Role of Rabbit Gut-Associated Lymphoid Tissue in Cell Replication. The Follicular Cortex as Primary Germinative Site* (33542)

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It has been suggested that in the rabbit, certain gut-associated tissues function as a site of differentiation for antibody forming cells and represent a homologue of the avian bursa of Fabricius (1). In both organs, the lymphoid follicles possess a close anatomical relationship to overlying epithelium of the gut and show a clear differentiation into a cortex and medulla. Extirpation of the bursa in birds (7) or the appendix, sacculus, and Peyer's patches in rabbits, causes antibody deficiency syndromes (2, 8). Neonatal thymectomy in either chicken or rabbit diminishes, but does not abolish antibody responses to many antigens (3, 4).

The avian bursa is characterized by having a very large percentage of cells in DNA synthesis (5) as befits an organ functioning as expander of a population of cells. We, therefore, assessed DNA synthesis in rabbit appendix, sacculus rotundus, and Peyer's patches by *in vivo* and *in vitro* methods in search of further similarities between these organs and the bursa of Fabricius.

Methods. Young New Zealand white rabbits of both sexes obtained from a local breeder were given an intravenous injection of tritiated thymidine (^{3}HT) (6.7 Ci/mmole), 0.5 μCi/g of body weight, and were killed 5 hr later. Sections of formalin-fixed tissues were dipped in Kodak NTB_2 emulsion, exposed for 1–4 weeks and developed. For *in vitro* labeling experiments organs were removed and washed repeatedly in normal saline containing penicillin, 200 U/ml, and streptomycin, 200 μg/ml. Single cell suspensions were prepared by gentle homogenization and the cells were suspended at a concentration of 3×10^6/ml in 3-ml aliquots in minimum essential medium, (Grand Island Biological) containing 20% rabbit serum, 100 U of penicillin, and 100 μg of streptomycin/ml. The cultures were incubated at 37° in an atmosphere at 4% CO_2 and room air. Thirty min after incubation, 4 μCi of ^{3}HT were added to each culture, 1 hr later an excess of cold thymidine was added, the cells were washed repeatedly, fixed in 25% glacial acetic acid in absolute alcohol, and placed on clean dry slides; autoradiographs were prepared as before. Background grain counts were determined over 4000 μ^2 cell-free areas immediately adjacent to the cells being counted; they varied from 5–15 in autoradiographs of cell suspensions, and from 15–30 in autoradiographs of fixed tissues. The number of labeled cells in the cortex and medulla of follicles was assessed by counting labeled cells in randomly chosen 4000 μ^2 areas in either cortex or medulla. Cells with 3 or more grains over the nucleus were considered labeled.

Results. In vitro labeling of suspensions of rabbit lymphoid organs with a 1 hr pulse of ^{3}HT produced a very high labeling index (percentage of cells labeled) in preparations of bone marrow, appendix, sacculus, thymus, and Peyer's patches (Fig. 1). The highest percentage of labeled cells was seen in bone marrow suspensions (27%), followed in order by appendix (17%), sacculus (14%), thymus and Peyer's patches (both 7%). The difference between appendix on the one hand and the Peyer's patches and thymus on the other hand, was significant ($p<0.05$). Cells from the peripheral lymph nodes and spleen had a much lower index than those of the

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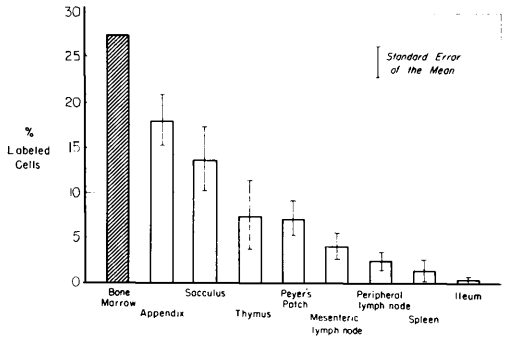


FIG. 1. Percentage of labeled cells in various organs of the rabbit; tracer: tritiated thymidine.

appendix ($p<0.01$), thymus, sacculus, or Peyer's patches. Small segments of ileum which lacked organized lymphoid tissue were included in the experiment to rule out significant participation of gut epithelial cells in the labeling index. As shown in Fig. 1, the percentage of labeled cells in these tissues was very low.

In an attempt to determine the source of the rapidly replicating cells in the appendix and other gut-associated lymphoid organs, autoradiographs prepared from histological sections after an *in vivo* pulse label of 5 hr were examined and compared to autoradiographs of sections of spleen, mesenteric and popliteal lymph nodes.

The number of follicles was much greater in appendix and sacculus than in any of the other organs examined. In appendix, sacculus, and Peyer's patches, the typical follicles extended to the mucosal surface of the gut and lacked the mantle zone of small lymphocytes so characteristic of the follicles in lymph nodes and spleen. In many well-developed follicles of the appendix, sacculus, and Peyer's patches, two separate populations of lymphocytes could be distinguished (Fig. 2). In the periphery of the follicle (follicular cortex) the cells were large and the nuclei had an open, lacy chromatin pattern while in the center of the follicle (follicular medulla), the cells were smaller and had nuclei with darker, more condensed chromatin. In the follicular cortex the number of grains per cell was low compared to the number of grains over labeled cells of the interfollicular areas; the labeling index, however, was very high and in all follicles examined, 40–60% of the cells in the follicular cortex were labeled. In the follicular medulla few cells were labeled and the number of grains over the nuclei of these cells was somewhat lower than over the cells of the follicular cortex. The difference in labeling index between the two groups was significant ($p<0.001$) (Table I).

In the interfollicular areas of appendix, sacculus, and Peyer's patches, fewer labeled cells were present than in the follicular cortex, but these cells were more heavily labeled than those in the follicle. The transition between follicle and interfollicular area was sharply demarcated by the follicular capsule and by the abrupt changes in labeling pattern and labeling index.

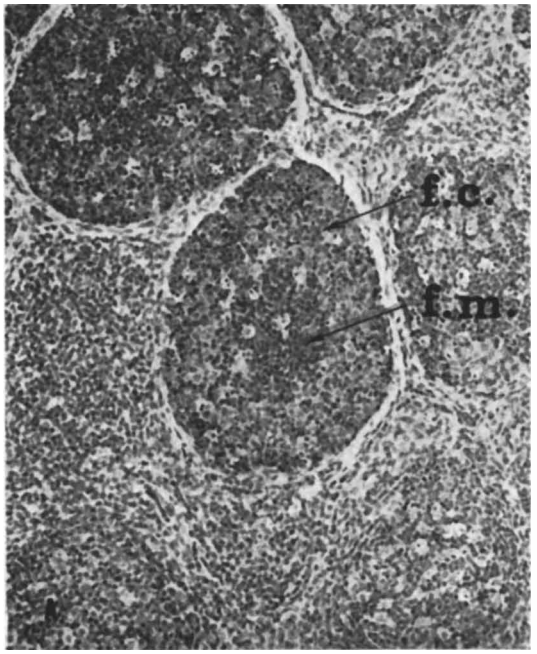
The follicles in the peripheral lymphoid tissues differed markedly from those of the gut-associated lymphoid tissues (Fig. 3). Well-developed follicles from the spleen, mesenteric and popliteal lymph nodes had a mantle zone of small lymphocytes with condensed nuclei; few of these cells were labeled, but the number of grains per cell was

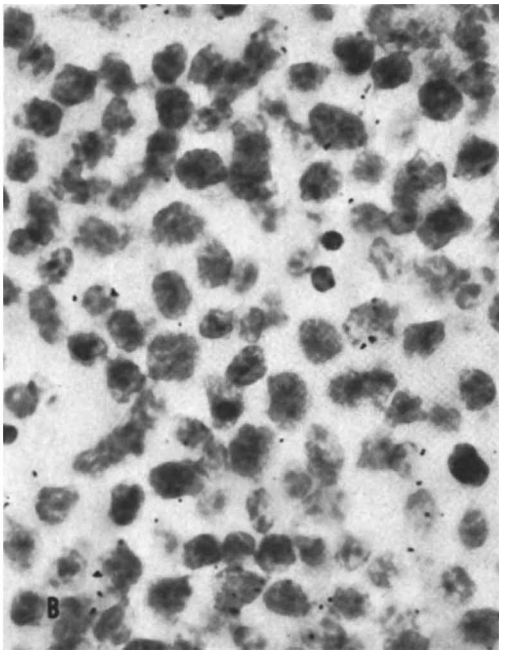
TABLE I. Percentage of Labeled Cells in Cortex and Medulla of Rabbit Appendiceal Follicles.

Rabbit no.	Percentage of labeled cells per follicle[a] in Cortex	Medulla
1	61	12
	50	18
	50	16
2	50	7
	53	12
	45	10
3	47	20
	67	10
	56	9
4	52	10
	50	7
	46	10
Mean[b]	52 ± 2.3	12 ± 0.9

[a] Average number of cells counted per follicle: in cortex, 106; in medulla, 123.

[b] Mean ± SE. The difference between the percentage of labeled cells in cortex and medulla is significant ($p<0.001$).





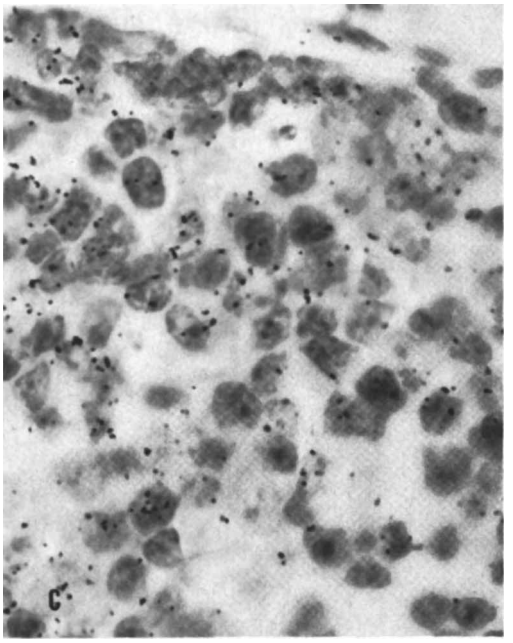


FIG. 2. Follicles in the rabbit appendix: (a) Many follicles contain a central zone of darker cells: follicular medulla (f.m.) and a peripheral zone of larger, lighter stained cells: follicular cortex (f.c.). In the follicular medulla few cells are labeled (b) while in the cortex the labeling index is high (c). Original magnification: (a) ×100; (b) ×1000; and (c) ×1000.

high. The majority of cells in the germinal center were large pale staining cells with pale staining nuclei, morphologically very similar to those of the follicular cortex. These cells also had a similar labeling pattern (distribution of grains in the tissue) and labeling index; many cells were labeled, but fewer grains were present over the nucleus as compared to the labeled cells in the mantle zone. In only one instance did we observe a small area of lightly labeled cells within the germinal center reminiscent of the cells in the medulla of appendiceal follicles.

Discussion. This study shows that gut-associated lymphoid tissues in the rabbit are sites of intense cell proliferation, far surpassing other lymphoid tissues in this respect. Of all organs examined only the bone marrow is a more active source of newly formed cells. As our *in vivo* labeling studies indicate, the follicles in appendix, sacculus, and Peyer's patches appear to be the primary locations for cell replication because in the follicles many more cells were in DNA synthesis than in the interfollicular areas.

We have also shown that within the follicles of gut-associated lymphoid tissues a distinction can be made between cortex and

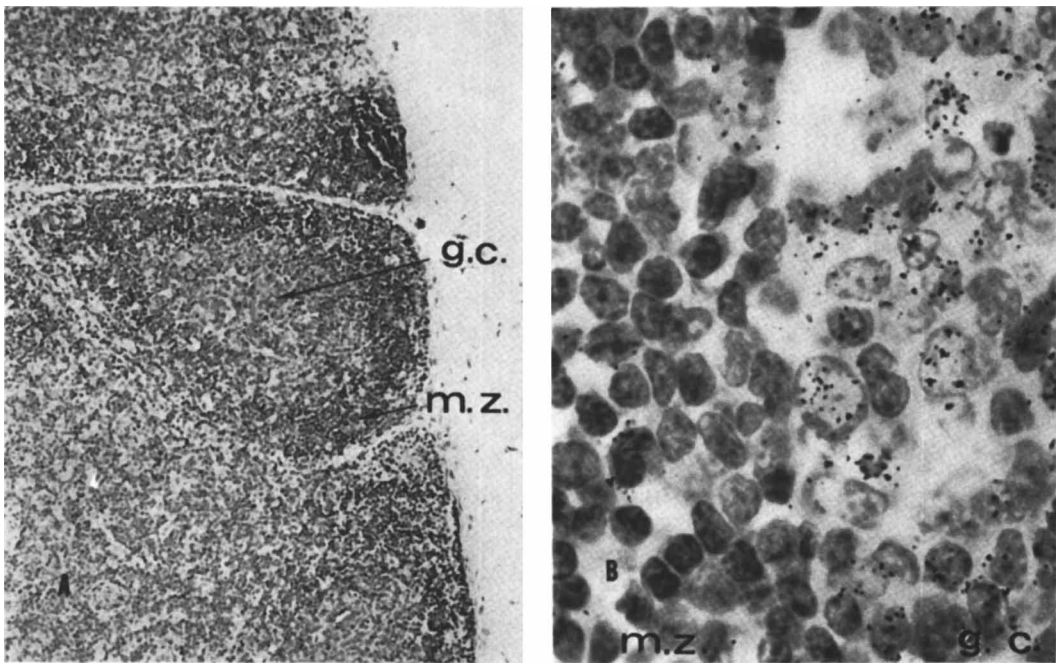


FIG. 3. Follicles in rabbit mesenteric lymph node: (a) The center of the follicle consists of the germinal center (g.c.), and is surrounded by a mantle zone of small lymphocytes (m.z.). (b) The cells in the germinal center are large and pale staining; many of these are labeled. Few cells are labeled in the mantle zone. Original magnification: (a) ×125; (b) ×1250.

medulla in the sense that by far the most active labeling was seen over the cells of the follicular cortex. The cells in the medulla differed from cortical cells by their distinct morphological appearance (and a significantly lower labeling index. The germinative function of the follicle, therefore, seems to be confined primarily to the layers of cells with blastoid appearance) constituting the cortex of the follicle, whereas the majority of cells in the medulla are not in active DNA synthesis. Previous studies showed that under certain circumstances the medulla of appendical follicles may be relatively devoid of cells; these studies are consistent with our present concept of these follicles as hollow organs in which active cell replication takes place in the wall of the follicular bag.

What the stimulus for the concerted cell proliferation in the follicular cortex is, remains unanswered by our studies so far. The fate of the newly produced cells is also unknown: they may die *in situ*, move directly to blood or lymph, or migrate first toward the gut mucosa, as seems to occur in another gut-associated lymphoid organ of the rabbit, the tonsil (6). Our studies indicate the possibility that before any of these migrations are undertaken, the progeny cells may assemble in the follicular medulla.

Although pronounced differences are found between follicles in spleen and lymph nodes on the one hand (and those in gut associated lymphoid tissues on the other hand) as indicated above, striking similarity nevertheless exists between the cells of the germinal center and cells of the follicular cortex in appendix, sacculus, and Peyer's patches. By light microscopy at least, these cells cannot be differentiated even when labeling characteristics are taken into account. At present, there are no data concerning the relationships between these cells. In the chicken, the bursa functions as an expander and differentiation site for germinal center cells in spleen and lymph nodes, as shown by failure of development of germi-

nal center cells after bursectomy at hatching followed by irradiation (7), or by embryonic bursectomy (9). Recent work in our laboratory has revealed the important role played by rabbit gut-associated lymphoid tissue in development of antibody production (2,8). Our present study underscores the exceptional character of these tissues in terms of their high rate of cell proliferation as compared to other lymphoid organs, and the unique nature of their follicular structure.

Future studies are needed to elucidate the destination of the cells produced in the rabbit appendix, spleen, and Peyer's patches, and to outline their role in the process of antibody formation. Comparative studies of the follicles of the bursa of Fabricius are also in order.

Summary. As shown by *in vitro* labeling techniques, the rabbit appendix and sacculus rotundus contained a higher number of cells in DNA synthesis than any other lymphoid organ examined. The bone marrow alone had a higher number of labeled cells. When labeled *in vivo,* gut-associated lymphoid organs of the rabbit contained a high number of cells in DNA synthesis. The follicles in these organs were distinguished from follicles of peripheral lymphoid tissue by separation into follicular cortex and medulla. The cortex contained large blastoid cells with a high labeling index; the medulla consisted primarily of small darker cells with a low labeling index. These findings support the hypothesis that the gut-associated lymphoid organs in the rabbit expand the lymphocyte population by rapid proliferation, and may play a role as primary lymphoid organs.

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