Oxidation of Extramitochondrial Diphosphopyridine Nucleotide by Various Tissues of the Mouse

Abstract. *Exogenous reduced diphosphopyridine nucleotide was not oxidized by mitochondria but by cytoplasmic hydrogen-accepting systems catalyzed by lactic, α-glycerophosphate, and malic dehydrogenases. The three enzymes were found in all tissues of the mouse; the amount of activity of each enzyme differed in the various tissues, however. It is suggested that each tissue has a unique pattern for oxidation of extramitochondrial diphosphopyridine nucleotide.*

During glycolysis extramitochondrial diphosphopyridine nucleotide (DPN^+ or NAD^+) becomes reduced, so that its continuous reoxidation is necessary for maintenance of the flow of carbon through the glycolytic pathway. Studies with isolated cell particulates and intact cells of normal and malignant tissues indicate that this reduced diphosphopyridine nucleotide (DPNH) is not oxidized directly by mitochondria (*1–5*); rather, oxidation is catalyzed by hydrogen-accepting systems localized in the extramitochondrial (soluble cytoplasmic) compartment of the cell (*2–7*). The hydrogen-accepting systems include the pyruvate-lactate, dihydroxyacetonephosphate-α-glycerophosphate, and oxaloacetate-malate oxidation-reduction systems. The second and third mentioned pathways are components of "metabolite shuttle" mechanisms for the passage of hydrogen through the cytoplasmic-mitochondrial barrier (*5*). Athough the measurements of the activities of the dehydrogenases, especially lactic dehydrogenase, in different mammalian tissues are numerous, a direct evaluation of the three pathways in a variety of tissues is wanting. Such a comparison is reported here. The data suggest that each tissue has a unique pattern for oxidation of extramitochondrial DPNH.

Excised tissues were minced in the cold in a medium containing 5 percent polyvinylpyrrolidine; 0.05*M* sodium phosphate buffer, *p*H 7.4; and 5 m*M* ethylenediaminetetraacetate. The minced tissue was washed by decantation and then homogenized in 3 ml of medium with two strokes of a low speed, loosely fitting Potter-Elvehjem homogenizer. Depending on the activity of the dehydrogenase, the homogenates were used without further dilution (α-glycerophosphate dehydrogenase in lung) or were diluted variously with medium to as much as 200 times (malic dehydrogenase in kidney). Dilutions were made so that the change in absorbancy at 340 mμ was limited to 0.060 per minute. Extramitochondrial preparations of tissues were obtained by centrifuging homogenates at 30,000*g* for 20 minutes. The supernatants were centrifuged again at the same force for 10 minutes and were then diluted appropriately.

Activities of the dehydrogenases were determined spectrophotometrically at room temperature (22° to 24°C) from initial (0 to 3 minutes) rates of oxidation of DPNH. The reaction mixture contained 0.1 m*M* DPNH; 0.033*M* triethanolamine hydrochloride, *p*H 7.45; 0.1 ml tissue preparation; and H_2O to 2.9 ml. The reaction was initiated by the addition of 0.1 ml of substrate; final concentrations were 1 m*M* pyruvate, 0.3 m*M* dihydroxyacetonephosphate, and 0.1 m*M* oxaloacetate.

In the absence of reducible substrate exogenous DPNH was not oxidized or, at most, was metabolized insignificantly by homogenates of the various tissues. Addition of cytochrome *c* to the reaction did not increase these rates. In the presence of reducible substrate DPNH was oxidized; antimycin A was without effect. Such findings indicate that the lack of direct DPNH oxidase activity was not due to leakage of cytochrome from mitochondria; rather, they suggest that mitochondria from mammalian tissues, when undamaged by preparative techniques, are unable to oxidize extramitochondrial DPNH, presumably because of the impermeability of the mitochondrial membrane to the reduced pyridine nucleotide (*1, 2, 4*). This brings into focus the importance of extramitochondrial hydrogen-accepting systems for the oxidation of DPNH formed glycolytically in many, if not all, mammalian tissues.

The three oxidation-reduction systems catalyzed by lactic, malic, and α-glycerophosphate dehydrogenases were found in all tissues of the mouse (Fig. 1). The amount of activity in the various tissues, expressed per unit of wet weight or of protein, differed, however. Lactic dehydrogenase activities ranged from about 350 μmole of DPNH per minute per gram of wet weight in leg muscle and kidney to less than 50 μmole in lung. Our values for liver, skeletal muscle, heart, and brain agreed reasonably well with those reported for these tissues of the rat or rabbit (*6, 8*).

Appreciable α-glycerophosphate dehydrogenase activity was found in liver, kidney, skeletal muscle, and testes, whereas it was barely measurable in heart. Relatively high α-glycerophosphate activities were noted previously in rat liver and muscle (*6*). The low α-

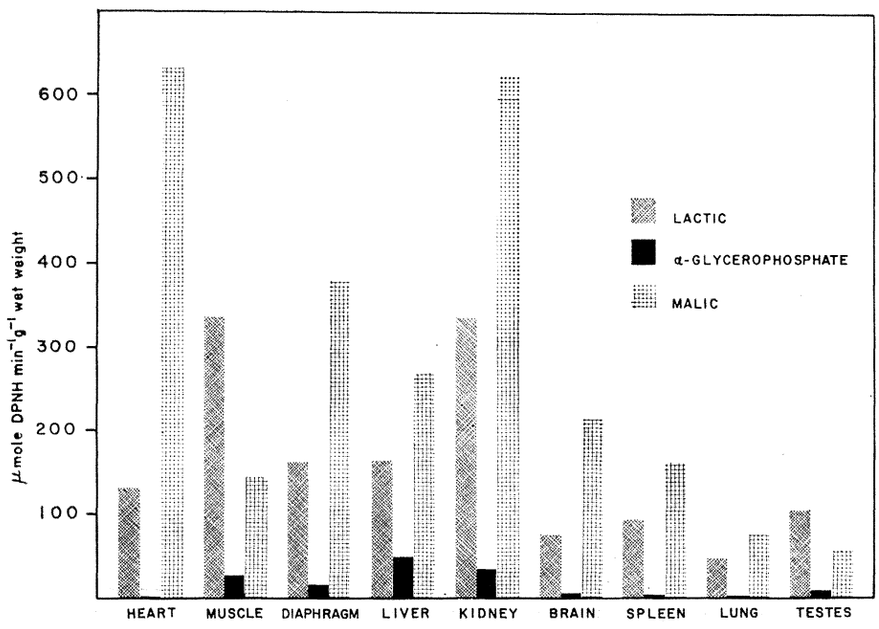

Fig. 1. Activities of lactic, α-glycerophosphate, and malic dehydrogenases in homogenates of various tissues of the mouse. The heights of the bars represent the average of two to four determinations.

glycerophosphate dehydrogenase activity in heart, coupled with the veritable absence of α-glycerophosphate oxidase from mitochondria of this tissue (*9*), suggests that the α-glycerophosphate cycle is of little significance in heart.

Malic dehydrogenase activity was remarkably high, especially in heart, kidney, diaphragm, and liver. In fact, malic dehydrogenase was more active than lactic dehydrogenase in all tissues except leg muscle and testis. The present values for malic dehydrogenase were two- to four-fold those reported for several tissues of other species (*6, 8*).

The mean values of 330, 30, and 150 μmole of DPNH oxidized per minute per gram of wet weight of skeletal muscle with the lactic, the α-glycerophosphate, and the malic dehydrogenases, respectively, were in marked contrast to titers of 0, 1230, and 400 for these same three enzymes in flight muscle of flies (*5*). Enzymatic activities of extramitochondrial preparations of the various tissues were not appreciably different from those of homogenates.

Although measurements of the optimum activities of the three dehydrogenases give estimates only of the potential of each pathway, a comparison of the values in the different tissues suggests that each tissue has a unique pattern for oxidation of extramitochondrial DPNH. Additional evaluation of the pathways was obtained by determining the concentrations of the redox metabolites in tissues at "rest" and while performing work. As an example, concentrations in skeletal muscle of α-glycerophosphate, malate, and lactate increased in a characteristic fashion with repeated isometric contractions (*10*). Other changes in concentration of metabolites during physiological activities of tissues are described elsewhere (*11*).

BERTRAM SACKTOR
ARTHUR R. DICK
Chemical Research and Development Laboratories, U.S. Army Edgewood Arsenal, Maryland 21010

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Inhibition of Antibody Plaque Formation by Sensitized Lymphoid Cells: Rapid Indicator of Transplantation Immunity

Abstract. *Spleen cell suspensions obtained from mice immunized with sheep erythrocytes form localized zones of hemolysis ("antibody plaques") when incubated in agar gel containing sheep red blood cells and complement. Plaque formation can be inhibited by prior incubation in vitro with spleen cell suspensions from another strain of mice previously sensitized to the first by spleen cell transplant. Suppression of plaque formation was found to be quantitatively related to the number of cells incubated and apparently reflected a homograft reaction in vitro of one spleen cell population against another. Plaque inhibition may be a useful indicator of transplantation immunity.*

Immunologic incompatibility between unrelated individuals within a species is often evaluated by determining the time required for overt rejection of grafted tissues (*1*). There have been several attempts to develop rapid and reproducible procedures for demonstrating either transplantation immunity or histocompatibility between graft donors and recipients. For example, "tissue typing" procedures in vivo involving intradermal injection of peripheral recipient leukocytes into prospective donors have been reported as relatively rapid and reliable indicators of tissue incompatibility (*2*). Similarly, a "tissue culture" method in vivo for assaying homograft reactions has been described by Celada and Carter (*3*). In their procedure, immunologically inert x-irradiated recipient mice were injected with lymphoid cell suspensions from two strains of mice, one of which had been previously immunized with sheep erythrocytes. Tissue incompatibility between the two strains was assessed by measuring the degree of suppression of expected agglutinin formation in the irradiated recipients several days after cell transfer.

However, a test for histocompatibility ideally should be performed completely in vitro within a short period of time. In this regard, the use of cytotoxic or agglutinating antiserums for "typing" in vitro of peripheral leukocytes has been reported as a possible method for detecting antigenic differences among individuals (*4*). Similarly, interaction in vitro of lymphoid cells from unrelated individuals in tissue culture media has been recently presented as a possible method for demonstrating homograft reactivity (*5*). In this procedure, incubation of peripheral leukocytes from two unrelated individuals in tissue culture medium resulted in a specific increase in the number of mitotic figures and large lymphoid cells during a period of about a week. No increased mitotic activity was observed after incubation of lymphoid cells from genetically identical individuals.

This report is concerned with detection of homograft sensitivity in mice by an adaptation of the recently described localized hemolysis techniques (*6, 7*). In the method developed by Jerne *et al.* (*6*), immunologically competent lymphoid cells from animals sensitized with foreign erythrocytes form localized zones of hemolysis when incubated at 37°C with erythrocytes suspended in solidified agar gel, and then incubated with guinea pig complement. In the procedure described independently by Ingraham and Bussard (*7*), culture medium is thickened with high-viscosity cellulose gum (carboxymethoxycellulose) instead of agar. Lymphoid cells from erythrocyte-immunized animals are suspended in the thickened medium, to which washed erythrocytes and guinea pig complement are added. This mixture is incubated directly on microscope slides. Small zones of hemolysis, presumably surrounding antibody-secreting cells, develop during incubation. These localized hemolysis procedures permit rapid scoring of the number of individual antibody-releasing cells present in a given population of lymphoid cells. The use of Jerne's antibody-plaque method in agar gel as a possible indicator in vitro of homograft reactivity between lymphoid cells from two strains of mice is presented here.

For these experiments, "target" cells