to the stabilizing influence of ketosubstrates, the aminosubstrates seem to exert an opposite effect. The increase in temperature of a transaminase solution containing aminosubstrates causes additional augmentation in the absorption maximum at 333 nm on the spectrum of the enzyme. In this respect elevation of temperature has the same effect as an additional amount of aminosubstrate (Table). This increase in absorption at 333 nm upon heating may explain the instability and inactivation of transaminase when treated with aminosubstrates during the heating step in the process of enzyme purification[1].

Some general conclusions can be drawn from these observations. Transaminase, when stored at low temperatures around or below zero, acquires a conformation similar to the one the enzyme takes in the presence of ketosubstrates. This conformation is expressed on the enzyme spectrum with a high absorption on its maximum in the area of 430 nm. In both cases the transaminase is stable and protected from inactivation. In contrast, high temperatures or the presence of aminosubstrates induce a conformation of the transaminase molecule which is expressed by an increase in the absorption at 340 nm (Table) At this configuration the transaminase is unstable and rapidly inactivated.

If the described spectral behaviour of transaminase is valid for other vitamin B_6 enzymes, then one might predict their stability towards temperature and propose optimal conditions to protect them from inactivation[6].

Zusammenfassung. Ein Zusammenhang zwischen optischen Eigenschaften und Stabilität der Transaminasen wird nachgewiesen. Temperaturabhängigkeit und Art der Partnerverbindung im Enzymsubstrat-Komplex (Amino- oder α-Ketoverbindung) einerseits und Konformation anderseits sind analog.

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Malate Dehydrogenase Isoenzymes in the Pancreatic Islets of Obese-Hyperglycemic Mice

It has been shown for a number of species that the pancreatic islets are characterized by a considerable malate dehydrogenase (MDH) activity[1,2]. It has also recently been demonstrated that the MDH in many cells can be segregated into different molecular forms, which have tentatively been assigned different physiological roles. Kaplan[3] suggested that a soluble MDH in the cytoplasm catalyses the formation of malate, which after the entrance into the mitochondria is oxidized by a mitochondrial MDH. The oxaloacetate produced may then be released into the cytoplasm, thus enabling the cycle to be repeated. The possibility of using disc electrophoresis[4,5] on polyacrylamide gels for the separation of enzymes from the small amount of tissue represented by the pancreatic islets of mammals[6] was taken advantage of in the present attempt at a further characterization of the MDH in mammalian B-cells with respect to molecular heterogeneity.

Material and methods. Adult American obese-hyperglycemic mice[7] of both sexes were used. Fresh pancreatic islets were isolated from the surrounding exocrine parenchyma as described by Hellerström[8] and homogenized in a 5% (v/v) aqueous solution of Triton-X-100 by means of a lucite micro-homogenizer. In addition, homogenates of exocrine pancreas and liver were similarly prepared. Electrophoresis was carried out as described by Ornstein[4] and Davis[5] at 3 °C (2.5 mA/gel; running time about 90 min) and the gels were subsequently incubated at 37 °C in the substrate-reagent medium given by Laycock et al.[9]. After extraction of the enzyme from the unstained central part of the gel[6], the activity of the different isoenzymes was fluorophotometrically assayed with oxaloacetate as substrate[10]. The total MDH activity was, in addition, assayed on crude tissue homogenates, the protein concentration of which was determined as described by Lowry et al.[11].

Results. The islets displayed an enzyme activity of at least the same magnitude as the exocrine pancreas (Table I). It appears from Figure 1 that 2 distinct MDH isoenzymes could be separated from the islet homogenates, as well as from the exocrine parenchyma and the liver. In all the tissues the more rapidly migrating fraction contained the highest activity towards malate, as estimated from the staining intensity. In an experiment, in which equal volumes of homogenate of all the 3 tissues were mixed prior to electrophoresis, the running distances

Table I. Malate dehydrogenase activity in the endocrine and exocrine pancreas and the liver of obese-hyperglycemic mice

Islets (6)	Acinar tissue (6)	Liver (5)
143.4 ± 18.1	116.1 ± 32.1	296.4 ± 37.7

1.1 m*M* of oxaloacetate was used as substrate and the figures denote moles of $NADH_2$ oxidized per kg protein and per hour. Mean values ± S.E.M. The number of animals studied is given in parentheses.

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Table II. Running distances in relation to bromphenol blue and relative activity in % for the 2 malate dehydrogenase isoenzymes in the endocrine and exocrine pancreas and the liver

	Islets		Acinar tissue		Liver	
	Rf (7)	Relative activity (6)	Rf (7)	Relative activity (6)	Rf (9)	Relative activity (7)
Anodal isoenzyme	0.49 ± 0.02	39.9 ± 4.2	0.49 ± 0.02	51.4 ± 2.7	0.50 ± 0.01	49.2 ± 2.6
Cathodal isoenzyme	0.10 ± 0.02	60.1 ± 4.2	0.09 ± 0.02	48.6 ± 2.7	0.15 ± 0.02	50.8 ± 2.6

1.1 m*M* of oxaloacetate was used as substrate. Mean values ± S.E.M. The number of animals studied are given in parentheses.

were found to be apparently identical for isoenzymes of different cellular origins. These distances, as calculated in relation to that of bromphenol blue, are given in Table II.

It was ascertained that a linear relationship existed between the amount of homogenate applied to the gel and the activity extracted after preceding staining. The quantitative distribution of enzyme activity between the different molecular forms of MDH in the islets, exocrine parenchyma and liver is presented in Table II. For all tissues, the enzyme activity towards oxaloacetate was quite uniformly distributed between the 2 isoenzymes. In further support of this, 2 peaks of about the same area were obtained when the MDH activity from the endocrine pancreas was measured in all parts of an unstained gel, sliced into discs of equal sizes (Figure 2).

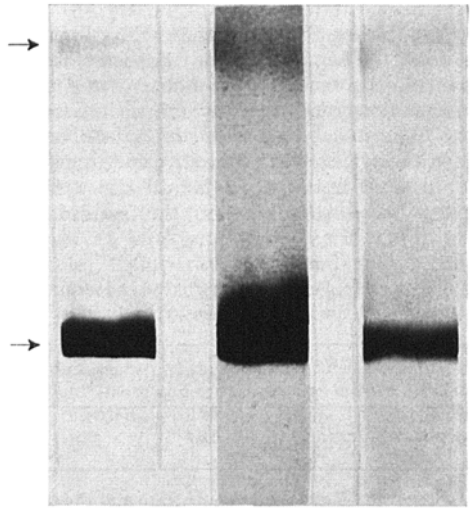

Fig. 1. Malate dehydrogenase isoenzymes from the endocrine (left) and exocrine (middle) pancreas and the liver (right). The cathodal ends of the gels are at the top.

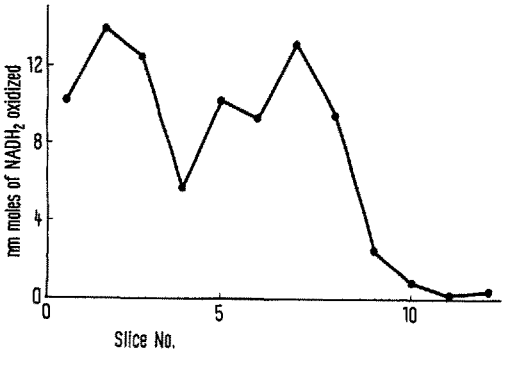

Fig. 2. Malate dehydrogenase activity in slices (3 mm each) of a polyacrylamide gel after electrophoresis of an islet homogenate prepared as described in the text. The cathodal end of the gel is to the left.

Discussion. The present data support the recent suggestion that disc electrophoresis may be a valuable analytical and preparative tool in studying the histochemistry of the B-cells in mammals[6,12]. The American obese-hyperglycemic mice were considered suitable for an electrophoretic study of the MDH, since their islets are composed of more than 90% B-cells[13] and, in addition, showed a considerable activity of this enzyme, like the endocrine pancreas of other species[1,2]. The molecular configuration of the MDH in the islets did not prove to be different from that of the enzymes in liver and exocrine pancreas, as judged from the electrophoretic mobilities. In evaluating the relative activity of the different isoenzymes, it should be remembered that the fluorophotometric measurements were performed with oxaloacetate as substrate, whereas the staining of the gels revealed activity towards malate. The interesting suggestion that the forward and backward reactions are catalysed by different molecular forms of MDH in the cells[3], receives some support from the present observation that the cathodal fraction, which exhibited the weakest staining with malate as substrate, was at least as active as the anodal one towards oxaloacetate. The fact that similar results were obtained for all 3 tissues indicates furthermore that this suggestion is as true of the islets as of the exocrine pancreas and the liver[14].

Zusammenfassung. Mit der Polyacrylamidgel-Elektrophorese lassen sich aus Langerhansschen Inseln fettsüchtig-hyperglykämischer Mäuse 2 Fraktionen von Malatdehydrogenase isolieren. Die rascher wandernde Fraktion scheint eine grössere Aktivität gegen Malat zu besitzen, während in der Aktivität gegen Oxalacetat kein Unterschied besteht.

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