One of the intestinal or cloacal glands overlying each submucosal lymphoid aggregation was widened and unusually elongated and extended vertically through the

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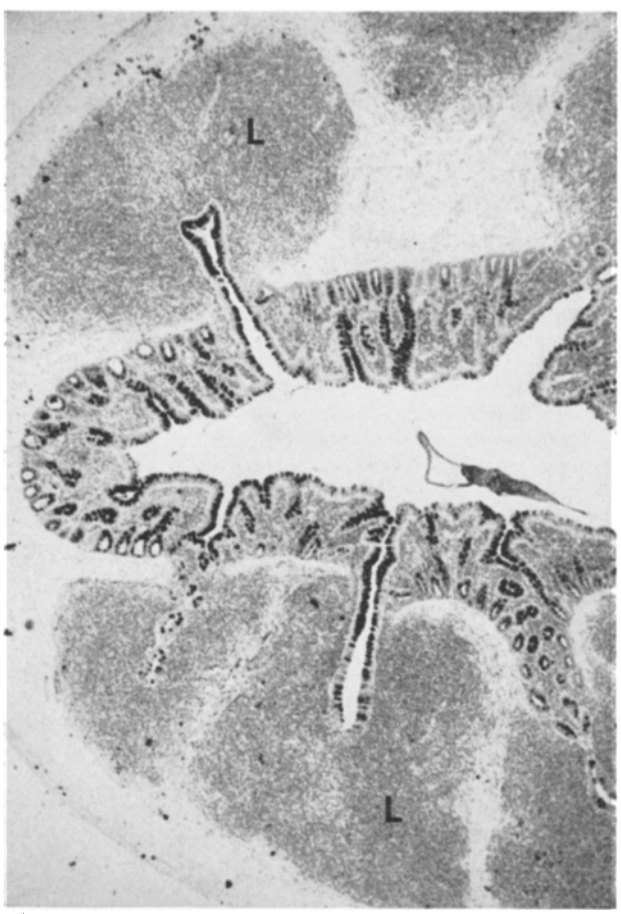

Fig. 1. Appendix showing unusually elongated intestinal glands projecting into submucosal lymphoid nodules (L) to form lymphoepithelial glands. Darkly stained goblet cells can be seen in the epithelium. (Toluidine blue stain: ×150.)

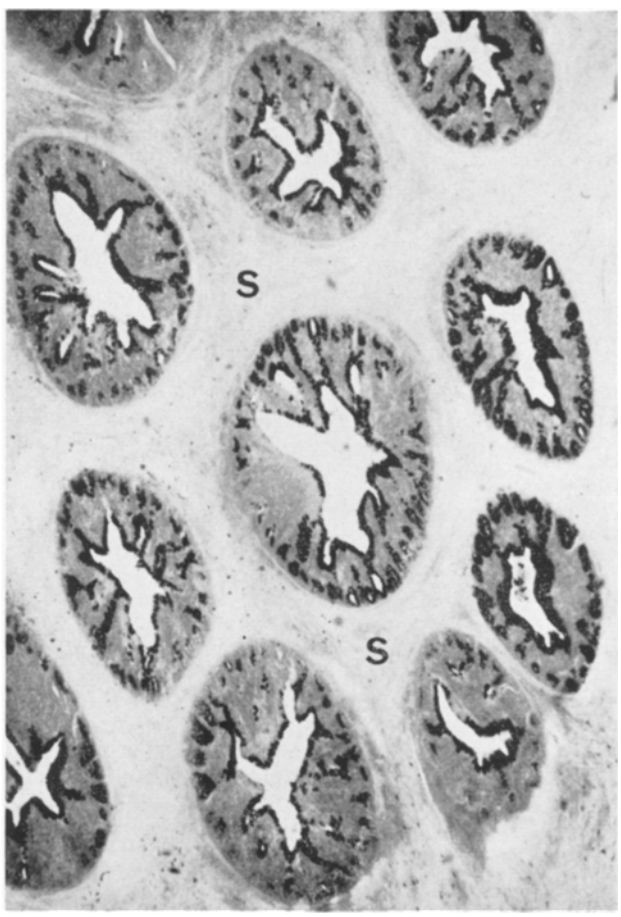

Fig. 2. Lymphoid nodules in the submucous layer (S) of the terminal colon. Each nodule is invaded by an elongated intestinal gland which gives rise to diverticula radiating to the periphery of the nodule. (Toluidine blue stain: ×150.)

muscularis mucosae into the subjacent lymphoid tissue; here it branched to form as many as 15 diverticula which radiated towards the periphery of the nodule (Figures 1 and 2). Both the elongated glands and the diverticula arising from them were lined by epithelium similar to that found elsewhere in the mucous membrane. It is suggested that an appropriate term of reference for the submucosal lymph nodule and the associated epithelium invading it is lymphoepithelial gland.

In the appendix lymphoepithelial glands were seen throughout the submucous layer. In the ileum, and also in the colon, they formed aggregations which macroscopically resembled the Peyer's patches found only in the ileum of most other mammals. In the cloaca, the lymphoepithelial glands were less numerous and more discrete.

The lymphoepithelial glands of the echidna, ranging in distribution from the ileum to the cloaca, were strikingly similar to the more discrete aggregations of lymphoid tissue and glands found in the bursa of Fabricius in the chicken. The possibility that, collectively, they are the homologue of the bursa requires further investigation in young animals. The lymphoepithelial glands of the adult echidna contain cortical and medullary lymphoid areas but nevertheless are clearly involved in immunoglobulin production for they also contain plasma cells and their precursors. From the present findings it would seem appropriate that further attempts to identify bursal homologues in the gut of more highly evolved mammals should include an examination of submucosal lymphoid aggregations for the presence of invading intestinal glands or their remnants, not only in the small intestine and appendix but also in the large intestine.

Zusammenfassung. In Darm und Kloake von Echidna (*Tachyglossus aculeatus*) wurden besondere Lymphdrüsen festgestellt, die Parallelen mit der Vogel-Bursa Fabricii zeigen.

G. C. SCHOFIELD and R. N. P. CAHILL

Department of Anatomy, Monash University, Clayton (Victoria, Australia), 20 September 1968.

Simian Virus 40 Infection of Uninoculated African Green Monkeys (*Cercopithecus aethiops*) Revealed by Repeated Cell Passages

Demonstration of Simian papovavirus (SV_{40}) contamination of uninoculated Rhesus cultures[1] posed the serious problem of inadvertent infection of humans by contaminated vaccines[2,3]. This became particularly acute when SV_{40} was shown to produce fibrosarcomas in hamsters[4,5], and that infected human diploid cells had the same tumor antigen found in sarcoma-bearing hamsters[6]. Investigations indicating that African green monkeys (AGM) were infected only by contact with Rhesus resulted in the substitution of African for Asiatic species[1,7,8]. Monitoring AGM cells for latent SV_{40} infection has emphasized serologic screening along with examinations of primary monolayers for typical SV_{40} cytopathology[9,10]. Our experiments reported here involved rapid passages of presumed uninfected AGM kidney cultures. Evidence of SV_{40} virus was found in all specimens, including those initially negative monolayers obtained from serologically negative monkeys. 14 monkeys (*Cercopithecus aethiops*) were studied over a period of about 1 year All primary suspensions were received from the same cell culture supplier and subcultivated in 6 oz dilution bottles and on 25×75 mm slides inserted in Leighton-type tubes. Anti-SV_{40} serum was never incorporated in the growth media. Bottle and tube cultures were grown in 10% calf serum in Eagles MEM (GIBCO) with conventional antibiotics. The bottle cultures were passaged every 3 days using 1×10^6 cell/bottle inocula during the earlier and more productive passages. The slide cultures were seeded with the same inoculum and received exactly the same treatment in order to monitor each bottle culture for viral cytopathology and analysis for SV_{40} tumor and viral antigen by immunofluorescence. Tumor antigen was detected by the direct method with hamster fluorescein labeled tumor anti-serum (Flow)[11]. The indirect method for viral protein employed calf anti-SV_{40} serum and rabbit fluorescein labeled anti-bovine globulin (BBL). Preparation of slides and processing of the anti-bovine conjugate were carried out as previously reported[12,13]. Serum was obtained from 7 monkeys at the importer 2–5 days prior to shipment to the cell culture supplier. SV_{40} antibody levels were measured with anti-SV_{40} calf serum, the Girardi strain of SV_{40} virus (Flow) and the BS-C-1 stable line of *Cercopithecus aethiops* kidney[14] (BBL). Eight of the primary suspensions were seeded as 1×10^6 cells/Leighton tube inocula on monolayers of BS-C-1 cells at the start of their respective passage. Uninoculated BS-C-1 cultures were used to monitor laboratory contamination by SV_{40} as well as uninoculated

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