L-GLUTAMATE DEHYDROGENASE IN RAT AND HUMAN FETAL LIVER SLICES

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Preliminary experiments showed that hepatic glutamate dehydrogenase (EC 1.4.1.2. L-glutamate : NAD oxidoreductase) appears almost simultaneously with the development of the liver in the earliest human fetuses studied. Glutamate dehydrogenase activity was readily evident at 12 weeks of development and had doubled by 16 weeks. Glutamate dehydrogenase was present on the 17th day of development in the rat liver and increased to a maximum some two weeks after birth, remaining at that level until at least eight weeks of age. The present paper shows that the activity of glutamic dehydrogenase is increased greatly when liver slices are incubated in vitro in the presence of glutamate or certain other substances.

METHODS

Sprague-Dawley female rats, 17-19 days pregnant, were obtained from the Charles River Breeding Laboratories in Wilmington, Mass. Human fetal liver tissue was obtained at therapeutic abortions. The livers were sliced into 50 mg portions and placed in 25 ml Erlenmeyer flasks containing 10 ml balanced salt solution (Earle, 1943) with or without 20 mg/ml of glutamate or malate. A portion (0.25 gm) of the liver was homogenized immediately in 2.0 ml of ice-cold 0.1 M Tris-HCl buffer, pH 7.6,

and frozen for subsequent assays to determine enzyme activity at zero time.

Slices were incubated from 2 to 8 hours at 37° C in a Dubnoff shaker. A mixture of 95% O_2 - 5% CO_2 was bubbled slowly through the solution for the duration of the incubation. At appropriate times liver slices were removed, rinsed, blotted dry, weighed, and homogenized in Tris buffer. The homogenate was rapidly frozen in dry ice-acetone and stored for subsequent assays of enzyme activity.

Previous experiments had shown that the combination of homogenizing, freezing and thawing released essentially all of the glutamate dehydrogenase activity. After thawing, the homogenate was centrifuged in a Servall refrigerated centrifuge at 20,000 x g for 20 minutes. The glutamic dehydrogenase activity of the resulting clear supernatant solution was measured in a Beckman DB spectrophotometer with a recording potentiometer by the increase in optical density at 340 mu during the first minute of reaction. Each cuvette contained, in total volume of 1 ml, 0.01 ml of supernatant, 4 umoles of NAD, 20 umoles potassium glutamate, and 59 umoles 0.1 M Tris buffer, pH 7.6. The conditions for the assays of other dehydrogenases were identical save for the replacement of glutamate by the proper substrate in the reaction vessel. Glutamate dehydrogenase was subjected to disc electrophoresis on 4% polyacrylamide gel using the technique of Van Bogaert et al (1967).

RESULTS

Incubation of slices of 20-day fetal rat liver in Earle's balanced salt solution resulted in no marked loss of activity of glutamate, lactate, malate or isocitrate dehydrogenase during a four-hour incubation (Fig. 1). The activity of these dehydrogenases in slices of adult liver was maintained for

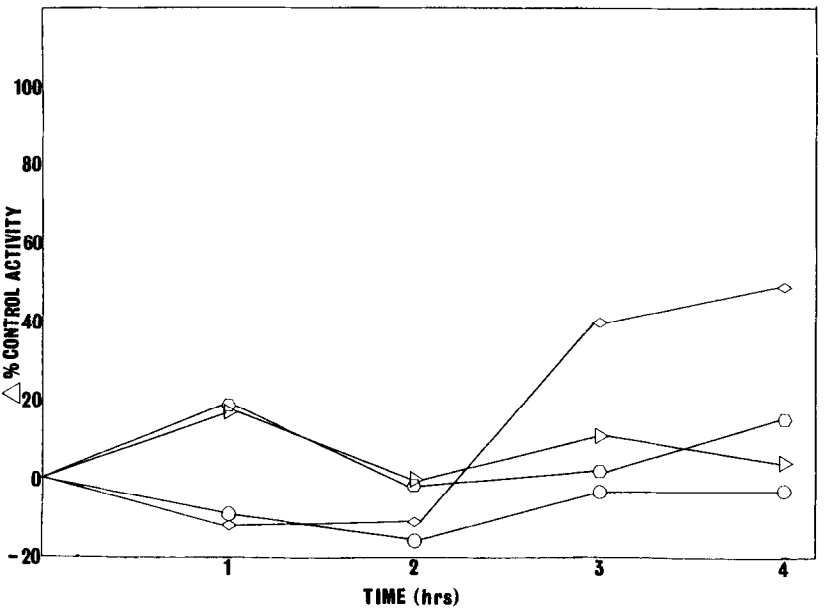

Fig. 1. Changes in the specific activities of glutamate dehydrogenase (◇——◇), malate dehydrogenase (⬡——⬡), lactic dehydrogenase (▷——▷), and isocitrate dehydrogenase (○——○) expressed as the percentage of activity at zero time. Fetal (21 day) rat livers were excised and incubated in 10 ml Earle's balanced salt solution at 37° C with 95% O_2-5% CO_2. The results represent the average of two fetal preparations.

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at least eight hours of incubation. The activity of glutamate dehydrogenase, but not of the other dehydrogenases, increased when either fetal or adult liver slices were incubated. An even greater increase in the specific activity of the glutamate dehydrogenase was noted when fetal liver slices were incubated in the presence of potassium glutamate, 20 mg/ml (Fig. 2). The decrease in the specific activity of GDH after four hours of incubation suggests that the fetal GDH is less stable than the GDH of adult liver which

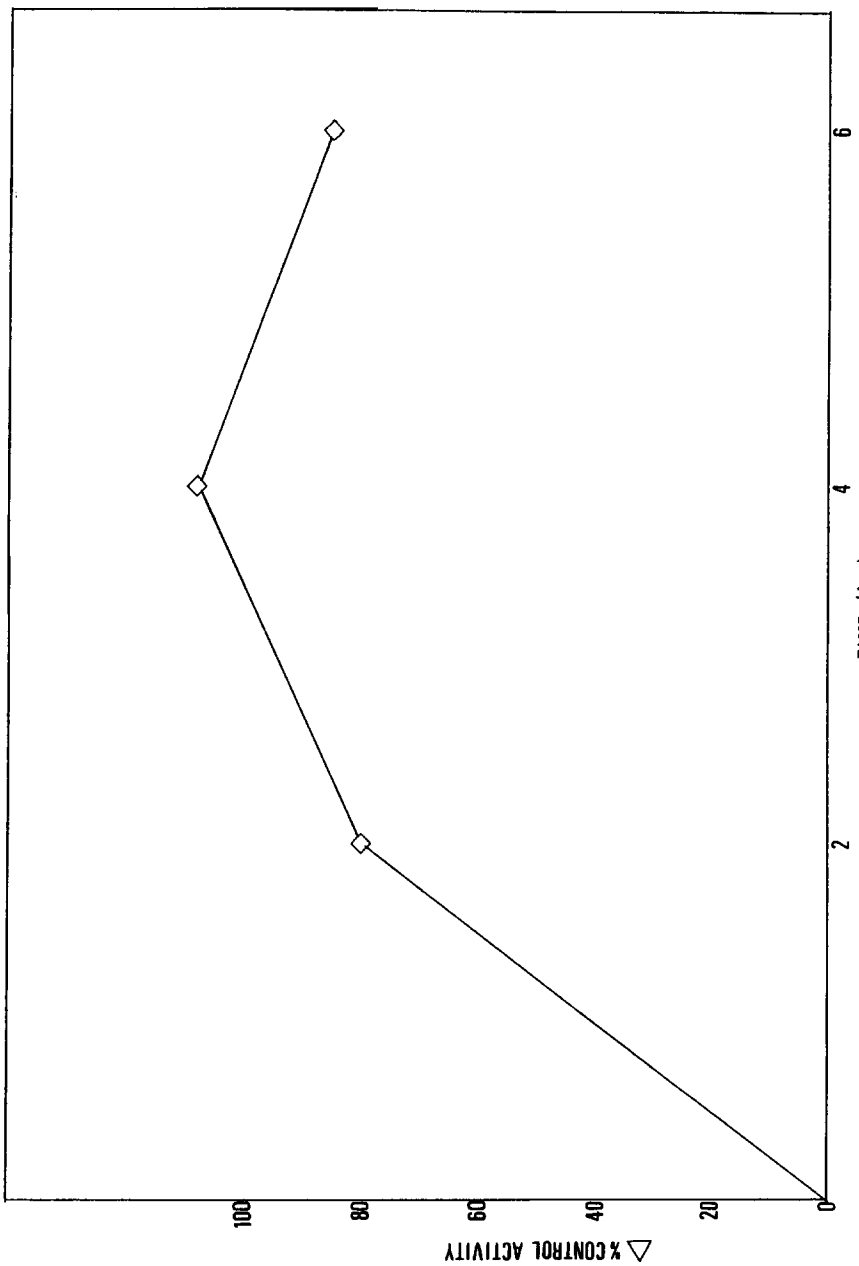

Fig. 2. Increase in specific activity of glutamate dehydrogenase of fetal rat liver during incubation of liver slices in Earle's solution containing 20 mg/ml potassium glutamate. Each point represents the average of two 20-day rat fetal liver preparations.

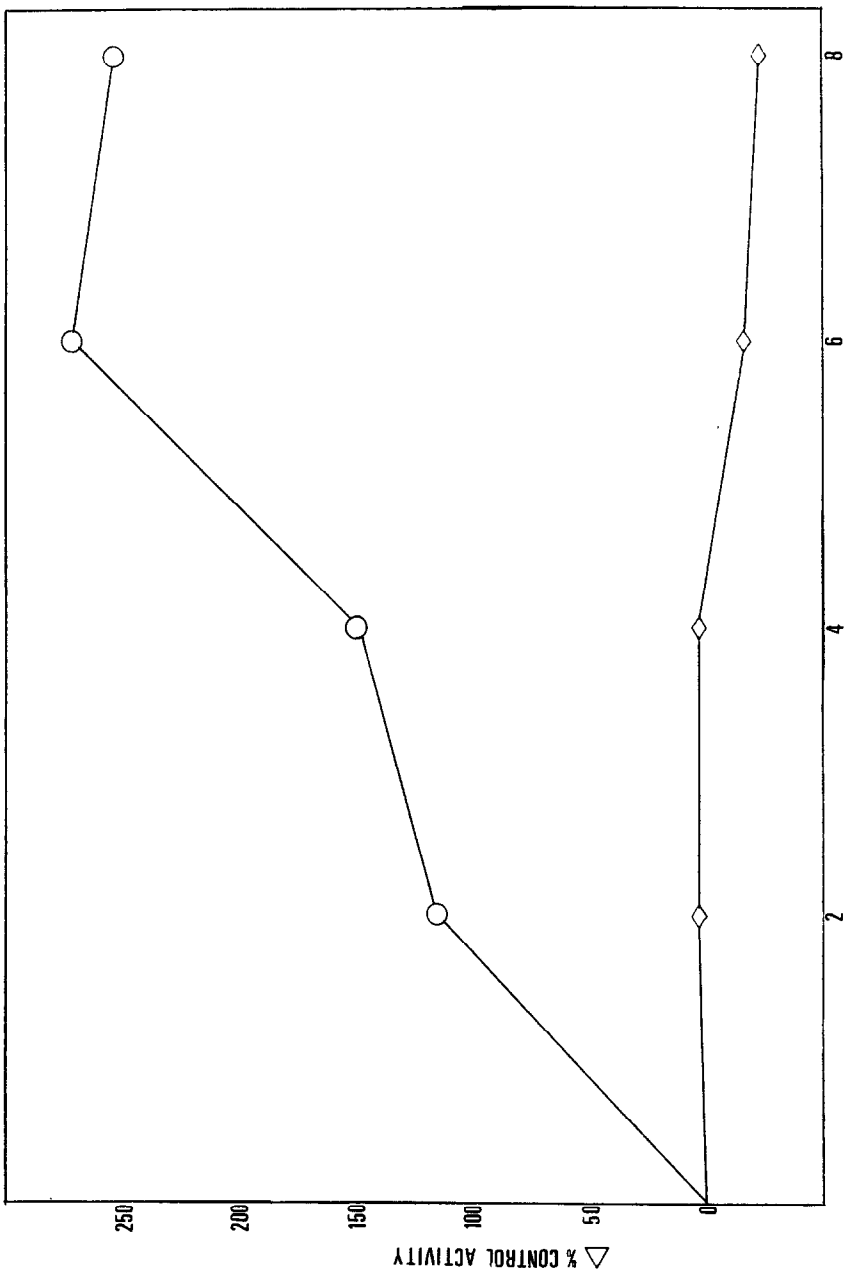

Fig. 3. Specific activities of glutamate dehydrogenase (○——○) and malate dehydrogenase (◇——◇) during incubation of slices of fetal (20 day) rat liver in the presence of 20 mg/ml sodium malate in Earle's solution.

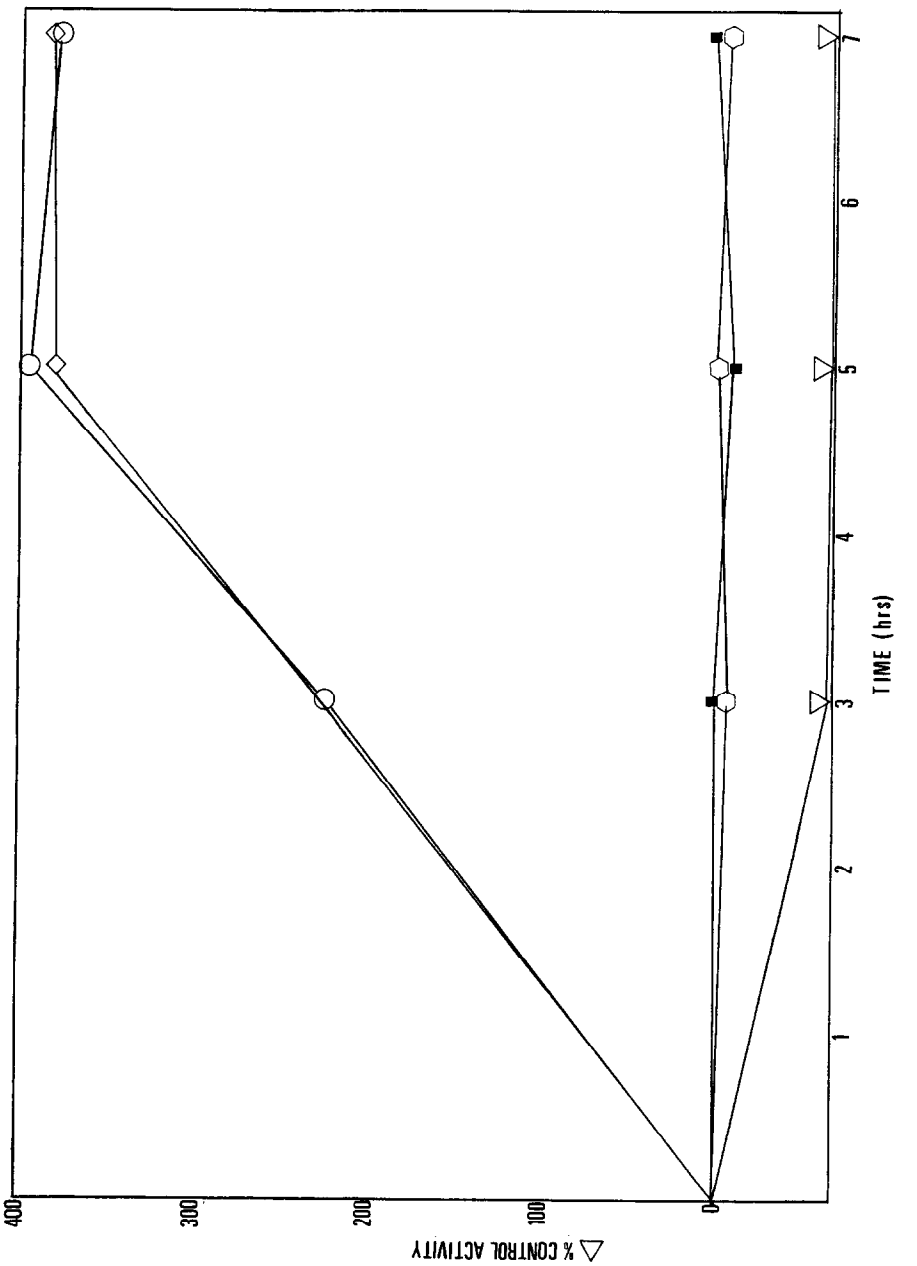

Fig. 4. Effect of the addition of sodium malate, 20 mg/ml, to the incubation medium upon glutamic dehydrogenase and malate dehydrogenase of human fetal liver slices. Glutamate dehydrogenase activity with (○——○) and without (▽——▽) added malate and glutamate

dehydrogenase activity with malate plus 20 ug actinomycin D per ml (◇——◇). Malate dehydrogenase with (○——○) and without (■——■) added malate. The fetus was a 16-week female measuring 12 cm crown-rump length.

maintained a high activity even after ten hours of incubation.

The activities of isocitrate, lactate and malate dehydrogenases were not increased when liver slices were incubated in a medium containing their respective substrates. However, malate was even more effective than glutamate in increasing the activity of glutamate dehydrogenase in fetal liver slices during prolonged incubation (Fig. 3). In the presence of 20 mg malate/ml of Earle's salt solution the GDH activity of rat fetal liver slices increased to 275% of the control values at six hours, whereas the malate dehydrogenase activity had decreased to 75% of the control value for MDH. A series of further experiments confirmed the finding that malate is even more effective than glutamate in increasing the activity of glutamate dehydrogenase in rat fetal liver slices.

Similar experiments with slices of human fetal liver showed that glutamate dehydrogenase activity was increased by the addition of glutamate or malate to the Earle's salt solution (Fig. 4). The GDH activity of the slices incubated in malate increased fourfold over control values in five hours. A comparable increase occurred when 20 mg of actinomycin D/ml of Earle's salt solution was present along with the malate.

The electrophoretic patterns of the glutamic dehydrogenase of fresh human fetal liver and from liver slices incubated four hours in the presence of malate were identical (Fig. 5). The greater activity of the GDH after incubation is reflected in the relatively larger size of the bands.

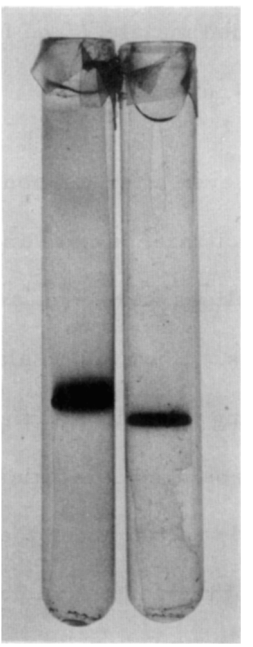

Fig. 5. Zymograms of glutamate dehydrogenase from human fetal livers (right) extracted immediately and (left) extracted from liver slices after four hours of incubation in Earle's solution containing 20 mg malate per ml.

DISCUSSION

That the activity of an enzyme may be regulated by its substrate has been documented for many enzymes. Thus, the injection of tyrosine into adult animals increased the activity of tyrosine-α-ketoglutarate transaminase sevenfold over a five-hour period (Lin and Knox, 1957), whereas the administration of tyrosine to fetal rats in utero failed to increase appreciably the low levels of tyrosine-α-ketoglutarate transaminase in fetal liver

(Sereni et al, 1959). The activity of tryptophan pyrrolase in fetal rat liver was also unaffected by injections in utero of L-tryptophan (Nemeth, 1959). The enzymes present in the livers of newborn and adult animals and in adults injected with substrate are chromatographically identical (Nemeth, 1961).

As controls for the present experiments both young and mature intact rats were injected with sodium glutamate or sodium malate but neither altered the activity of glutamate dehydrogenase in the liver. This might indicate simply that neither substance was accumulated by the hepatic cells in the intact animal. The concentrations of the free amino acids are greater in the livers of 22-week human fetuses than in the adult liver (Ryan and Carver, 1966).

The possibility that the increase in activity represents de novo synthesis of protein requiring the de novo synthesis of DNA-dependent RNA appears unlikely, since the addition of actinomycin D (20 ug/ml) did not prevent the increased activity. Other experiments showed that puromycin (100 ug/ml) did not prevent the increased activity of glutamate dehydrogenase in slices of adult rat liver. The possibility that the increase in activity in glutamate dehydrogenase results from the stabilization of the enzyme or from a decreased turnover of the enzyme in the presence of the substrate seems unlikely. In long-term incubations the activity of glutamate dehydrogenase after eight hours of incubation decreased to a greater extent in media containing substrate than in media without substrate. In other experiments the addition of glutamate to extracts of either fetal or adult liver did not protect the enzymatic activity; the fraction of activity remaining in extracts without substrate after 24 hours of storage in the cold was as great as that in extracts to which substrate was added.

Glutamate dehydrogenase is a tetramer with a molecular weight of 10^6 (Olson and Anfinsen, 1952) which undergoes dissociation to monomers of 2.5×10^5 (Frieden, 1958). The addition of estrogens, ATP or 1,10-phenanthroline increases alanine dehydrogenase activity and inhibits glutamate dehydrogenase activity. Conversely, ADP, DPN or TPN increases GDH activity and decreases alanine dehydrogenase activity (Tomkins et al, 1961). To test the possibility that incubation of tissue slices might be affecting the molecular configuration of the enzyme, the alanine dehydrogenase activity of several preparations was investigated along with the glutamic dehydrogenase activity. In all experiments the ratio of alanine dehydrogenase and glutamic dehydrogenase activities remained constant, which suggests that the enzyme was not undergoing a change in association during the incubation.

ACKNOWLEDGEMENT

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