

HISTOCHEMICAL STUDY OF SOME DEHYDROGENASES IN THE ORGANS OF GUINEA PIGS WITH HYPERSENSITIVITY OF DELAYED TYPE

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Succinate and α -glycerophosphate dehydrogenases in the heart, liver, kidney, and spleen were determined by a quantitative histochemical method in guinea pigs sensitized by intradermal injection of penicillin, on the 2nd day after injection of the allergen. The state of hypersensitivity of delayed type is accompanied, not so much by changes in the mean values of activity of oxidation-reduction enzymes of various organs, as by disturbances of the coordination of their metabolism.

Previous investigations have shown that the process of immunogenesis is accompanied by intensification of oxidation and by increased coordination between the functions of the organs, especially the lymphoid organs [1]. However, the stage of hypersensitivity of delayed type (HDT) is one of modified immunological reactivity to an antigen or hapten, leading to consequences harmful to the organism, and the modified metabolism of the peripheral blood lymphocytes differs qualitatively from that found during the normal course of immunogenesis [2, 3, 7].

The object of the present investigation was to study the energy metabolism in various organs during sensitization of guinea pigs with penicillin. Penicillin is a substance of low molecular weight which, when combined with tissue proteins, acquires allergenic properties and, if injected intradermally, can induce HDT [10-13, 14].

TABLE 1. Cytochemical and Histochemical Indices of Activity of Succinate and α -Glycerophosphate Dehydrogenases in Lymphocytes and Organs of Guinea Pigs

Group of animals	Succinate dehydrogenase					α -Glycerophosphate dehydrogenase				
	lymphocytes	spleen	liver	kidney	heart	lymphocytes	spleen	liver	kidney	heart
Control	14,2 $\pm 1,29$	20,2 $\pm 1,32$	43,6 $\pm 1,96$	60,5 $\pm 2,48$	72,3 $\pm 2,48$	12,3 $\pm 1,15$	28,2 $\pm 1,16$	19,6 $\pm 1,02$	25,9 $\pm 1,18$	25,8 $\pm 0,86$
Experimental: before sensitization	17,3 $\pm 1,10$	—	—	—	—	14,1 $\pm 1,29$	—	—	—	—
2nd day after	13,7 $\pm 1,16$	20,8 $\pm 1,06$	45,7 $\pm 3,0$	58,3 $\pm 1,99$	68,0 $\pm 2,58$	11,0 $\pm 1,28$	28,4 $\pm 1,44$	20,1 $\pm 1,63$	24,9 $\pm 1,26$	23,2 $\pm 0,72$

Note: Incubation medium contained as substrate for the enzymes:
50 mmoles sodium succinate and 50 mmoles sodium α -glycerophosphate.

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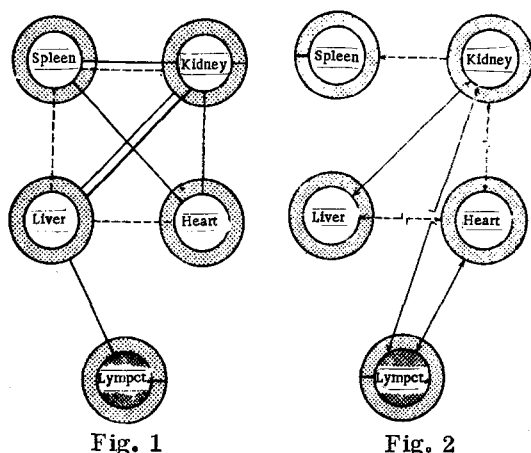


Fig. 1

Fig. 1. Correlation of enzyme activity between organs of control guinea pigs. Inner circle) α -glycerophosphate dehydrogenase; outer circle) succinate dehydrogenase. Broken and continuous lines represent correlations with different levels of statistical significance: broken line) significance of correlation coefficient $P < 0.1$; thin continuous line) $P < 0.05$; thick line) $P < 0.01$.

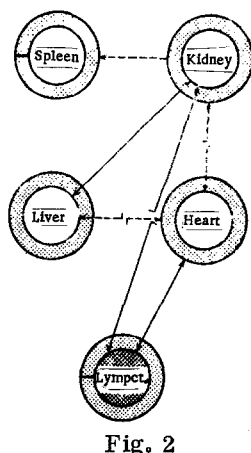


Fig. 2

Fig. 2. Correlation of enzyme activity between organs of guinea pigs during sensitization. Interrupted line with strokes on opposite sides — feedback (negative coefficient of correlation). Remainder of legend as in Fig. 1.

The numerical results were subjected to statistical analysis, the coefficients of correlation were calculated, and the degree of significance of the differences between the mean values of activity of the enzymes were compared by the criteria of Student and White. Many of the calculations were carried out by a Minsk-22 computer.

EXPERIMENTAL RESULTS

As has already been pointed out, intradermal injection of penicillin into the sensitized animals caused the appearance of a skin reaction, in the form of infiltration and necrosis, within 48 h at the site of injection. In some animals, only infiltration was observed after skin tests if the interval between sensitizations was long. The positive skin tests in all the sensitized animals were evidence of a state of HDT to penicillin.

The main results of the cytochemical and histochemical investigations are summarized in Table 1.

As Table 1 shows, the indices of enzyme activity in the organs of the animals of the control group and the sensitized guinea pigs were virtually indistinguishable. Only a very slight tendency toward a decrease in oxidation was found in the heart (the decrease was statistically significant for α -glycerophosphate dehydrogenase). However, it must be noted that higher values of activity of the dehydrogenases were observed in the peripheral blood lymphocytes of the sensitized guinea pigs before injection of the preparation than in the control animals. The subsequent injection of the allergen led to a decrease in the intensity of energy-forming processes in these cells. Intensification of succinate oxidation after the end of sensitization has been observed previously by the writers. Having regard to the connection between metabolism of the lymphocyte and the internal organs (see below), indices of oxidation would be expected to be higher in the organs of the sensitized guinea pigs before injection of the reacting agent and that this would be followed by a decrease on the 2nd day of sensitization. However, this hypothesis requires further experimental confirmation.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs weighing 400–600 g. The control consisted of 13 animals, and another 13 animals were sensitized by intradermal injection of 0.1 ml of a penicillin solution containing 300,000 units/ml [4, 9]. The animals were used in the experiment after systematic appearance of a skin reaction which was always of the type characteristic of HDT and reached its highest intensity after 48–72 h. As a rule the guinea pigs were sensitized no fewer than 5 times. On the 2nd day of sensitization, i.e., at the time of maximal development of the skin reactions, the animals were killed by electrocution (220 V) and the spleen, liver, kidney, and heart were extracted. Activity of succinate dehydrogenase [succinate: (acceptor)-oxidoreductase, 1.3.99.1] and α -glycerophosphate dehydrogenase [L-glycerol-3-phosphate: (acceptor)-oxidoreductase, 1.1.2.1] was determined in these organs histochemically by the method of Nartsissov et al. [6]. Activity of the enzyme was expressed in microunits. The unit of activity of the enzyme was taken to be the number of micromoles of formazan formed by 1 mg protein in 1 min at 37°C:

$$1 \text{ unit} = \frac{1 \mu \text{mole formazan}}{1 \text{ mg protein} \times 1 \text{ min}}$$

In parallel experiments the activity of succinate- and α -glycerophosphate dehydrogenases was determined in the peripheral blood lymphocytes [5]. The tests were carried out before injection of the allergen and on the 2nd day after sensitization. The mean number of granules of reaction product (formazan) per lymphocyte was calculated after analysis of 100 cells in blood films.

By comparing the mean levels of enzyme activity in the organs it was thus impossible to detect any difference between the animals of the experimental and control groups. Nevertheless, the use of a more delicately informative criterion – the degree of correlation between the metabolism of the organs – it was possible to establish certain general principles governing the changes in the degree of functional association between the organs in this state. For example, in the guinea pig whose condition was the severest during sensitization, discoordination was found between the functions of the organs. In this case, a slight increase in activity of the dehydrogenases was found in the liver and kidney, while the intensity of energy processes in the heart was appreciably reduced. It was therefore decided to make a mathematical analysis of the correlation between enzyme activity of the various organs of the animals belonging to the control and experimental groups. The results of this correlation analysis are given in Figs. 1 and 2.

It is clear from Fig. 1 that in the control animals coordination of enzyme activity is found to a varied degree in all organs tested. Correlation of activity of the various enzymes between individual organs and also the interweaving of the links between them are evidence of a complex mechanism regulating the overall metabolism of the body. It is a striking fact that the peripheral blood lymphocytes of guinea pigs have a correlative link with the internal organs through the liver. Coordination of metabolism in the organs has also been demonstrated by the writers in other species of laboratory animals (mice and rabbits) [1]. This coordination is not very rigidly controlled and is subjected to various influences and changes. For instance, the state of immunogenesis causes an increase in the functional correlation between the organs, which can be regarded as a physiological adaptive response to antigenic stimulation.

A different picture is observed when the functions of organs are studied in a state of HDT (Fig. 2). In this case coordination of metabolism of the organs of the sensitized animals differs from that of the control group of guinea pigs both qualitatively and quantitatively. During sensitization there is a decrease in the closeness of direct coordination between the organs as found in healthy animals, and new links appear which are not normally found, i.e., discoordination of metabolism is observed between individual organs. It is interesting to note the appearance under these conditions of correlations with a negative sign (a reciprocal relationship). The existence of a connection between lymphocytes and the heart is also noteworthy, for it was not observed under normal conditions.

Simultaneously with the disturbance of coordination between the organs, an increase in coordination between the two dehydrogenases in the spleen was discovered. Whereas in the intact spleen succinate and α -glycerophosphate dehydrogenases function relatively freely with respect to each other, during development of the HDT a strong correlation arises between oxidation of the metabolites in this organ. An increase in coordination of this type within the lymphoid organ has also been found in rabbits and mice during immunization [1]. These results can be considered together with the intensification of function of that organ accompanied by proliferation of large, pyroninophilic cells. These cells are at present regarded as the immediate precursors of immune lymphocytes [8].

Consequently, the state of HDT is accompanied not so much by changes in the mean levels of activity of oxidative enzymes in various organs, as by disturbances of the coordination of their metabolism.

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