

## A RADIOAUTOGRAPHIC STUDY OF THE UTILIZATION OF DEOXYCYTIDINE FOR THE FORMATION OF DEOXYRIBONUCLEIC ACID-THYMINE IN LYMPHOCYTES

BUNSUKE OSOGOE and AYAKO UEKI. From the Department of Anatomy, Okayama University Medical School, Okayama, Japan. Dr. Osogoe's present address is the Department of Anatomy, Kyoto University Faculty of Medicine, Kyoto, Japan

The lymphoid tissues show marked regional differences in the pattern of incorporation of tritiated thymidine (TdR-<sup>3</sup>H) by lymphocytes (1-5). Thymic lymphocytes and germinal-center cells of lymph nodes are characterized by weak labeling with TdR-<sup>3</sup>H, whereas the large majority of lymphocytes in lymph nodes which occur outside of germinal centers are intensely labeled with TdR-<sup>3</sup>H radioautographically. The reasons for the observed differences in labeling intensity between the two types of lymphocytes are not apparent. However, it seems probable that they might be related to the differences in the pathways of formation of DNA-thymine. Sugino et al. (6) have demonstrated by chemical methods that, in the thymus, deoxycytidine (CdR) which is circulating in a considerable amount (7) is utilized for the formation of DNA-thymine. In this study, we have attempted to gain further information on the above-noted utilization of CdR by lymphocytes by means of radioautographic techniques.

### MATERIAL AND METHODS

18 male mice of a subline of the DBA strain, weighing around 25 g, were divided into three groups: five were injected intraperitoneally with TdR-<sup>3</sup>H alone; five with TdR-<sup>3</sup>H together with unlabeled CdR; and eight with CdR-<sup>3</sup>H alone. The specific activity of TdR-<sup>3</sup>H was 5 Ci/mmol, and that of CdR-<sup>3</sup>H 500  $\mu$ Ci/mmol. Both TdR-<sup>3</sup>H and CdR-<sup>3</sup>H were obtained from Radiochemical Center, Amersham, England, and unlabeled CdR from Sigma Chemical Company, St. Louis, Mo. Since the concentration of circulating CdR in rodents is estimated to be as high

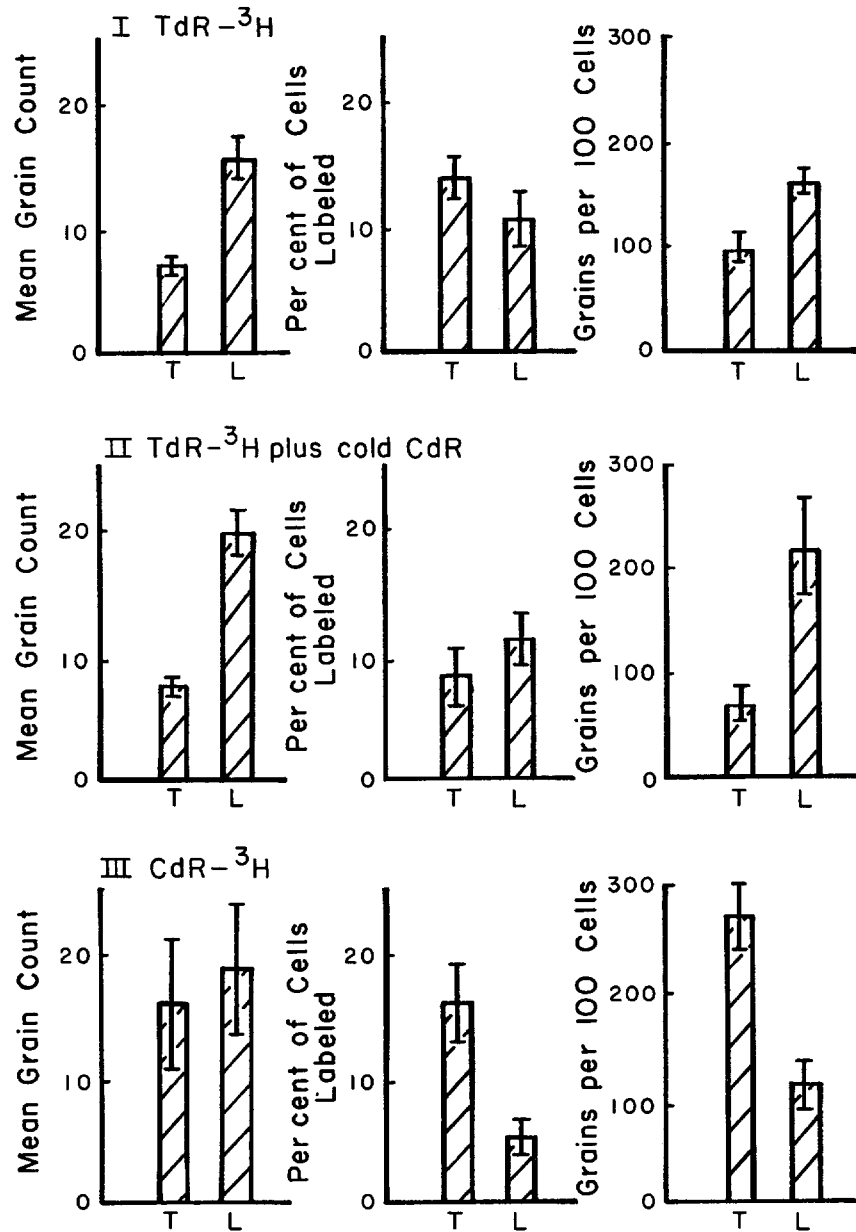
as 10  $\mu$ g/ml (7), CdR-<sup>3</sup>H was injected in a single dose of as much as 400  $\mu$ Ci/mouse (or in a concentration of about 13  $\mu$ g/ml if the total volume of free water is assumed to be 15 ml/mouse). Corresponding to such a high dose of CdR-<sup>3</sup>H, TdR-<sup>3</sup>H was given in a single dose of 200  $\mu$ Ci/mouse. The dose of unlabeled CdR given together with TdR-<sup>3</sup>H was 0.5 mg/mouse. This dose is about 50 times as great as that of TdR-<sup>3</sup>H.

Animals were sacrificed 24 hr after the injection, and smears were made from the thymus and mesenteric lymph nodes, so that each slide had two smears, one from the former and the other from the latter. After fixing in Carnoy's fluid for 8 min, smeared samples were prepared for radioautography, using NR-M3 dipping film (Konishi-roku Shashin-kogyo Co., Tokyo, Japan), and were exposed for 14 days at 4°C. For examination of radioautograms, at least 1000 lymphocytes were counted in each smear and the mean number of grains of all labeled cells (mean grain count), the percentage of cells labeled, and the number of grains per 100 cells (mean grain count  $\times$  percentage of cells labeled) were determined. Cells with less than three grains were not counted as labeled.

### RESULTS AND DISCUSSION

The results are illustrated in Fig. 1.

In the first group of mice that received TdR-<sup>3</sup>H alone, the mean number of grains per cell was considerably less in thymic lymphocytes than in lymph node lymphocytes. Consequently, the number of grains per 100 cells, a more reliable measure of labeling intensity, was significantly smaller in thymic lymphocytes than in lymph-node



T: Lymphocytes in thymus  
 L: Lymphocytes in mesenteric lymph nodes

FIGURE 1 Diagrams illustrating the patterns of labeling in lymphocytes 24 hr after a single intraperitoneal injection into mice of (I) TdR-<sup>3</sup>H alone; (II) TdR-<sup>3</sup>H together with cold CdR; and (III) CdR-<sup>3</sup>H alone. The injected doses of TdR-<sup>3</sup>H, CdR-<sup>3</sup>H, and cold CdR are given in the text. Each column in the diagrams represents mean  $\pm$  standard error of the values from five or eight mice.

lymphocytes, although labeled cells occurred more numerously in the former than in the latter.

In the second group of mice that was injected with TdR-<sup>3</sup>H together with unlabeled CdR, the labeling intensity of thymic lymphocytes was reduced to a remarkable extent, whereas the effect on lymph-node lymphocytes was not conspicuous. These findings reflect dilutions of injected TdR-<sup>3</sup>H by unlabeled CdR in thymic lymphocytes, but not in lymph-node lymphocytes.

In the third group of mice that received CdR-<sup>3</sup>H alone, not only the percentage of cells labeled but also the number of grains per 100 cells was strikingly greater in thymic lymphocytes than in lymph-node lymphocytes. The finding is in sharp contrast to the pattern of incorporation of TdR-<sup>3</sup>H by lymphocytes. It is interesting to note here that no significant difference in the mean number of grains per cell was observed between the two types of lymphocytes. This may be caused by the presence of germinal-center cells and other lymph-node lymphocytes which are similar to thymocytes in their utilization of CdR-<sup>3</sup>H.

The above-mentioned results indicate that, in thymic lymphocytes, CdR which is circulating in a considerable amount (7) is utilized for the formation of DNA-thymine and hence the thymic lymphocytes are weakly labeled with injected TdR-<sup>3</sup>H, whereas such a utilization of CdR is not the case in the majority of lymph-node lymphocytes and hence they are intensely labeled with injected TdR-<sup>3</sup>H radioautographically.

For the biosynthesis of thymidine nucleotides, two possible pathways have been considered (8), namely: *Pathway I*: uridine monophosphate → deoxyuridine monophosphate → thymidine monophosphate; and *Pathway II*: uridine monophosphate → cytidine monophosphate → deoxycytidine monophosphate → deoxyuridine monophosphate → thymidine monophosphate.

In *Pathway II*, an enzyme which is involved in the deamination of deoxycytidine monophosphate to deoxyuridine monophosphate (deoxycytidylate deaminase) is expected to play an important role (8–10). It has been reported by Sugino et al. (6) that a high specific activity of this enzyme is found in the thymus, but not in the lymph node (6). This might be related to the observed differences in utilization of CdR between thymic and lymph-node lymphocytes.

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