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A Cytoenzymatic Study on Murine Lymphoblastic Lymphatic Leukaemias

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SUMMARY

A comparative study was made of the activities of lactate and succinate dehydrogenases in axillary lymph node, thymus, spleen and liver in normal and two types of virally induced leukaemic mice. The results clearly indicate an increased activity of both the enzymes studied in the leukaemic lymph node and thymus. Slight increase was also noted in spleen of leukaemic host, but no difference could be detected between normal and leukaemic condition in liver cells. The increase in enzyme activities are indicative of enhanced rates of glycolysis and oxidative metabolism as a consequence of higher cell proliferation demanding supply of extra energy.

INTRODUCTION

Many biochemical changes observed in tumors have been attributed to alterations in mechanisms of cellular control.[1] Localization of enzymes at the cellular and sub-cellular levels using histo- and cytochemical methods have provided information concerning intermediary metabolism which formed the basis for further biochemical investigation. The present study is also an attempt to proceed along this direction.

Lactate dehydrogenase, an enzyme of the glycolytic system is responsible for the effective conversion of pyruvate to lactate reversibly. The relationship between the enzyme and neoplasia has been studied from time to time and alterations, mostly in the form of increased activity of this enzyme in serum, plasma and different tissues in neoplastic condition have been reported[2-8].

Succinate dehydrogenase occupies a key position in the Krebs cycle because of its important link in the chain of enzymes that are responsible for biological oxidation. It reversibly converts succinate to fumerate in the cycle and has been shown to be correlated with sites of active cellular function, including growth[9]. Succinate dehydrogenase activity has been found to vary considerably under malignant condition. Some workers[10-16] observed that the activity was low or totally absent in malignancy, others[17-19] found

higher activity, while still, others[20-22] reported an irregular behavior of this enzyme. Reports on this enzyme in leukaemic tissues are considerably few. Kazanova and Terent'eva[23] studied the activity of succinate dehydrogenase in cells of circulating blood and bone marrow cells of human leukaemic patients and concluded that the increased or decreased activities of the enzyme seemed to depend on the form, state and degree of severity of the disease.

Previous reports by Mitra and his colleagues[24-25] dealt with these enzymes in some tissues of mice bearing ascitic Schwartz leukaemia. The present report furnishes our findings on lactate and succinate dehydrogenase activities in axillary lymph node, thymus, spleen and liver of host bearing Schwartz and Moloney leukasmia as compared to their normal counterparts.

MATERIALS AND METHODS

Schwartz and Moloney lymphoblastic leukaemia are being maintained in our department in Swiss and Strain A mice respectively by serial passage of leukaemic tissue filtrates. Both these leukaemic strains are established lymphoblastic leukaemia, characterized by massive enlargement of the spleen, thymus, lymph nodes, mesenteric glands, liver and in very advanced stages — kidney also, due to extensive proliferation of lymphoblast cells which almost completely replaces and obliterates the normal structure of these organs.

In the present study, imprint smears which yield thin uniform cell layers of the thymus, lymph node, spleen and liver of normal and leukaemic mice were prepared quickly after dissecting out the organs and very briefly fixed in chilled 60% acetone in distilled water. The slides were then washed in distilled water and incubated at 37°C for 30 minutes and 1 hour in the substrate mixtures for lactate and succinate dehydrogenases. Activities of lactate and succinate dehydrogenase were localized by histochemical means using Nitro-BT as the electron acceptor[26-27]. The specificity of the staining reaction was determined by comparing these with slides incubated without the substrate or Nitro-BT and also incubating smear slides which were subjected to 60°C.

The reaction products of these enzymes consisted of blue formazan granules at the site of activity.

Rating Method:

The enzyme activity was analysed by subjective grading according to the intensity of the coloured products of the reaction and were assigned scores of 1+(very weak) to 4+(strong). About 200–500 lymphoblastic cells were scanned before an average grading was done. In case of liver large number of parenchyma cells were present in the smear and they were also taken into account while grading.

RESULTS AND DISCUSSION

The spectrum of lactate and succinate dehydrigenase activities in the tissues studied are shown in Tables 1 & 2. The activities of both lactate and succinate dehydrogenases, as expressed by the staining intensity, were more or less similar. However, the succinate dehydrogenase reaction seemed to produce slightly coarser granules than lactate dehydrogenase. The localization of both the enzymes in the form of either very fine or slightly coarser blue formazan granules were restricted to the cytoplasm, sometimes being more prominent in particular region of a cell, surrounding a clear unstained nucleus at the centre.

Table 1. *Lactate Dehydrogenase Activity in Some Tissues of Normal and Leukaemic Mice*

Tissues	Normal Swiss Mice	Schwartz Leukaemia Bearing Swiss Mice	Normal Strain A Mice	Molony Leukaemia Bearing Strain A Mice
Lymph Node	1+	3+	1+	1+
Thymus	2+	3+ to 4+	2+	3+ to 4+
Spleen	2+	3+ to 4+	2+	2+ to 3+
Liver	2+ to 4+	2+ to 4+	2+ to 4+	2+ to 4+

Table 2. *Succinate Dehydogenase Activity in Some Tissues of Normal and Leukaemic Mice*

Tissues	Normal Swiss Mice	Schwartz Leukaemia Bearing Swiss Mice	Normal Strain A Mice	Molony Leukaemia Bearing Strain A Mice
Lymph Node	1+	2+ to 3+	1+	3+
Thymus	2+	3+	2+	2+ to 3+
Spleen*	2+	2+ to 3+	2+	2+ to 3+
Liver	2+ to 4+	2+ to 4+	2+ to 4+	2+ to 4+

* In both types of leukaemic spleen, occasional blast cells were found to be very strongly positive for both dehydrogenases (greater than 4+).

The highest activity of both the enzymes studied were found in the liver parenchyma cells and the staining intensity was more or less the same in both non-leukaemic and leukaemic condition (Fig. 1). Apart from these, the other cells visible had a much lower activity. The lowest activity observed was in the normal lymph node cells. Generally, the activities of both lactate and succinate dehydrogenase were comparatively higher in the leukaemic subjects studied (Fig. 2, 3).

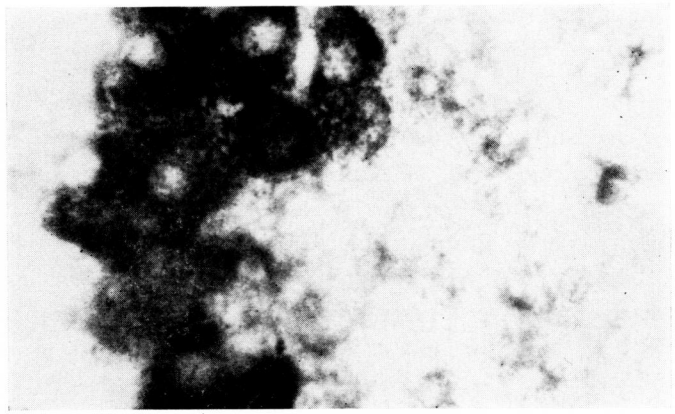

Fig. 1. Succinate dehydrogenase activity in normal mouse liver parenchyma cells, showing strong reaction. (× 400).

(a)

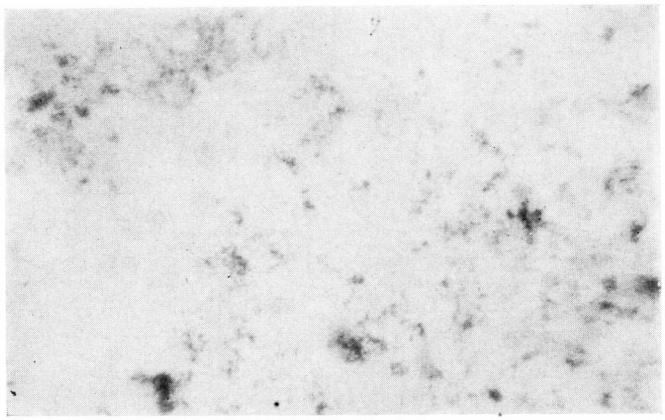

Fig. 2 (a). Localization of succinate dehydrogenase activity in imprint smear of normal mouse thymus showing weak activity. (× 400).

(b)

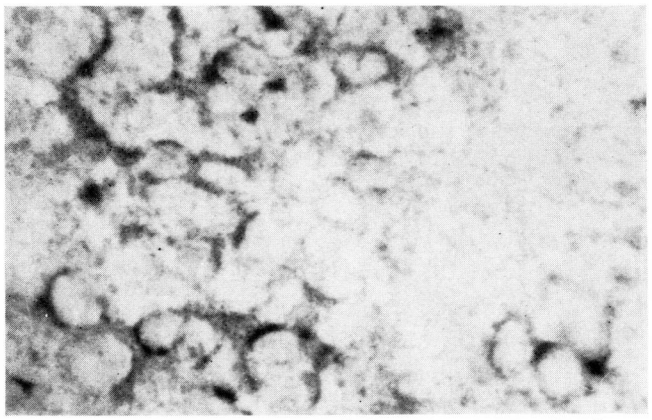

Fig. 2 (b). Increased activities of the same enzyme in thymus of leukaemic mouse. (× 400).

(a)

Fig. 3 (a). Lactate dehydrogenase activity in imprint smear of nomal thymus. (× 400).

(b)

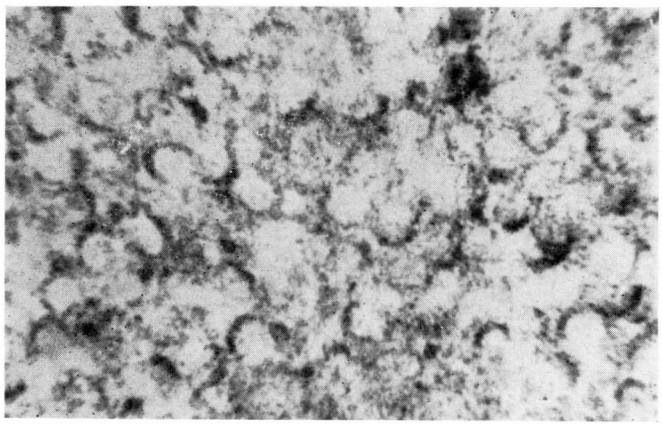

Fig. 3 (b). The same as 3 (a) in leukaemic condition showing enhancement of activity. (× 400).

Reports in literature usually point towards an increased activity of lactate dehydrogenase in malignant condition and it has been suggested that the high activities observed probably reflect increased glycolytic function[28-31]. Bierman and co-workers[32] had earlier provided evidences to show the conneciton between increased lactate dehydrogenase activity and the growth processes.

Observations on succinate dehydrogenase activities in malignant condition, however, seem to be rather contradictory[10-22]. Schwartz and co-workers[33] believe that such differences probably are dependent on many factors including cytological character of the tumour as well as their state of differentiation. Das *et al.*[34] also revealed an association of high succinate dehydrogenase with high rates of cellular growth. Mitra and co-workers[24-25], in a previous report, demonstrated increased activities of these two dehydro-

genases in ascitic Schwartz leukaemic tumour cells, as well as in various organs of the host bearing the tumour, as against their normal counterparts.

In the present investigation on Schwartz and Molony leukaemia we have encountered increased activity of the enzymes concerned in all the lymphatic tissues under leukaemic condition, but there seemed to be no demonstrable difference in the liver of leukaemic and normal mice. Studies on the influence of tumour growth (hepatoma) on the activities of these two enzymes show a decrease in succinate dehydrogenase activity[35] with no change in the activity of lactate dehydrogenase.

As in most malignancies, in leukaemia also, the cells concerned (blast cells in the present case) proliferate profusely at various sites of the body

(a)

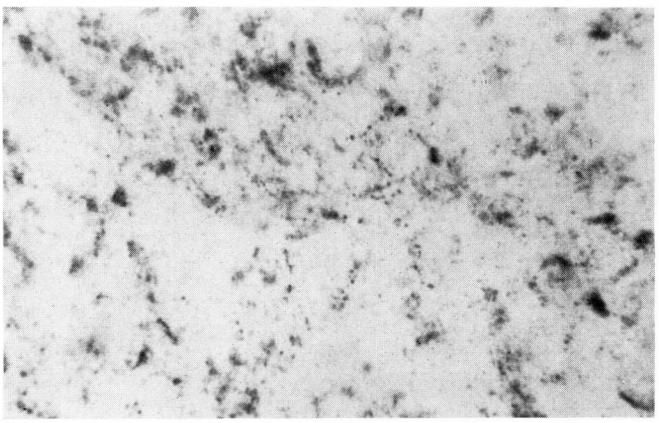

Fig. 4 (a). Succinate dehydrogenase activity in imprint smear of normal spleen. (× 400).

(b)

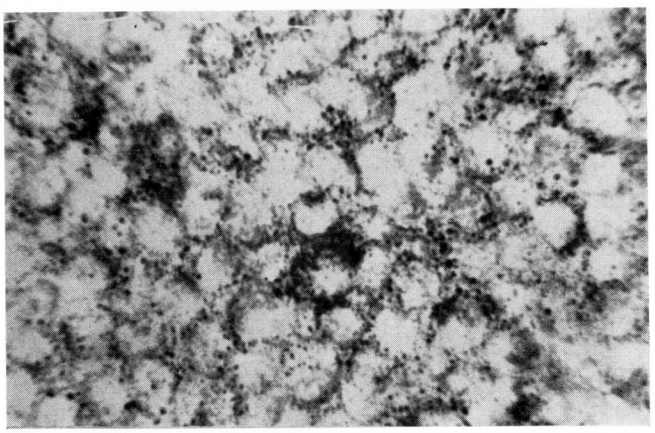

Fig. 4 (b). Same as 4 (a) in leukaemic spleen, showing enhanced activity. × 400).

of the host. It should seem natural to suspect that to cope with such abnormal and enhanced rate of cell proliferation, there will be greater demand for energy. In order to be able to supply this demand there would be an obvious rise in metabolic activity of the cells which would eventually be reflected as increase in the activity of the enzymes concerned. Our present observation seems to fall in line with such speculation.

Since succinate dehydrogenase is one of the key enzymes of the Krebs cycle and lactate dehydrogenase of the glycolytic pathway, their enhanced activity, as seem here, evidently points towards the increased respiratory capacity of the cells which makes possible the availability of extra energy for the proliferating cell population.

(a)

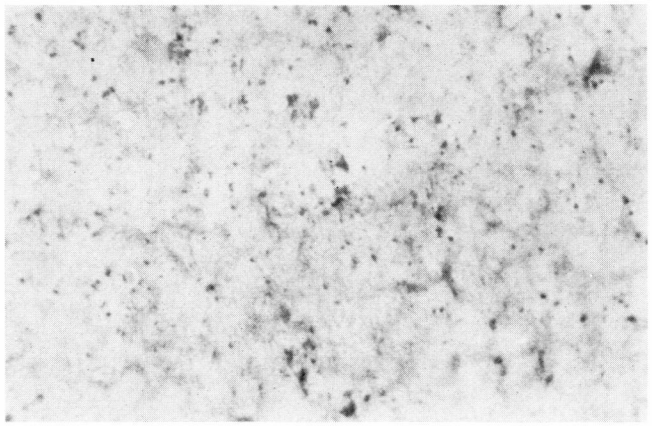

Fig. 5 (a). Succinate dehydrogenase activity in imprint smear of normal thymus. (× 400).

(b)

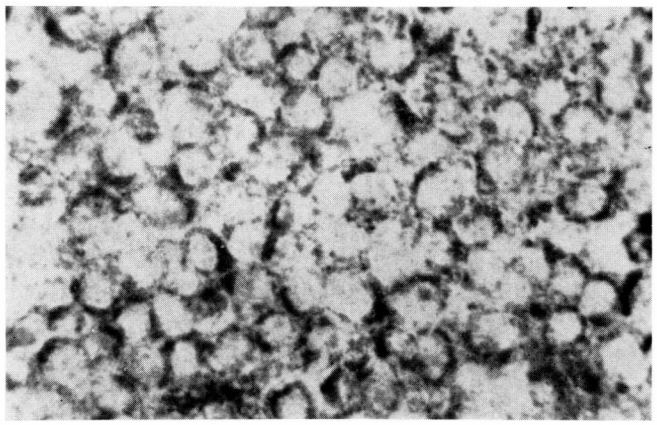

Fig. 5 (b). Increased activity of the same as in 5 (a) in leukaemic condition. (× 400).

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