

tions with 1 g/100 ml albumin. When lipolysis was activated with 10^{-5} M NE, a marked acidosis (final pH = 6.6) occurred in the medium of low buffer capacity and the lipolytic activity was significantly inhibited as compared to lipolysis in the medium with normal buffer capacity. This inhibition was partially reversed when theophylline 10^{-2} M was added. When fat cells were incubated in these two media, but with glucose present or an albumin concentration of 5 g/100 ml, lipolysis proceeded at a similar rate independently of the buffer capacity of the medium.

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Significance of Increased Alkaline Phosphatase Activity in Viral-Induced Thymic Lymphoma.* (32226)

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Murine thymic lymphoma cells have been shown to have a high concentration of alkaline phosphatase enzymatic activity, whereas normal thymic lymphocytes have none(1,2). This finding is of interest because of the possibility that it might represent a specific metabolic alteration related to the neoplastic change. Further, the histochemical alkaline

phosphatase staining of these cells might offer a way to identify positively these neoplastic cells in tissue sections for study of cell proliferation kinetics(2). Smith, studying the progression of leukemogenesis in irradiated C57BL mice, noted a positive correlation between the regions of phosphatase activity and the development of lymphoma in the thymuses, and suggested that the phosphatase activity was related to the lymphoma(1). Metcalf *et al* correlated alkaline phosphatase activity in AKR leukemogenesis, and found that the phosphatase levels

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were usually elevated in cases of thymic lymphoma, although the levels in individual mice were highly variable(2). Most recently, Lagerlöf and Kaplan, reporting on radiation-induced and viral-induced leukemogenesis in C57BL mice, have reported that the alkaline phosphatase activity has appeared concomitantly with the first morphological signs of tumor development, although the correlation was not perfect(3).

To determine whether the phosphatase activity observed in the thymic lymphoma of mice is an integral part of the actual neoplastic process, concerned with fundamental metabolic alteration, or whether it represents merely a secondary change or attribute of lymphoma tissue, it is necessary to determine at what point in the neoplastic process the increased alkaline phosphatase activity appears. If the phosphatase increased before or concurrent with the appearance of overt thymic lymphoma, then this enzymatic alteration may form part of the neoplastic process. On the other hand, if phosphatase activity appears only after the tumor is already well established, then the enzymatic alteration may be regarded as a secondary change, resulting from, rather than being part of, the causal sequence in thymic neoplasia. Accordingly, this paper reports on the temporal relation between the appearance of the lymphoma and the appearance of alkaline phosphatase activity in the affected tissues.

Materials and methods. A murine leukemia virus (Rich)(4) was employed as previously described(5). Newborn Ha/ICR Swiss mice random-bred in our colony were inoculated intraperitoneally with 0.1 ml of undiluted virus stock. The animals were chosen at random for sacrifice between 52 and 145 days after inoculation. Previous studies had established that most animals sacrificed before this time would show no histologic changes, whereas most animals killed after this time would show only far-advanced lymphoid leukemia(5). Uninoculated animals were provided for a staining control. At autopsy, the right thymus, left thymus, and spleen were separately weighed and fixed in cold acetone at 4°C for 24 hours. Following paraffin embedding, 4 micron alternate serial sections

were stained for alkaline phosphatase by Gomori's azo dye method(6) and with a hematoxylin-eosin stain. The histologic appearance of the thymuses stained with hematoxylin-eosin was evaluated by criteria developed previously(5), and given briefly below. On the basis of the histology and the individual thymic weights, animals were placed into one of 4 arbitrary groups:

1) *No gross or histologic change from normal.* In this group were placed animals which showed no pathologic changes in the thymuses.

2) *Unilateral thymus depletion and lymphoma-in situ.* Mice which had one thymus (right or left) which showed more than a 30% weight loss relative to the opposite thymus were placed in this category. In addition, mice which had a thymus showing histologic changes indicative of malignancy but in which the neoplastic-appearing cells had not yet proliferated sufficiently to enlarge that thymus at least 3 times compared to the opposite thymus, were also placed in this category.

3) *Unilateral lymphoma.* Criteria for overt unilateral lymphoma included histologic replacement of the entire thymus by large cells of uniform appearance resembling primitive lymphoblasts and at least a 3× increase in weight of the tumor bearing thymus compared to the opposite thymus. Cases in which the thymic cells histologically appeared neoplastic, but in which these cells had not yet proliferated sufficiently to enlarge that thymus at least 3× were designated "lymphoma-in situ" and placed in Group 2, above.

4) *Disseminated lymphoma.* Animals were placed in this group when, in addition to tumor in at least one thymus, tumor was also present in the spleen and other organs.

Alkaline phosphatase-stained tissue sections adjacent to those stained with hematoxylin-eosin were examined and the thymic cells were graded positive or negative. In all cases, each AP-stained section was considered satisfactory only if the luminal border of the endothelial cells of the thymic arterioles stained densely black, providing an internal positive stain control (Fig. 2).

Results and discussion. The conventional

TABLE I

Histologic group	No. of animals	No. with positive thymic alkaline phosphatase
1. No change from normal	7	0
2. Unilateral depletion and lymphoma <i>in situ</i>	19	0
3. Unilateral lymphoma	4	0
4. Disseminated lymphoma	7	5

histologic appearance of the hematoxylin-eosin stained tissues was correlated with the appearance of the staining for alkaline phosphatase, and the results are given in Table I. Thymuses which showed no changes by conventional criteria (Group 1) were uniformly negative for AP-staining, as were the thymuses of 5 uninoculated control mice. No alkaline phosphatase staining was found in the thymic cells of animals in Group 2, representing the nascent lymphomas, nor in Group 3, representing early but definite lymphoma (Fig. 1, 2). Of the 7 cases of disseminated lymphoma (Group 4), 5 cases stained for alkaline phosphatase (Fig. 3), and 2 did not.

Of critical importance in this study was the temporal relation of the thymic alkaline phosphatase staining to the appearance of overt lymphoma judged by examination of hematoxylin-eosin sections and by thymic weight. Animals in Group 3 had both sub-

jective evidence of lymphoma development, based on histologic examination (Fig. 1), and objective evidence of lymphoma in that the tumor-bearing thymus had already enlarged to more than $3\times$ the weight of the opposite, tumor-free thymus. Fig. 1 and 2 illustrate a representative specimen of a 63 mg lymphomatous left thymus from an animal which had a 19 mg tumor-free right thymus. Fig. 1, stained with hematoxylin-eosin, illustrates the uniform appearance of the lymphoma cells. Fig. 2, an adjacent section stained for alkaline phosphatase, shows that these tumor cells are free of alkaline phosphatase activity. Proof that the tissue preparation and staining technique are satisfactory for this slide is given by the positive alkaline phosphatase staining of the intima of a capsular arteriole (Fig. 2, arrow). The majority of cases of disseminated lymphoma (Group 4) had positive alkaline phosphatase staining of the lymphoma cells, both in the thymuses and in

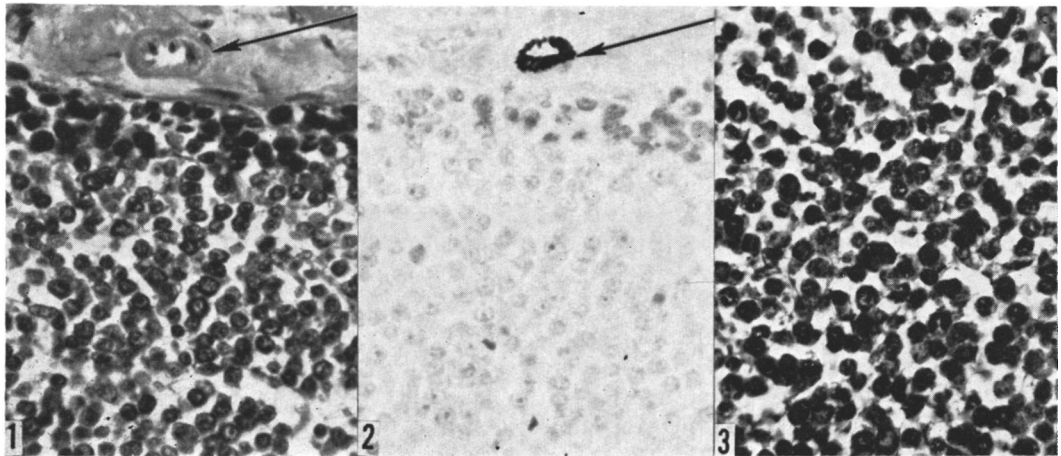


FIG. 1. 63 mg left thymus showing cortex filled with lymphoma cells. Arrow indicates capsular arteriole, for comparison with FIG. 2. Right thymus of this animal, not illustrated, weighed 19 mg, and was histologically normal. (400 \times). H & E stain.

FIG. 2. Section adjacent to that of FIG. 1, stained with Gomori's azo dye method for alkaline phosphatase. Note that whereas the intima of the capsular arteriole stains densely black, (arrow), indicating that the alkaline phosphatase stain is effective, the tumor cells are negative. (400 \times).

FIG. 3. Section of far-advanced, thymic lymphoma mass in which tumor cells stained positive for alkaline phosphatase. (400 \times).

the other organs (Table I). Fig. 3 illustrates the positive staining of thymic lymphoma cells from a representative far advanced lymphomatous thymus.

Although difficult to illustrate by photographic means, under direct examination with the microscope the stain precipitate appeared in the lymphoma cells as a very fine black particulate deposition localized at the cytoplasmic borders and in occasional tumor cells at the nuclear membrane as well (Fig. 3).

The data in the present study indicate that the increase in alkaline phosphatase activity observed in thymic lymphoma arises only *after* definite lymphoma cells are present in one thymus and does not even appear until these tumor cells have already proliferated sufficiently to enlarge that thymus at least $3\times$ in weight. These data, together with the variability in staining response reported by Metcalf *et al.*, and confirmed in this study (Group 4 animals), suggest that the increased level of alkaline phosphatase in lymphoma tissue is a consequence rather than a cause of the neoplasia. This interpretation, based on histochemical evidence, is compatible with that reached by Metcalf *et al.*, who concluded that the "alkaline phosphatase levels became elevated in mouse lymphocytes only after the cells become frankly neoplastic". The data

from the present study differ from those reported by Lagerlöf and Kaplan(3), who found that the alkaline phosphatase appeared concomitantly with the first morphological signs of tumor development. Possibly differences in the staining techniques, in the host-virus system used, and in histologic interpretation, may explain the lack of agreement of results.

Summary. Mice inoculated with a leukemogenic virus (Rich) were studied to determine when alkaline phosphatase appears in thymic cells relative to the developing thymic lymphoma. The findings indicated that the enzyme increase occurs only after the tumor is definitely established, hence this change cannot be causally related to the neoplasm.

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Effect of Age and Species on Sensitivity of Lymphocytes to Prednisolone and Phytohemagglutinin.* (32227)

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Prednisolone has been shown to have a delayed cytotoxic effect on blood and splenic lymphocytes of rats, mice and rabbits. In contrast, lymphocytes from the blood and spleen of men and guinea pigs were found to be relatively resistant to the hormone(1). Similarly the blood lymphocytes of man and lower animals differed in sensitivity to phy-

tohemagglutinin(2). As these reagents produce interesting differential biologic effects, we studied the effects of the reagents on lymphocytes from the thymus, spleen and appendix of neonatal, young and adult rabbits and from the thymus of neonatal man, rat and mouse. Of particular interest was the finding that thymocytes of neonatal rabbits are resistant to prednisolone.

Methods. The tissues used were the thymus,

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