An Assay for L-Alanine-Glyoxylate Amino-transferase

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We have adopted an assay method for L-alanine-glyoxylate aminotransferase that measures pyruvate formation colorimetrically by the salicylaldehyde method of Berntsson (1955). Incubations contain 0.02 M-L-alanine, 0.02 M-glyoxylate, 0.05 M-phosphate buffer, pH 7.4, and tissue homogenate prepared in phosphate buffer. Samples are taken into an equal volume of 10% (w/v) trichloroacetic acid and pyruvate is measured after centrifugation. The other materials present in the denatured extract do not interfere.

For the same assay pyruvate concentrations are equivalent whether measured by the salicylaldehyde method or by a modification of the lactate dehydrogenase method of Thompson & Richardson (1968).

Glycine formation measured by separation on paper is equivalent to pyruvate for incubations with rat and rabbit liver. For these experiments assay samples were inactivated in perchloric acid. After neutralization with potassium hydroxide, supernatant samples were incubated at 25° for 30 min. with 1% (w/v) hydrogen peroxide to remove glyoxylate before paper chromatography; otherwise, non-enzymic transamination occurred, apparently on the paper.

The salicylaldehyde method is not very sensitive; 1 μ mole of pyruvate gives an extinction of 0.15 and the reagent blank is high (extinction 0.3). Nevertheless, we obtain linear progress curves and proportionality between initial rate and tissue concentration, and the method is simple.

If it were easy to dispose of glyoxylate specifically in extracts one could take advantage of the greater sensitivity of the dinitrophenylhydrazine method for pyruvate determination. A procedure based on this principle, reported by Rao & Ramakrishnan (1962), removes glyoxylate by reaction with cysteine before pyruvate analysis. In our experience much pyruvate is also removed by cysteine.

From assays with salicylaldehyde we previously reported especially high activities for the enzyme in rabbit and pig livers (Rowsell, Carnie & Taktak, 1966). We suggested this might signify a large requirement for glycine synthesis for glycocholate formation. However, this cannot be the only factor involved. Extension of this work has reproducibly revealed high activities in cat liver (1550 μ moles of pyruvate/g./hr.), and in frog and toad liver (4690–7120), and glycine conjugates do not occur in the bile of these species (Haslewood, 1967).

We find this enzyme does not occur in brain, heart, skeletal muscle or spleen of mouse, rat, guinea pig or rabbit. Kidney activity varies with species, but in every case the total kidney contribution is a small fraction of the total liver enzyme.

- Berntsson, S. (1955). *Analyt. Chem.* **27**, 1659.
Haslewood, G. A. D. (1967). *Bile Salts*. London: Methuen and Co. Ltd.
Rao, N. A. N. & Ramakrishnan, T. (1962). *Biochim. biophys. Acta*, **58**, 262.
Rowsell, E. V., Carnie, J. A. & Taktak, B. (1966). *Biochem. J.* **101**, 42P.
Thompson, J. S. & Richardson, K. E. (1968). *Analyt. Biochem.* **24**, 197.

The Separation of Rat Liver Mitochondria into Two Morphologically Different Fractions by Density-Gradient Centrifugation

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Apparent separation of rat liver mitochondria into different populations by centrifugation has been ascribed either to abnormalities caused by high sucrose concentration or to contamination of mitochondrial fractions by other cell organelles such as lysosomes or microbodies (Beaufay *et al.* 1964).

Hackenbrock (1966, 1968) showed that mitochondria in different metabolic states differ in their ultrastructure.

Mitochondria prepared as described by McMurray & Dawson (1969) were placed on a continuous sucrose gradient ranging from 0.9 M to 1.9 M, containing 0.001 M-EDTA, and centrifuged at 130 000 g_{av} for 90 min. in the 59589 MSE swing-out rotor. Three distinct bands were formed: the top band consisted mainly of damaged mitochondria, but the middle band (sp.gr. 1.184) and the lowest band (sp.gr. 1.216) consisted of intact mitochondria. The 1.184-sp.gr. mitochondria resembled the 'condensed' type described by Hackenbrock (1968), and the mitochondria in equilibrium with the 1.216-sp.gr. medium resembled 'orthodox' mitochondria. Both types had identical protein:phospholipid ratios and showed no significant qualitative or quantitative differences in the fatty acid composition of their neutral lipids.

Both types had similar specific activities for cytochrome oxidase, glutamate dehydrogenase, malate dehydrogenase and monoamine oxidase, but glucose 6-phosphatase and acid phosphatase were virtually absent.

In normal rat liver the lower-density mitochondria predominated, but in starved rats both