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.

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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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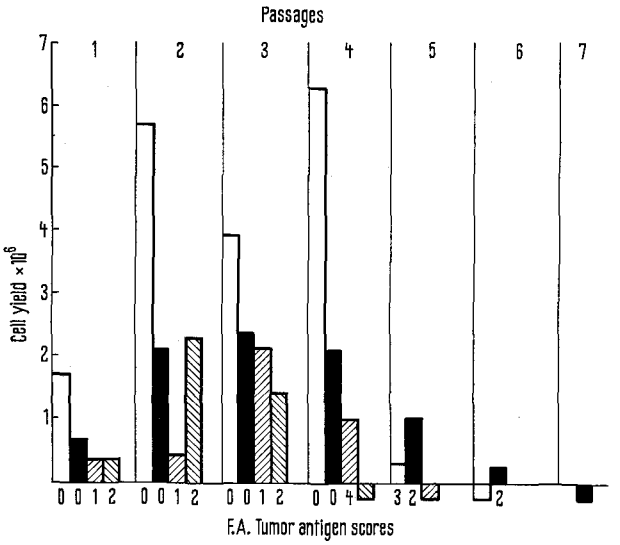
Table I. Relation between detection of tumor (T) and capsid (V.C.) antigen in first passage monolayers and manifestation of cell lysis

Monkey	First monolayer immunofluorescence T	V.C.	Abrupt drop in cell yield (Passage)	Terminal lysis (Passage)
1[a]	0	0	8	10
2[a]	0	0	5	6
13[a]	0	0	5	8
14[a]	0	0	3	5
15[a]	0	0	8	9
18[a]	0	0	7	8
4[a]	0	0	6	7
7	+	0	2	2
5	+	0	6	8
12	+	0	1	1
3	+	0	2	3
10	+	+	1	1
8	++	++	4	5
6	+++	+++	3	4

[a] SV_{40} confirmed in later passages. T and V.C. scores: 0 monolayer negative, + 1–5% positive cells, ++ 6–25% positive cells, +++ 26–50% positive cells.



Correlation between cell yield, number of passages and first evidence of tumor antigen.

Table II. Relation between monkey SV_{40} antibody level, initial detection of tumor antigen and appearance of spontaneous lysis

Monkey	Monkey SV_{40} antibody	T antigen scores First cell suspension	First monolayer	Passage showing terminal lysis
13	< 1:2	0	0	8 (7)[a]
14	< 1:2	0	0	5 (5)[a]
15	< 1:2	0	0	9 (9)[a]
18	< 1:2	0	0	7 (7)[a]
5	1:64	++	+	8
4	1:2	+++	+	7
6	1:64	+++	+++	4
8	na	++++	++	5

[a] Passage with first indication of tumor antigen. na, serum not available. 0 monolayer negative, + 1–5% positive cells, ++ 6–25% positive cells, +++ 26–50% positive cells, ++++ 51–75% positive cells.

control mates for SV_{40} virus controls in the antibody and immunofluorescence assays.

As seen in Table I all cultures eventually showed the typical SV_{40} vacuolated degeneration regardless of evidence for virus in the first passage. Abrupt reduction of cell yield prior to spontaneous cell lysis was obtained both with frankly infected cells and those cultures devoid of any evidence of SV_{40} virus during their first passage. The correlation between initially detectable SV_{40} infection, cell yield and serologic evidence of virus is shown in the Figure. Both initially negative cultures required 5 passages before manifestation of viral cytopathology or SV_{40} antigen by immunofluorescence. The importance of frequent passages for revealing latent SV_{40} infection in serologically negative monkeys is seen in Table II. All cell suspensions without detectable virus and obtained from monkeys devoid of infectious antibody nevertheless developed both typical cytopathology and SV_{40} antigens following repeated passages.

These findings raise the possibility that African green monkeys are infected in their natural environment or that present shipping procedures may be less than foolproof. Regardless of the source of virus, it appears that low levels of infection in primary African monkey kidney cultures require successive cell divisions for SV_{40} virus manifestation.

The demonstration of low levels of SV_{40} following repeated passages is not surprising in view of the report of induced AGM kidney cultures-SV_{40} carrier systems[15]. The necessity of cell division for expression of SV_{40} infection has been demonstrated both by the need to grow out explants from inoculated kidney biopsies[8] and the resistance of non-dividing cells to SV_{40} induced transformation[16,17].

Résumé. Nous avons trouvé le virus SV_{40} chez le singe *Cercopithecus aethiops* presumé non infecté. Par la méthode de culture des cellules du rein et en effectuant des passages rapides nous avons pu démontrer l'existence du virus même chez les animaux dépourvus d'anticorps et même dans le cas où le virus n'apparaissait pas durant le premier passage.

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