Replication of Spleen Lymphocytes and Ileal Crypt Cells in Conventional and Germfree Young Rats[1] (36501)

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During studies of the effects of DNA labeling with tritiated thymidine (^{3}HTdR) upon the replication of spleen lymphocytes in the young rat, it was found that their mitotic labeling curve was markedly skewed. This occurred with both 0.4 and 1.0 μCi/g (1). At the latter labeling dose relatively symmetrical curves had been observed earlier with hepatocytes (2) and with ileal crypt cells (3).

Since the spleen may act as a lymphoid organ which responds to antigenic stimulation, it seemed possible that the asymmetrical mitotic labeling curve might have resulted from antigenic stimulation of large and/or small lymphocytes, with consequent perturbation of their cycling (4). Accordingly, studies were made in germfree rats. The replication of coexistent ileal crypt cells was also examined.

Methods. Germfree and conventional rats of the Wistar strain, aged 3.5 to 5.5 weeks received 0.4 μCi/g body weight of ^{3}HTdR (sp act 13.0 Ci/mmole) sc. They were killed at frequent intervals of 0.5 to 2 hr and their ileal and splenic tissues were fixed in acetic acid–alcohol, 1:3. Paraffin sections 5 μ in thickness were prepared as autoradiographs using NTB-3 emulsion. These were stored for 30 days, and then developed. The autoradiographs were stained with Delafield's hematoxylin.

Several thousand cells were examined for interphase labeling and mitotic indices, in each slide. At least 75 metaphase mitoses were scored for labeling in each specimen. In the case of the spleen, large and small lymphocytes were counted together.

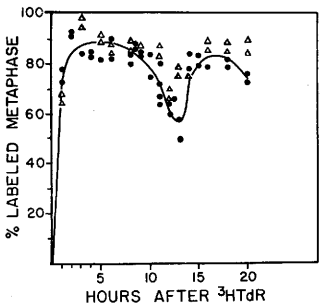

FIG. 1. Metaphase labeling curve of spleen lymphocytes from germfree (△) and conventional (●) rats.

Results. Spleen. The curve for the germfree rats is skewed and there is a more shallow trough than is seen in the conventional rats (Fig. 1). It is difficult to calculate meaningful cycle time compartments from such a curve. However, it has the general configuration of that of the conventional animals. In both cases, the peak is broad and fails to reach 100%. In addition, the curve rises sharply and at the 50% intercept of the ascending limb, the time interval for $G_2 + (M/2)$ is less than 1 hr. The ascending limb marking the beginning of the second cycle, cannot be measured at the 50% intercept since the troughs occur at 67 and 54%, respectively, in the germfree and conventional rats. However, measurement of the interval between the initiation of labeling in the first and second cycles suggests a cycle time of about 12 hr for the germfree and 13 hr for the conventional. These curves are similar to

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TABLE I. Labeling Data from Germfree and Conventional Rats.

	% Interphase labeling	% Mitosis
	Spleen lymphocytes	
Germfree	14.1	0.6
Conventional	47.3	1.9
	Ileal crypt cells	
Germfree	49.3	7.0
Conventional	50.7	7.6

those reported by Fliedner *et al.* (5) in adult rats.

One significant difference between germfree and conventional rats is that the interphase labeling index of the former two hr after ^{3}HTdR, is 14.1% and that of the latter is 47.3%. The respective mitotic indices are 0.6 and 1.9% (Table I). The cytoarchitecture of the spleens from these two different populations of rats is the same.

Ileum. The data for ileal crypt cells are similar in both groups (Fig. 2). The mitotic labeling curve in the germfree animals shows two well-defined cycles during the 20 hr period. The cycle time is about 10.5 hr when measured through the 50% intercepts of the two ascending limbs and when estimated from the initiation of labeling in the first and second cycles. The S time is about 6 hr in each cycle. Using these interval measurements on the mitotic labeling curve, the conventional animals show a cycle time of about 9.5 hr and an S time of about 6 hr. The interval for $G_2 + (M/2)$ is less than 1 hr in both groups.

The interphase labeling and mitotic indices, 2 hr after ^{3}HTdR, are 50.7 and 49.3%, and 7.6 and 7.0%, respectively, in conventional and germfree rats, Table I. The histological appearance of the ileum in both groups is the same.

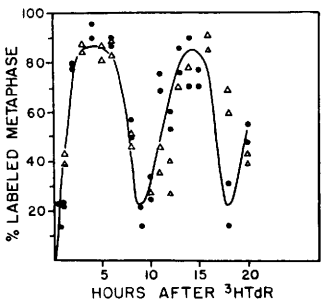

FIG. 2. Metaphase labeling curve of ileal crypt cells from germfree (△) and conventional (●) rats.

Discussion. Spleen lymphocytes. In published studies (1) on the replication of spleen lymphocytes in the 3-week-old rat, a markedly skewed mitotic labeling curve was found after 1.0 μCi ^{3}HTdR/g of body weight. It was similar to that reported earlier by Fliedner *et al.* (5) in the adult rat. Several possible explanations were presented. First, inasmuch as the evidence indicated that large and small lymphocytes replicated as separate populations, and since all mitoses from large and small lymphocytes were scored together, a skewed mitotic labeling curve could represent the resultant curve of two or more populations of lymphocytes with different generation times and component intervals (1). Second, the skewing could be due to a wide spread in the distribution of cell transit times of a single population. Third, the curve could be a result of the effect of internal radiation from the ^{3}HTdR labeling of DNA, since similar distortions have been found in the mitotic labeling curves of liver (2) and of ileal crypt cells (3) after 2 μCi/g. Fourth, the curve might represent the perturbation of the cycling pattern induced by antigenic stimulation. This could arise through a loss of asynchrony in DNA replication with variations in the flux of labeled and unlabeled cells through mitosis. This possibility was suggested because the spleen participates as a lymphoid organ in response to antigenic stimulation.

The data show that the splenic lymphocyte mitotic labeling curves of the germfree and conventional animals are similar (Fig. 1). Hence, the skewing of the curves may not be

ascribed to bacterial antigenic stimulation. However, the interphase labeling index of spleen lymphocytes in conventional rats is about three times that of the spleen lymphocytes in germfree rats. Their respective mitotic indices also show a threefold difference. These data indicate that spleen lymphoid tissue in these young rats, responds to the presence of bacterial stimulation with increased cellular replication. Olson and Wostmann (6) found greater incorporation of ^{3}HTdR into lymphocytes and plasma cells of lymph nodes of conventional mice than in germfree animals.

Thus, while the presence of antigenic stimulation does not alter the cycling pattern of spleen lymphocytes, it is associated with a larger cohort of proliferating lymphocytes. With regard to the skewed mitotic labeling curve, while the label dose of ^{3}HTdR may have altered its configuration, it seems more likely that the cause may be related to the difference between the cycle times and component intervals of large and small lymphocytes.

Ileal crypt cells. The mitotic labeling curves and the time intervals derived therefrom, are similar in both germfree and conventional rats. The slight widening of the second cycle mitotic labeling curve of the germfree rats, is not significantly different from the conventional. The labeling and mitotic indices of the crypt cells of both groups are similar. Hence, the proliferation of these cells is the same in the germfree and in the conventional animals.

Lesher, Walburg and Sacher (7) reported slight lengthening of the S times (6.5 to 8.0 hr) in duodenal crypt cells of germfree mice, compared with conventional animals. In addition they found fewer labeled crypt cells among germfree mice.

Abrams, Bauer and Sprinz (8) observed that the rate of migration of ileal crypt cells to the villus tip was more rapid in conventional mice. This was associated with a greater mucosal thickness among these animals, compared with the germfree. Matsuzawa and Wilson (9) also reported faster migration of crypt cells among conventional mice. The present study confirms their findings (9) of similar labeling and mitotic indices of crypt cells among germfree and conventional animals. Khoury, Flock and Hersh (10) showed that the introduction of the "conventional" bacterial flora into the gastrointestinal tract of germfree mice hastened the migration of crypt cells to the villus tip.

The present studies were not carried out long enough to make significant observations on the migration rate. In addition no conclusive differences between mucosal thickness in the two groups of rats could be established.

Summary. Studies were made of the replication of spleen lymphocytes and of coexistent ileal crypt cells in conventional and germfree young rats. There are no significant differences in the mitotic labeling curves of these two cell populations, among conventional and germfree rats. However, among the splenic lymphocytes of conventional rats, there are more cells engaged in replication than in the germfree animals.

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