CYTOCHEMICAL AND ELECTROPHORETIC INVESTIGATION OF MACROPHAGE LACTATE DEHYDROGENASE ISOENZYMES

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A cytochemical and electrophoretic investigation was made of the isoenzyme composition of lactate dehydrogenase (LDH) in macrophages from the lungs and peritoneal cavity of guinea pigs. Five LDH isoenzymes were found in both populations of macrophages; in the macrophages of the peritoneal cavity with a predominantly glycolytic type of metabolism and in those of the lungs, in which respiration is dominant, the LDH isoenzymes were chiefly of the muscular type (LDH-4 and LDH-5), characteristic of tissues with the glycolytic type of metabolism.

Previous investigations showed that the lung macrophages of mice have a predominantly oxidative, while the peritoneal macrophages have a predominantly glycolytic type of metabolism [1, 5, 9].

Following observations reported in the literature [6, 11] that the slowly migrating isoenzymes of lactate dehydrogenase (LDH) of muscular type are predominant in tissues with a high level of anaerobic glycolysis and that fast-migrating LDH isoenzymes of cardiac type are predominant in tissues with an oxidative type of metabolism, it was natural to suppose that fast-migrating LDH isoenzymes would be predominant in macrophages of the lungs and slowly migrating LDH enzymes in peritoneal macrophages. The lack of information on the isoenzyme composition of LDH in the macrophages from the lungs and peritoneal cavity in the literature justified the investigation described below.

EXPERIMENTAL METHOD

Macrophages from the lungs and peritoneal cavity of guinea pigs were used. A suspension of lung macrophages was obtained by Myvrik's method [8], and a suspension of peritoneal macrophages by Rowley's method [10]. Medium No. 199 containing 1% EDTA solution and heparin (5 units/ml) was used to wash out the lungs and peritoneal cavity. In the cytochemical investigations the suspension of macrophages was centrifuged at 400 g for 15 min, and the residue was resuspended in medium No. 199 containing 10% bovine serum; the number of cells per ml was counted with a Goryaev's chamber, the suspension of macrophages was diluted to a concentration of 500,000 cells/ml, and the macrophages were then allowed to settle for 30 min at 37°C on coverslips. The resulting preparations of macrophages were dried in air and incubated for 10 min at 37°C in medium intended for the demonstration of LDH activity: 1 ml distilled H_2O, 10 mg NAD, 1 ml 0.2 M phosphate buffer, pH 7.2, 1 ml Krebs' solution, 1 ml nitro-blue tetrazolium solution (2.5 mg/ml), 1 ml 0.2 M sodium lactate, 750 mg polyvinylpyrrolidone (mol. wt. 12,000), and 0.2 ml phenazine methosulfate (1 mg/ml). For the cytochemical differentiation of the LDH isoenzymes urea was added to the incubation medium in a final concentration of 1 M, 2 M, 3 M, and 4 M.

For the electrophoretic analysis of the LDH isoenzymes the suspension of macrophages was sedimented by centrifugation for 15 min at 1600 g, the residue was diluted with Tris–EDTA–borate buffer, pH 9.2, in the ratio of 1 : 5, and the resulting suspension was carefully homogenized (under microscopic control). The homogenate was then centrifuged for 15-20 min at 10,000 g, and the LDH isoenzymes tested

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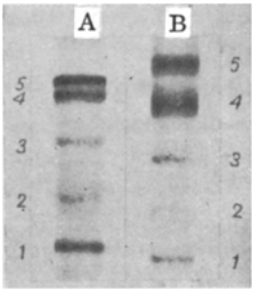

Fig. 1. LDH isoenzymes in alveolar (A) and peritoneal (B) macrophages.

in the supernatant by electrophoresis. The LDH isoenzyme spectrum was determined by vertical electrophoresis in polyacrylamide gel [2]. Fractionation of LDH was carried out for 70 min at 0°C in Tris–EDTA–borate buffer, pH 9.2, with a current of 5 mA applied to each tube. The reaction of reduction of the nitro-blue tetrazolium in the presence of sodium lactate, NAD, and phenazine methosulfate was used to detect the LDH isoenzymes.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of electrophoresis (Fig. 1) showed that the alveolar macrophages of the lungs and the peritoneal macrophages of guinea pigs contain five LDH isoenzymes; both the peritoneal and the alveolar macrophages contained high activity of the slowly migrating LDH isoenzymes (LDH-4 and LDH-5) and low activity of the fast-migrating LDH isoenzymes (LDH-1 and LDH-2).

The results of the cytochemical investigation of the LDH isoenzyme composition in the macrophages of the lungs and peritoneal cavity (Fig. 2) show that, on the addition of urea to the incubation medium used for detecting LDH activity, that activity was reduced in the macrophages of both populations. The presence of urea in the incubation medium in a final concentration of 1 M (Fig. 2: 3, 4) led to a marked decrease in LDH activity in both peritoneal and alveolar macrophages. On increasing the urea concentration in the incubation medium to 2 M, traces of LDH activity were still found only in solitary peritoneal and alveolar macrophages (Fig. 2: 5, 6), while if urea was present in the medium in concentrations of 3 M and 4 M, LDH activity in both populations of macrophages was completely suppressed.

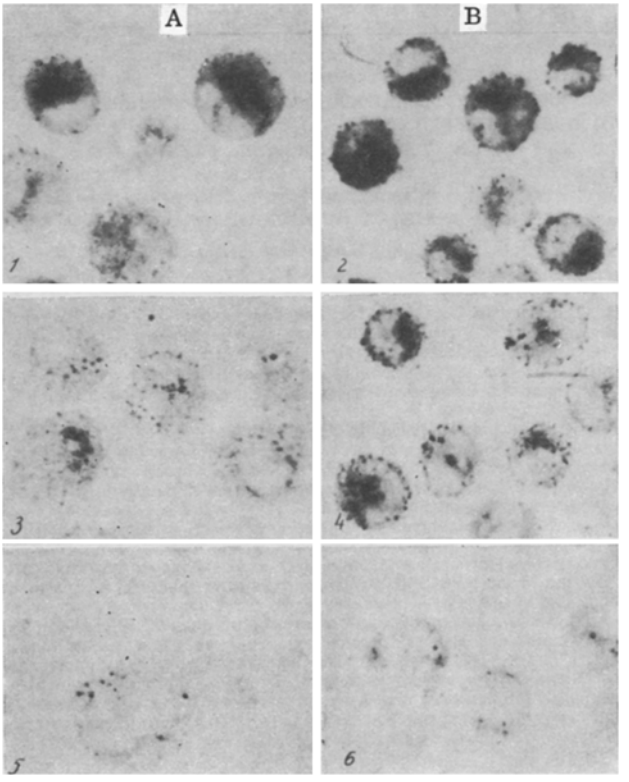

Fig. 2. Inhibition of activity of LDH isoenzymes of muscular type in alveolar (A) and peritoneal (B) macrophages on addition of urea to the incubation medium (objective 60×, ocular 6.3×): 1, 2) control; 3, 4) 1 M urea; 5, 6) 2 M urea.

The cytochemical observations showing a decrease in total LDH activity in both populations of macrophages under the influence of urea show that the total LDH activity in both peritoneal and alveolar macrophages is due principally to isoenzymes of the muscular type, whose activity is selectively inhibited by urea [3, 4, 6, 7]. Meanwhile, LDH isoenzymes of cardiac type are present in only very small proportions in both the alveolar and peritoneal macrophages.

Contrary to expectation, the results of electrophoretic and cytochemical determination of the isoenzyme composition of LDH in the alveolar and peritoneal macrophages thus demonstrate unequivocally that both in the peritoneal macrophages with a high level of anaerobic glycolysis and in the alveolar macrophages with dominant respiration, slowly migrating LDH isoenzymes of muscular type characteristic of tissues with a glycolytic type of metabolism are predominant. The presence of slowly migrating LDH isoenzymes in the macrophages of the lungs suggests that these cells are capable of anaerobic glycolysis despite their high activity of respiratory enzymes. A similar picture is found in the red muscles, which have a predominantly oxidative type of metabolism but, nevertheless, contain slowly migrating LDH isoenzymes [4]. The similarity between the LDH isoenzyme composition of the alveolar and peritoneal macrophages is presumably indirect evidence of their common origin, and it shows that although the alveolar macrophages have acquired a predominantly oxidative type of metabolism in the course of their differentiation in an oxygen-rich medium, they still preserve some metabolic features characteristic of the phylogenetically older pathway of energy production – glycolysis.

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