

Occurrence of Lymphocytes within the Gut Epithelium of Normal and Neonatally Thymectomized Mice* (32974)

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The percentage of lymphocytes within the epithelium of the small intestine of mammals is high, 5–10%, and sometimes the figure is even higher¹ (12). Most of the lymphocytes within the gut epithelium, here called theliolymphocytes, are situated between the row of epithelial nuclei and the basement membrane (2,3,4). They are usually situated between the epithelial cells (5). Many authors state, however, that theliolymphocytes at the crypts of Lieberkühn are located within the epithelial cells (1,5). The more or less disintegrated nuclei, which are seen in this location, and stated to be lymphocytes, may however be something else; 100% of these “tingible bodies” are labeled 10 hours after injection of thymidine-³H.² The ultrastructure of theliolymphocytes is different from that of propria-lymphocytes; the theliolymphocytes have less endoplasmatic reticulum and are nearly lacking mitochondria (5).

The kinetics of the theliolymphocytes has been studied with the aid of thymidine-³H labeling (2,4). It is evident from Fichtelius' study in rats (4) that at least 2–3% of the theliolymphocytes synthesize DNA *in situ*. As many as 12% of the theliolymphocytes at the tip of the villi of the small intestine are less than 3 days old. These data can be best explained by the assumption that theliolymphocytes are to a large extent immigrants from the blood, and that these immigrants constitute a selection of young lymphocytes compared to the ordinary mixture of mostly old blood lymphocytes.

The question about the function of the theliolymphocytes is, of course, intimately

connected with the question of their fate. Do they just slide together with the epithelial cells towards the tips of the villi to be shed off into the lumen of the gut? Or do they reenter lamina propria after a short stay within the epithelium?

Regarding it very unlikely that the theliolymphocytes were lost in the lumen, Fichtelius advanced a theory (6) that the epithelium of the whole gut in, e.g., fishes, is a first level lymphoid organ, the epithelium having the same influence of lymphocytes and lymphoid tissue as the bursa Fabricii has in birds. The mammals may be in the process of developing a special bursa equivalent, the epithelium covering the gut-associated lymphoid tissue.

The theory was partly based on the assumption that fishes, amphibians, and reptiles did not show demonstrable gut-associated lymphoepithelial organs to which a bursa function could be ascribed (7). During a study on the occurrence of lymphocytes within the gut epithelium of vertebrates, however, looking at the tissues from a new perspective, a series of lymphoepithelial microorgans have been discovered that might be regarded as bursa equivalents³. The theory about the diffuse bursa equivalent had to be modified to include some of the lymphoid cells in the lamina propria close to the epithelium. This does not, however, make the theliolymphocytes less interesting. All new information about theliolymphocytes is considered important. Our present study deals with the occurrence of theliolymphocytes in normal mice of different ages, and with the influence of neonatal thymectomy on occurrence of theliolymphocytes.

Material and Methods. The inbred strain A mice of both sexes used were kept in a room with artificial light from 6 a.m. to 6 p.m.

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¹ Fichtelius, K. E., Finstad, J., and Good, R. A., to be published.

² Fry, R. J. M., personal communication.

³ Fichtelius, K. E., Finstad, J., and Good, R. A., to be published.

Thymectomies were performed by the technique of Sjodin *et al.* (8). In sham operations the thorax was opened but the thymus was left intact. One group of thymectomized animals and one group of sham-operated animals was sacrificed at the age of 14 days. Another pair of groups were sacrificed at 21–23 days, a third pair at 42–61 days and 2 months, respectively, and a last pair at 5 months. Each group consisted of 7–10 animals. The thymectomized animals did not show symptoms of wasting when sacrificed at the age of 14 days or 21–23 days of age. The thymectomized animals sacrificed at 42–61 days of age all showed clear signs of wasting. The last group of thymectomized animals, killed at the age of 5 months, did not show signs of wasting.

Before sacrificing the animals between 9 a.m. and noon, a blood sample was taken from the tail vein for leukocyte count and preparation of a smear. At autopsy a number of organs were saved for microscopic examination. In this article we are concerned only with the upper part of jejunum. The examination of the other organs will be described elsewhere. In the thymectomized animals the absence of mediastinal thymic tissue was verified under the dissecting microscope and by microscopic examination of the mediastinal contents. Any animals having detectable thymic tissue were discarded.

The number of lymphocytes per cubic millimeter in peripheral blood was obtained from the total leukocyte count in a Bürker chamber and the differential count of the smears. One thousand epithelial cells were counted in the sections of jejunum from every animal and the number of theliolymphocytes among these 1000 epithelial cells was recorded. Only perpendicularly sectioned epithelium of the villi was examined, and in every instance, the uttermost tips of the villi were avoided. All sections were examined without knowledge of the slide numbers by the same, specially trained technician.

The number of theliolymphocytes per 1000 epithelial nuclei is recorded in Fig. 1. This value was very low in 2-week-old mice, being about the same in sham operated and thymectomized animals (mean \pm SD 5.0 \pm 2.9 and

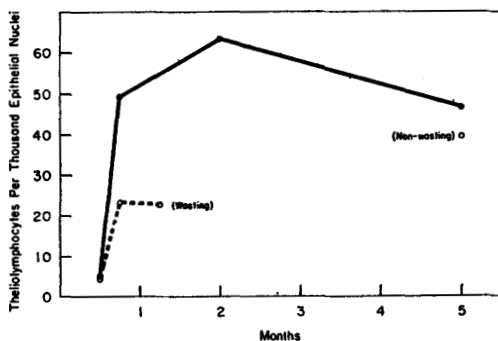


FIG. 1. The occurrence of theliolymphocytes in sham-operated and thymectomized mice. Each point represents the mean value 7–10 animals) of theliolymphocytes per 1000 epithelial nuclei in the villi of the jejunum; (○) groups of neonatally thymectomized mice of A strain at 15, 21, 42–61, and 150 days; (●) sham-operated control groups at 14, 21, 60, and 150 days of age.

4.7 \pm 2.7, respectively). In the 3-week-old sham-operated animals the number of theliolymphocytes was already 10 times higher than the number observed in the 2-week-old mice (mean \pm SD, 49.2 \pm 20.9). By contrast the increase in thymectomized animals was much less (mean \pm SD, 23.4 \pm 15.6). The difference between sham operated and thymectomized mice at 3 weeks of age fell between 14.6 and 44.2.⁴

The number of theliolymphocytes continued to increase in sham-operated animals to a mean of 63.5 \pm 22.8 per 1000 epithelial nuclei in 2-month-old mice. There was no corresponding increase in thymectomized mice (mean \pm SD, 22.8 \pm 16.2), and the difference between sham-operated 2-month-old mice and thymectomized wasting animals fell between 9.7 and 25.5.⁴

The value for 5-month-old sham-operated mice was slightly lower than for 2-month-old sham-operated animals (mean \pm SD, 46.9 \pm 18). The difference here fell between 2.0 and 71.1.⁴ This difference may indicate a beginning age involution. The 5-month-old non-wasting animals had nearly as large a number of theliolymphocytes (mean \pm SD, 39.6 \pm 17.9) as the 5-month-old sham-operated mice.

The number of blood lymphocytes in sham-

⁴ Ninety-five percent confidence intervals.

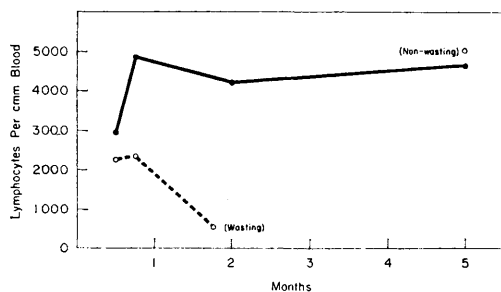


FIG. 2. The occurrence of blood lymphocytes in sham-operated and thymectomized mice. Each point represents the mean value (7-10 animals) of blood lymphocytes per mm³; (○) groups of neonatally thymectomized mice of A strain at 14, 21, 42-61, and 150 days, (●) sham-operated control groups at 14, 21, 60, and 150 days of age.

operated and thymectomized mice differed from each other in the same direction as the number of theliolymphocytes (Fig. 2). The value for 3-week-old thymectomized mice was lower than for the 3-week-old sham-operated animals (mean \pm SD, 2302 \pm 1064 and 4859 \pm 1822). The difference fell between -2746 and 3826.⁴ The value for 2-month-old thymectomized mice was very low compared to the 2-month-old sham-operated animals (mean \pm SD, 4204 \pm 1474 and 962 \pm 695). The difference fell between 1913 and 4150.⁴ By contrast to the decrease in numbers of theliolymphocytes the numbers of blood lymphocytes did not decrease in sham-operated animals between 2 and 5 months of age. The 5-month-old animals not undergoing wasting had normal blood lymphocyte counts.

Discussion. The results show that the number of theliolymphocytes is very low in 2-week-old mice, only about 0.5% and that there is a tenfold increase in number of these elements during the following week. This late occurrence of theliolymphocytes in mice is interesting with regard to the late appearance of an immune response in mice. The newborn pig, relatively mature with respect to immune response at birth, has relatively more theliolymphocytes at this time than does the mouse 2 weeks later.¹

Neonatal thymectomy causes a decrease of the number of theliolymphocytes of the gut which parallels the decrease of the number of blood lymphocytes. This finding may indicate

that the theliolymphocytes are thymus dependent in one way or another, but it also may be that the decrease in the number of theliolymphocytes is simply a function of the wasting disease in thymectomized animals whatever its cause may be.

The theliolymphocytes can, according to the original theory of Fichtelius, be compared with lymphocytes of the bursa of Fabricius. From this point of view, it is interesting to mention that thymectomy in the chicken causes a striking depletion of small lymphocytes in the bursa (9). Superficial comparison between the development of Peyer's patches in our material did not reveal any striking difference between thymectomized animals and controls at 2 and 3 weeks of age.

The primary lymphoid organs, the thymus and the bursa Fabricii, are known to involute earlier than do the other lymphoid organs. It is interesting to note that the number of theliolymphocytes decreases with age in our experiment while the number of blood lymphocytes does not undergo much change.

Summary. Lymphocytes located within the gut-epithelium of mammals, here called theliolymphocytes, are interesting but hitherto rather neglected. The kinetics of the theliolymphocytes suggests that they do not leave the organism together with the epithelial cells and that they may be part of a diffuse "primary lymphoid organ" comparable to the bursa Fabricii of birds. In this study it is shown that theliolymphocytes appear relatively late; there are as few as 0.5% in 2-week-old mice. A tenfold increase of theliolymphocytes occurs during the third week of life which might be correlated with immune responses which also appear at this time. Neonatal thymectomy causes a decrease of the number of theliolymphocytes in parallel with a decrease of the total number of blood lymphocytes. The latter finding, however, could be a consequence of wasting disease.

1. Trowell, O. A., Intern. Rev. Cytol. 7, 235 (1958).
2. Darlington, D. and Rogers, A. V., J. Anat. 100, 813 (1966).
3. Kelsall, M. A., Anat. Record 96, 391 (1946).
4. Fichtelius, K. E., Exptl. Cell Res. 49, 1 (1967).

5. Andrew, W. J., *J. Natl. Cancer Inst.* **35**, 113 (1965).

6. Fichtelius, K. E., *Exptl. Cell Res.* **46**, 231 (1967).

7. Good, R. A. and Finstad, J. in, "Germinal Centers in Immune Response," Cottier, H., Odartchenko, N., Schindler, R., and Congdon, C. C., eds., p. 4.

Springer, New York, 1967.

8. Sjodin, K., Dalmaso, A. P., Smith, J. M., and Martinez, C., *Transplantation* **1**, 521 (1963).

9. Jankovic, D. and Isokovic, K., *Inter. Arch. Allergy Appl. Immunol.* **24**, 278 (1964).

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Influence of Epinephrine upon Plasma Potassium Concentration: Changes with Time during Constant Infusion* (32975)

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Many investigators have observed that injection of epinephrine in the dog under hydrocarbon anesthesia will produce cardiac arrhythmia (1,2). D'Silva (3) showed that injection of epinephrine also increased the serum potassium concentration, $[K^+]$, and that most of the extra potassium came from the liver. O'Brien and co-workers (4) found that when the liver was isolated from the circulation, thereby preventing hyperkalemia, a significant number of animals anesthetized by hydrocarbon was protected from arrhythmia during injection of epinephrine. Subsequently, Davis *et al.* (5) reported that animals thus protected developed arrhythmia when potassium chloride was given along with the epinephrine injection. They concluded that hyperkalemia contributed to the development of cyclopropane-epinephrine ventricular tachycardia in many of the animals. These studies indicating a possible relationship between the hyperkalemic effect of epinephrine and the arrhythmia all involved only single injections of epinephrine.

Other studies have utilized continuous infusion of epinephrine to study changes in serum or plasma $[K^+]$. Many investigators (6-9) have shown that under these circumstances the hyperkalemia is only temporary,

occurring significantly in the first 1-3 min of the infusion, with $[K^+]$ falling to less than control values after 3-20 min, despite continued infusion of epinephrine.

In 1966, Vick (10) noted that animals anesthetized by chloroform and infused continuously with epinephrine for 30 min or longer often sustained arrhythmia for the entire period of the infusion. This finding was difficult to explain in terms of the hypothesis of Davis *et al.*, since it is apparent that arrhythmia continuing beyond the point where plasma $[K^+]$ returns to control levels cannot be dependent upon an elevated plasma $[K^+]$. However, this conclusion was based upon the studies of others showing that plasma $[K^+]$ was only temporarily elevated, and might not parallel the time course of the cardiac arrhythmia.

It became apparent that the available studies on serum or plasma $[K^+]$ changes with continuous epinephrine infusion were not adequate because of insufficient information about levels of epinephrine infused with respect to body weight (6); use of only one rate of infusion, which was two to three times that dose applicable to the chloroform-anesthetized animal (7-9); or results not complete enough to support a precise statement of the temporal course of the change in $[K^+]$ during continuous infusion of epinephrine (6-9).

The work reported here was undertaken to delineate the changes in plasma $[K^+]$ during

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