L-Glutamate Dehydrogenase Isoenzymes

SEVERAL dehydrogenases have been resolved into isoenzymes by electrophoresis in starch gel. Attempts to fractionate L-glutamate dehydrogenase in this way, however, have so far failed. Markert and Moller¹ found that this enzyme was electrophoretically immobile in starch gel, and Tsao² obtained negative results in attempts to fractionate and render glutamate dehydrogenase visible.

Using a technique developed for determination of lactate dehydrogenase isoenzymes in clinical routine^{3,4}, however, it proved possible to demonstrate multiple forms of glutamate dehydrogenase in homogenates of human organs.

Fractionation was effected by electrophoresis in agar gel, and the dehydrogenase activity was made visible by incubation of the gel in a solution containing sodium glutamate, diphosphopyridine nucleotide, phenazine methosulphate and a tetrazolium salt.

Human tissues were obtained by autopsy less than 12 hr. after death. The tissues were stored at -20°C . for one night. Tissue extracts were prepared by homogenizing 1 gm. of tissue with 1 ml. water by means of a motor-driven Potter-Elvehjem tissue grinder. The extracts were clarified by centrifugation. Electrophoresis was carried out with 8 μl . of the extract, using the technique on microscope slides of Wieme⁵. The slide was then incubated for 1 hr. at 37°C . in the following medium: sodium-DL-glutamate (M) adjusted to pH 7.4 (1 ml.); diphosphopyridine nucleotide (10 mgm./ml.) (1 ml.); sodium cyanide (0.1 M) (1 ml.); phosphate buffer (0.5 M , pH 7.4) (2.5 ml.); nitroblue-tetrazolium (1 mgm./ml.) (2.5 ml.); phenazine methosulphate (1 mgm./ml.) (0.25 ml.).

At the sites of dehydrogenase activity purple deposits of formazan are formed during incubation. When glutamate is omitted from the incubation fluid, no formazan bands are formed. After incubation the slide is washed with water and, if desired, densitometry can be carried out with the wet slide. The proteins can also be fixed in the following fluid: ethanol 70 vol., acetic acid 5 vol., distilled water up to 100 vol. The plate on which the protein fractions become visible during fixation is afterwards dried while covered by filter paper. Densitometry can then be carried out on the dry plate. The slides must be stored protected from daylight; in the light, a background slowly develops. The distance between human albumin and dextran, run on the same plate, was taken as unit of measurement for the determination of relative mobilities.

The result obtained by this technique with an extract of human heart muscle is shown in Fig. 1. Five glutamate dehydrogenase fractions are visible, and some activity is retained in the application slit. The results of electrophoretic separation of glutamate

Fig. 1. Distribution of glutamate dehydrogenase isoenzymes in the myocardium

Table 1. DISTRIBUTION OF GLUTAMATE DEHYDROGENASE ISOENZYMES IN HUMAN TISSUES

Fraction	I	II	III	IV	V	VI
Relative mobility	0.95	0.74	0.46	0.20	0.00	-0.12
Liver	+	+++	+	+	++	+
Heart	++	+++	+	+	+	+
Kidney	+++	+++	+	++	++	+
Spleen	+++	+++	+	++	++	+

+, Weak; ++, medium; +++, strong band.

dehydrogenase isoenzymes of other tissues are given in Table 1. A total of six isoenzymes has been found. Also it appears that there are differences in the isoenzyme contents of the different tissues.

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¹ Markert, C. L., and Moller, F., *Proc. U.S. Nat. Acad. Sci.*, **45**, 753 (1959).

² Tsao, M. U., *Arch. Biochem. Biophys.*, **90**, 234 (1960).

³ Helm, H. J. van der, *Lancet*, 108 (1961).

⁴ Helm, H. J. van der, *Clin. Chim. Acta*, **7**, 124 (1962).

⁵ Wieme, R. J., *Clin. Chim. Acta*, **4**, 317 (1959).

Heat-Coagulation of Edestin

PROTEINS in aqueous solution are denatured by heating; few separate proteins resist the temperature of boiling water for more than a few minutes. It is therefore surprising to find that under certain conditions a protein molecule as large as that of edestin can be heated for a long time at 100°C . without causing any change. This was observed during experiments being carried out to establish the best conditions for complete heat-coagulation of edestin.

For this purpose we selected conditions similar to those used by M. and S. P. L. Sørensen¹ for the heat-coagulation of ovalbumin, namely, an acidity very near the isoelectric point and a high ionic strength (1/3 saturated sodium sulphate). The isoelectric point of edestin being unknown, it was necessary to establish the optimal pH under the conditions which were as follows: to one volume of a solution in 10 per cent sodium chloride and 0.1 M sodium bicarbonate of a carefully purified edestin was added one volume of a buffer mixture and one of a saturated solution of sodium sulphate. The mixture was heated in a boiling water bath (98°C .) for 20 min. and filtered hot on steam-heated funnels. The amount of uncoagulated protein in the filtrate was determined by the Kjeldahl procedure. Measurements of pH were made at room temperature before heating.

The results (Fig. 1) show that coagulation is practically complete at pH 4.6 and lower. It may be remarked that at these low pH values the protein precipitates before heating. Only an insignificant amount of clot is formed at pH 5.8–7.8, 95 per cent or more remaining in solution. At higher pH values partial coagulation occurs with a maximum around pH 10.

The interesting pH-range is that where practically no coagulation occurs. At first, we thought that denaturation did occur but that coagulation had been prevented by the high temperature. Cooling of the filtrates did show indeed the formation of a precipitate, but only at pH values of less than 6.3, as indicated by the broken curve in Fig. 1. It is interesting to note that at pH 5.5 the protein is soluble when heated but precipitates when cooled, and this may be repeated at will, imitating the behaviour of Bence-Jones protein.