

INCREASED SUCCINATE DEHYDROGENASE ACTIVITY OF LYMPHOCYTES IN ECZEMA

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Abstract. Succinate dehydrogenase activity has been studied in the peripheral blood lymphocytes from controls and patients with a variety of skin and other diseases. Increased activity has been found in eczematous dermatitis and dermatographism, and also in one patient with chronic lymphatic leukemia. Normal levels were found in psoriasis. The enzyme activity is broadly correlated with the extent and activity of the disease process.

Key words: Lymphocytes; Eczema; Succinate dehydrogenase; Psoriasis

During the course of a study on succinate dehydrogenase activity in human basophil leukocytes (13) one of us noted the presence of the enzyme in other cells of the same preparation (14). These cells were smaller than red cells, and after a number of counter staining procedures had been performed (Janus green, methylene green, methylene blue, Wright's Giemsa), they revealed themselves as members of the lymphocyte series. When using a modification of the published technique for iodonitrotetrazolium staining of basophils (13), dark blue granules appeared within the cytoplasm of these lymphocytes during the one hour incubation period, readily distinguishing them from the large majority of lymphocytes which developed a more diffuse reddish-brown staining with acicular crystals on the surface only at a later stage, viz. after 4-6 hours (Figs. 1 and 2). Despite the ordinary microscopical appearances after counter-staining, which led us at first to suspect an extracellular site of deposition of the enzyme, phase contrast and dark ground procedures clearly demonstrated that the formazan granules we observed were within the plasma membrane of the cell. The chief modification of the original method consisted in using a buffer with greater concentration of magnesium

ions (70 mg NaCl, 22 mg Na_2HPO_4 , 2 mg KH_2PO_4 , 10 mg MgCl_2 , 136 mg Na-succinate, 10 mg iodonitrotetrazolium, 10 ml H_2O adjusted to pH 7.4). We have found flat, open and rectangular glass capillary tubes (Vitro Dynamics, Inc., Rockaway, N. J., USA) most useful for cell counting. These seal at both ends with a vinyl plastic putty. Those used had a viewing path length of 0.05 mm, and outside dimensions of 50 mm $\times$ 15 mm $\times$ 0.6 mm, giving an internal volume of 1 cu mm.

A number of morphological types of granule formation are recognizable in the succinate dehydrogenase stained lymphocytes (S.D. lymphocytes) and are illustrated in Figs. 1 and 2. These are readily distinguished as a sub-population from the majority of lymphocytes which are not stained after one hour's incubation in the substrate medium. With the technique employed the S.D. lymphocytes appear to be of the small variety (5-7 μm diameter), but whether they are T- or B-cells is not yet established.

The specificity of the succinate dehydrogenase procedure was indicated by the lack of staining in the presence of sodium malonate or in the absence of sodium succinate as substrate. The numbers of cells staining in this manner were a small proportion of the total lymphocyte count, and in a series of controls (either healthy adults or adult patients with trivial skin complaints such as warts or moles) the mean count was 40 ± 24 (1 S.D.) per cu mm, and the mean as a percentage of the absolute lymphocyte count was 2.05 ± 1.11 (1 S.D.). The absolute and relative proportions of the S.D. lymphocytes varied considerably in disease states and appeared to depend both upon the diagnosis and the extent of the disease process. A study of the S.D. lymphocytes is summarized in Tables I and II. The highest count

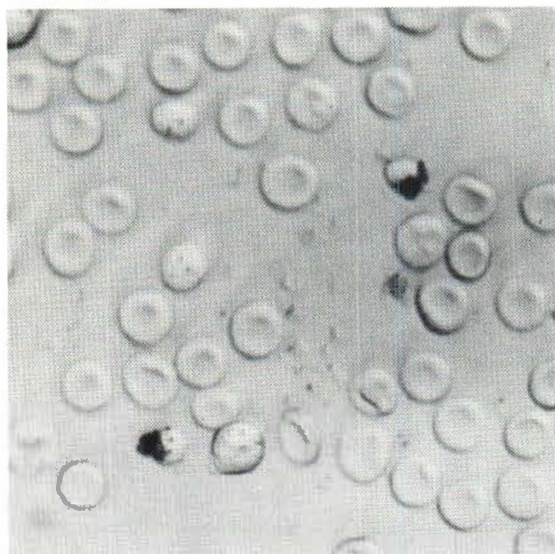


Fig. 1. Two lymphocytes showing succinate dehydrogenase activity. Note typical "capping" distribution of enzyme and size of lymphocytes relative to red cells. $\times 345$.

was seen in a patient with chronic lymphatic leukemia, but counts considerably greater than normal were seen in dermatographism, widespread eczema (mean $\pm$ 1 S.D. = 128 ± 18 per cu mm) and in some other dermatoses.

DISCUSSION

There has been some discussion as to whether succinate dehydrogenase is present within the mitochondria of normal leukocytes (2, 7, 10) or is non-mitochondrial (1) but the present consensus appears to favour the intramitochondrial localization of this enzyme. Ackerman (1) noted that only occasional lymphocytes showed dehydrogenase activity, unless there was prior damage to the mitochondrial membrane by addition of a non-ionic surface active agent (renex 20) when staining became more obvious. As already recorded by Wachstein (15), Ackerman (1) has also observed that a much greater proportion of lymphocytes from normal human lymph glands were stainable than those found in peripheral blood samples. Patients with chronic lymphatic leukemia usually showed increased dehydrogenase activity and when using a procedure for endogenous non-specific dehydrogenase Wachstein (15) found that 9 of 10 such patients had increased activity. Suspensions from tonsils and

lymph nodes from surgically removed specimens found to be normal on microscopic examination showed 46–54% of lymphocytes to be positive.

Apart from these and other scattered observations, mainly in the Russian and Eastern European literature, there have been no studies of succinate dehydrogenase activity in lymphocytes in human disease states. In particular there have been no such investigations directed at lymphocytes from patients with dermatological disease, despite the fact that many dermatoses such as lichen planus, Sezary's syndrome, mycosis fungoides, have as a feature marked infiltration with cells of the lymphocyte series. Quaglini et al. (11, 12), Gough & Elves (4), Nadler et al. (8) and Parkes & Howell (9), have all demonstrated increased enzyme activity, including succinate dehydrogenase activity, in lymphocytes undergoing transformation to large lymphoblasts and it is conceivable that the S.D. lymphocytes we have observed may represent a population of cells at an early stage in this process. Their site and morphology, however, would indicate that they are not fully transformed cells; indeed many of the cells we observed as having succinate dehydrogenase activity were perceptibly smaller than the average small lymphocyte. Experiments using stimulation with phytohaemagglutinin (P and M), and with antigens such as PPD in skin test positive reactors, have so far not revealed any increase in the proportion of S.D. lymphocytes in cultures maintained for

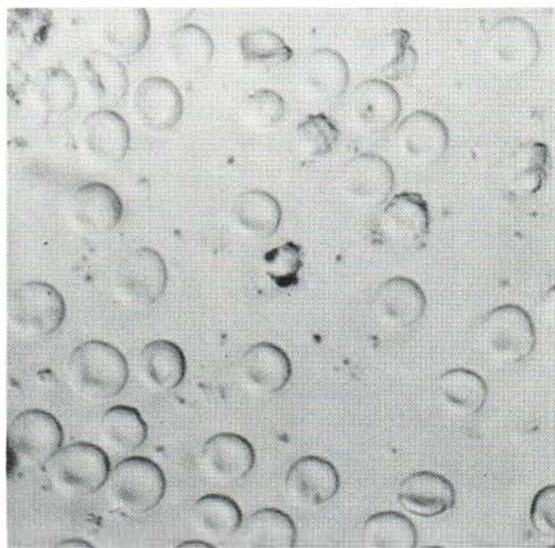


Fig. 2. One small lymphocyte showing peripheral distribution of succinate dehydrogenase activity. $\times 345$.

Table I. *Peripheral blood lymphocytes showing succinate dehydrogenase activity in controls and patients with eczema of psoriasis*

	Controls	Eczema	Psoriasis
Absolute count per cu mm (Mean $\pm$ 1 S.D.)	40 $\pm$ 24 (n=42)	128 $\pm$ 18 (n=17) (p<0.001)	54 $\pm$ 44 (n=8) (p>0.2)
Per cent of total lymphocyte count (Mean $\pm$ 1 S.D.)	2.05 $\pm$ 1.11 (n=21)	3.61 $\pm$ 1.14 (n=7) (p<0.01)	1.89 $\pm$ 2.28 (n=4) (p>0.5)

periods varying from one hour to 5 days. The discrepancy between our results and those of Quaglino et al. (12) may be explained by technical factors, or simply by accepting that the S.D. lymphocytes we have observed are not indicative of the process of transformation.

An intriguing possibility is that the S.D. lymphocytes represent all those lymphocytes available with the potential of transforming. Thus, phytohaemagglutinin (PHA) or antigenic stimulation might simply select the S.D. cells for further morphological development, and consequently a lower than normal count of these (S.D.) lymphocytes would ultimately be found in those cultures. Similarly the poor response of lymphocytes from chronic lymphocytic leukemia patients to PHA noted by Quaglino & Cowling (11) might also represent the existence of already maximally stimulated cells which cannot respond further in this regard. This stands in contrast with normal lymphocytes having S.D. activity and which we suggest might be part of the mobile pool of primitive and probably multi-potential progenitor cells which in appropriate circumstances would give rise to large numbers of dividing cells. The results given in Table I support this concept, for it can be seen that eczema subjects not only had a greater number of S.D. lymphocytes (absolute and as a percentage of the lymphocyte count), but there was also a rise in the total number of circulating lymphocytes.

In the patients we have studied it seems evident that certain conditions are associated with a significantly higher than normal count of S.D. lymphocyte, whether these are calculated as absolute numbers per cu mm, or as a percentage of the total lymphocyte count (Tables I, II). In particular, dermatographism and eczema are worthy of note. In the cases of eczema the area of skin involved seemed to correlate roughly with the number of S.D. lymphocytes. We suggest that widespread eczema represents a state of continued antigenic stimulation and that this is the main factor in inducing an increased number of succinate dehydrogenase-active lymphocytes. In this regard it is also of interest to observe the rise in S.D. lymphocytes in a patient (H. C.) with Kaposi's syndrome, as he became sensitized to unilaterally applied topical nitrogen mustard—a rise from 77 to 97 in venous blood from the untreated area, and to 114 in blood from the area showing eczematization. Subsequently this clinical reaction settled and S.D. lymphocyte counts were 28 and 19 per cu mm of blood from the untreated and treated areas respectively.

This and our other cases of eczema with high S.D. counts can be compared with the patient reported by Dahl et al. (3), an atopic subject suffering from acute vaccinaria whose lymphocytes showed an intense reactivity to stimulation with phytohaemagglutinin, coincident with a very high spontaneous

Table II. *Peripheral blood lymphocytes showing succinate dehydrogenase activity in other disease states*

Diagnosis	Myeloid leukemia	Lymphatic leukemia (1)	Dermatographism (2)	Urticaria (3)
Absolute count per cu mm.	56	465	146 103	17 70 17
Per cent of total lymphocyte count	1.87	8.27	n.d.	0.94 0.96

reactivity in the control cultures. The authors attributed these findings to the heavy demand on their patients' immune system due to vaccinia virus. Other authors (5, 6), however, have found less than normal reactivity to phytohaemagglutinin stimulation of lymphocytes in atopics. As noted above in regard to leukaemic lymphocytes, this could be due to a number of factors, one being the possibly already maximally stimulated state of these cells.

ACKNOWLEDGEMENTS

We are indebted to Mrs Lynne Todoroff for technical assistance. We thank the Haematology Service at Philadelphia General Hospital (Dr Miriam Dahlke) for total lymphocyte counts.

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Received March 8, 1976

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