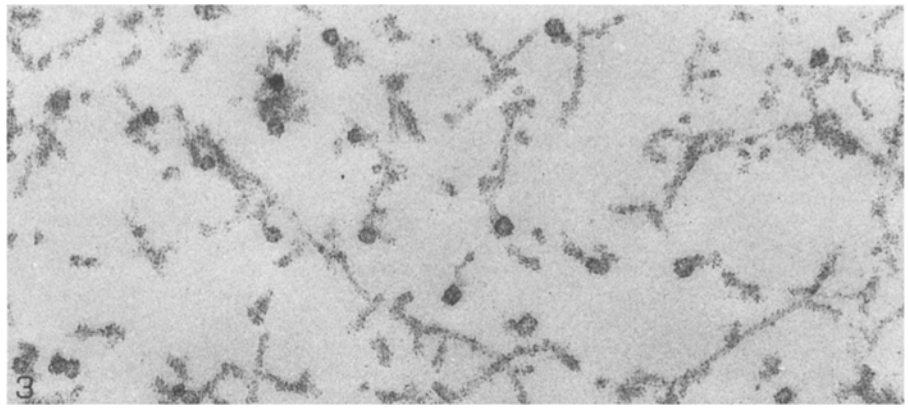

Fig. 3. A higher magnification of an area of the cytoplasm permits to see clearly the wall and the lumen of microtubuli. × 62,500.

ation only in Ringer containing 1% OsO_4, and c) fixation with a mixture of glutaraldehyde and formaldehyde followed by OsO_4. With all fixatives, microtubules were not observed in the cytoplasm of the electrocyte, although they were found in the axon of nerves localized near the posterior face of the electrocyte.

During studies carried out in our laboratory related with cytochemical localization of surface components of the electrocyte[5], microtubules were observed when alcian blue and lanthanum nitrate were used (figures 2, 3). The microtubules have a diameter of 40 nm, being composed by a wall 10 nm thick and a central clear zone with a diameter of 20 nm. The microtubules are wider than those observed in other cells. However, it is possible that alcian blue and/or lanthanum nitrate, interacting with the outer surface of the microtubules, precipitate some substances, thus increasing their diameter. The microtubules were observed in all the cytoplasm of the electrocyte, appearing more evident at the cell periphery. This fact can be related to the tissue penetration of the reagents used.

Alcian blue, lanthanum hydroxyde and ruthenium red have been used by several authors to stain microtubules in axons[8–11]. However, in the axons microtubules can be observed in usual preparations. These substances have been used in cytochemistry to detect acid mucopolysaccharides, and normally they do not penetrate through the membrane. The observation of microtubules inside the cell is probably a consequence of lesions of the cell membrane during specimen preparation, thus facilitating the penetration of the reagents used. It is possible that the visualization of microtubules by these substances results from their interaction with mucopolysaccharides of the microtubule wall.

The microfilaments also seem to react with alcian blue/lanthanum nitrate (figure 3). Similar results were observed in crayfish ventral axons[9]. Microtubules have been considered as a structure which may play a role in the transport of substances through the cell, maintenance of the form of the cell, etc.[12,13]. It is possible that in the electrocyte they are involved, in association with the network of filaments, in the maintenance of the form of the electrocyte.

Our results, associated with those described by Burton and Fernandez[8], Hinkley[9] and Lane and Treherne[10], show that substances used in cytochemistry to detect acid mucopolysaccharides may be useful to visualize microtubules.

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Differences in utilization of tritiated thymidine and tritiated deoxycytidine by rat lymph node cells

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Summary. Large lymphoid cells and plasma cells in antigenically stimulated rat lymph nodes retain less label after injection of ^{3}H-CdR than after injection of ^{3}H-TdR. There is no difference amongst small lymphocytes. The data are consistent with a defect in the utilization of ^{3}H-CdR in the late stages of B cell maturation in the rat.

This work started as a study of the migration of cells from lymphoid germinal centers. This has not been possible by classical autoradiographic methods because tritiated thymidine (^{3}H-TdR) labels deoxyribonucleic acid (DNA) in germinal center cells very weakly in comparison to cells outside germinal centers[1–3]. The report that tritiated deoxycytidine (^{3}H-CdR) provided intense labeling of germinal center cells in rats[4] raised the possibility that this DNA

precursor could be used to follow germinal center cells moving to other parts of lymph nodes.

Methods. 5-week-old female Sprague-Dawley albino rats (Simonson Labs, Gilroy, CA.) fed on Purina Rat Chow and tap water ad libitum were injected in each hind footpad with 50 µg of polymerized flagellin of proven antigenicity made from *Salmonella adelaide* by the method of Ada et al.[5].

For labeling, either ^{3}H-CdR, 5.0 Ci/mmole, or ^{3}H-TdR, 6.0 Ci/mmole, (Schwarz-Mann, Orangeburg, N.Y.) was injected twice into each hind footpad in doses of 25 µCi with an interval of 6 h between injections. For morphologic studies 2 rats were injected 1, 2, 3, 4, 5, 6, or 9 days after the flagellin and were killed 30 min after the 2nd injection. For kinetic studies, the nucleosides were injected on either the 1st or 4th day after the antigen, and pairs of rats were killed after intervals from 30 min to 10 days.

One half of each popliteal lymph node was fixed in formalin and processed as sections for autoradiography. For kinetic studies, the other half of each popliteal lymph node was teased into a single cell suspension, smeared, and fixed in 1% acetic acid-80% methanol. Autoradiographs were prepared using Kodak NTB 3 nuclear emulsion. Smears were stained with Giemsa and sections with methyl green-pyronin.

The autoradiographs of the smears were counted for: a) differential count of 1000 cells, classifying cells as small lymphocytes, large lymphoid cells or plasma cells, b) percent of each cell type labeled, and c) the grain counts of the labeled cells[6]. The data are expressed as mean and SD of the values from the 4 individual popliteal nodes from 2 identically treated rats.

Autoradiographs of sections were studied for location and degree of labeling of cells in germinal centers, diffuse cortex (thymus-dependent areas), and plasma cell areas such as medullary cords[7].

Results. The distribution of labeled cells in popliteal nodes immediately after injection of ^{3}H-CdR was the same as after ^{3}H-TdR. Cells in germinal centers had equivalent degrees of labeling, but the cells of the medulla and diffuse cortex were less heavily labeled by ^{3}H-CdR than by ^{3}H-TdR. However, this appearance was reversed within 1 day and thereafter germinal center cells in rats receiving ^{3}H-CdR were very lightly labeled relative to cells elsewhere.

The percent of cells labeled and the mean grain counts of cells on smears made from the popliteal lymph nodes of rats receiving the 2 nucleosides on the 1st day after the antigen were the same, although large lymphoid cells and plasma cells retained slightly more label 6 and 10 days after injection of ^{3}H-TdR than of ^{3}H-CdR.

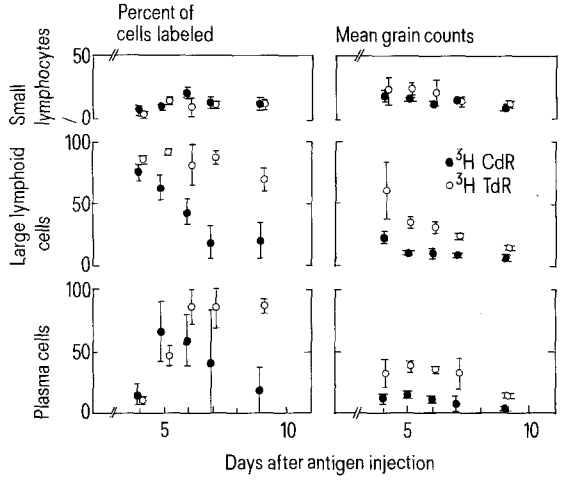
In contrast, when the nucleosides were given on the 4th day after the antigen, when germinal centers were first seen in sections, the large lymphoid cells and plasma cells consistently incorporated and retained more ^{3}H-TdR than ^{3}H-CdR (figure). The proportion of the cells initially labeled did not differ, but the percent of labeled large lymphoid cells aod labeled plasma cells decreased more rapidly after incorporation of ^{3}H-CdR than after incorporation of ^{3}H-TdR.

The percent of small lymphocytes labeled, and the mean grain counts of small lymphocytes were the same after injection of either nucleoside, and no difference appeared with time after injection.

Discussion. These studies confirm the morphologic observations of Osogoe et al.[4] that ^{3}H-CdR provides the appearance of relatively more intensive labeling of germinal center cells than does ^{3}H-TdR. However, the initial difference in patterns of labeling did not allow study of germinal center cells per se because label disappeared too rapidly from cells incorporating ^{3}H-CdR.

The kinetic data provide more information about the differences in behavior of cells labeled by ^{3}H-CdR or ^{3}H-TdR. There was little difference when the nucleosides are given on the 1st day of a primary immune response. However, on the 4th day of a primary response ^{3}H-TdR produces higher grain counts in large lymphoid cells than the same dose of ^{3}H-CdR. This increased efficiency of incorporation of ^{3}H-TdR is only reflected in mature plasma cells. Small lymphocytes do not show differences in either the proportion labeled or in their grain counts. Since most of the small lymphocytes in rat nodes are T cells, the decreased ability of large lymphocytes and plasma cells in rats to use ^{3}H-CdR represents a defect in the late stages of B cell development.

In studies of rat lymphoid cells labeled after multiple injections of ^{3}H-CdR and ^{3}H-TdR, Amano and Everett[8] found differences in incorporation which they believed related primarily to the life spans of the cells. We believe that our results are consistent with theirs, but that the differences are more directly related to differences in rats between T cells and late stage B cells.



Graph showing the mean and SD of the values for percent of each cell type labeled and for mean grain count of each cell type in 4 popliteal nodes of rats injected with either nucleoside on the 4th day after antigenic stimulus. The values for both percent of cells labeled and for mean grain counts are on the same scale at the left. The percent of large lymphocytes and plasma cells, and the mean grain counts of these cells were smaller after injection of ^{3}H-CdR than after injection of ^{3}H-TdR and there is less retention of label with time in these cells. This difference is not seen in small lymphocytes.

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