INTRACELLULAR DISTRIBUTION IN THE RAT LIVER OF CERTAIN ENZYME SYSTEMS OF SERINE METABOLISM

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ENZYME experiments in mammalian tissues are conducted with slices, homogenates or extracts. Studies with slices may introduce difficulties because of permeability factors, while results with extracts may be misleading because of variability in the amount of material discarded and lack of knowledge concerning the relation between the activity of the extract and that of the discarded portion. It is therefore, generally advantageous to use whole homogenates in which permeability factors are ruled out and no part of the activity is discarded.

During recent years, there has been considerable interest in investigations concerning localization of enzyme activities in tissue cells. It is now well recognised that cell metabolism is closely co-ordinated and organised within the constituent cell fractions, the nuclei, the mitochondria and the soluble phase (Dounce, 1950; Bradfield, 1950; Claude, 1950; Holter, 1952). This ' chemical geography ' of the living cell has become increasingly apparent with studies on the intracellular localization of different enzyme systems.

It was of interest to extend the observations reported in earlier communications (Nadkarni and Sreenivasan, 1957 *a, b*) on the enzyme system involved in the metabolism of serine to a study of their intracellular distribution.

EXPERIMENTAL

Separation of Cell Fractions.—Adult albino rats maintained on the laboratory stock diet were decapitated and the livers were quickly removed and chilled over cracked ice. After removing the connective tissues, the liver pieces were weighed and homogenised in a glass homogeniser with cold isotonic (0·25 M) sucrose solution (Schneider and Hogeboom, 1950) to make a 20 per cent. suspension. 15 ml. of the sucrose homogenate were centrifuged in an International Refrigerated Centrifuge (PR-2) at 700×*g*, for 10 minutes for separation of nuclei and unbroken liver cells. The supernatant was kept aside in the cold for further separation. The sediment was

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washed with 5 ml. of isotonic sucrose by resuspending and recentrifuging. The sediment was suspended in 15 ml. of cold isotonic sucrose and was used as the nuclear fraction (N). The supernatant and washings were mixed together and recentrifuged at $5600 \times g$, for 10 minutes. The sediment was resuspended in 15 ml. of isotonic sucrose and was used as the mitochondrial fraction (M). The supernatant translucent part of the homogenate from which most of the large size granules were removed, was used as the supernatant fraction (S). The procedure was essentially that of Schneider and Hogeboom (1948).

The procedures for serine decarboxylation, glycine to serine conversion, ethanolamine to serine conversion, and serine deamination, were as detailed in earlier communications (Nadkarni and Sreenivasan, 1957 *a, b*).

For study of serine deamination, fractionation was carried out with isotonic saline (0·9 per cent.) (Schneider *et al.*, 1948). The procedure was otherwise the same as for sucrose homogenates. It was found that, with sucrose as medium, there was interference in the determination of NH_3 by the Nessler's Reagent. The pattern of separation of the cell constituents was the same as with isotonic saline or sucrose as revealed from succinic dehydrogenase activity determinations (see results).

RESULTS AND DISCUSSION

It was experienced that under the conditions of the foregoing fractionation, some mitochondria were sedimented along with the nuclei. The extent of such contamination by mitochondria of the nuclear fraction was assessed from determinations of succinic dehydrogenase activity which is associated only with mitochondria (Schneider *et al.*, 1948; 1950). Succinic dehydrogenase activity was followed manometrically in Warburg flasks. The main compartment contained 1·0 ml. of 0·1 M phosphate buffer pH 7·4, 0·2 ml. of 0·01 M methylene blue, 1·0 ml. of liver homogenate or its equivalent liver fraction and 0·6 ml. of water. The side arm contained 0·2 ml. of 0·1 M sodium succinate and to the centre cup was added 0·2 ml. of 10 per cent. KOH. The liver homogenate (or the corresponding fraction) was diluted to make a 1 : 50 suspension of the whole liver (Table I).

The results with the different enzymes concerned in serine metabolism are given in Table II.

Serine decarboxylase activity resides largely in the mitochondrial fraction. This is also the case with the system that brings about conversion of ethanolamine to serine. The presence of a CO_2-fixing enzyme in mitochondria has been reported recently (Lardy and Adler, 1956).

TABLE I

Distribution of Succinic Dehydrogenase activity in liver fractions as obtained

	Succinic Dehydrogenase	
	Activity Q_{O_2} $\mu l./$mg. N/hr.	Recovery per cent.
Whole liver homogenate ..	60	100
Nuclei ..	16	26·0
Mitochondria ..	46	76·6
Supernatant ..	nil	nil

TABLE II

Serine Metabolism : Intracellular distribution of enzymes

	Serine Decarboxylation		Ethanolamine to serine conversion		Serine dehydrase		Glycine to serine conversion	
	Activity $\mu l.$ $CO_2/$mg N	Recovery per cent.	Activity μ moles of serine/mg.N	Recovery per cent.	Activity μ moles NH_3 per mg. N	Recovery per cent.	Activity μ moles of serine/mg. N	Recovery per cent.
Whole liver homogenate	3·54	100	0·93	100	3·41	100	1·10	100
Nuclei ..	0·65	21	0·26	28	2·83	83	0·38	32
Mitochondria ..	2·65	71	0·70	75	0·66	19	0·80	71
Supernatant ..	0·40	12	nil	nil	0·08	2·3	nil	nil

Glycine to serine conversion is also brought about in mitochondria. Mitoma and Greenberg (1952) studied incorporation of formaldehyde into serine with mitochondria. Mackenzie *et al.* (1953) observed that both the washed sediment and the supernatant were required for the conversion of dimethylaminoethanol to formaldehyde. On the other hand, formaldehyde was isolated when methanol was incubated with the supernatant alone or when dimethylglycine or sarcosine were incubated with the washed sediment only. Thus, the enzyme system for formation of formaldehyde is associated

with the supernatant as well as mitochondria. It would seem that whether the 1-C fragment is formate or formaldehyde, mitochondria provide the enzyme system for its condensation with glycine to form serine. This is also indicated in the cyclophorase system in rabbit liver (Sarkar et al., 1952).

Deamination of serine is maximal with the nuclear fraction. Serine deamination has been reported only in micro-organisms and in tissue extracts. The present observation that nuclei are the active centre of this enzyme is therefore of interest.

SUMMARY

The activities of the enzyme systems for serine decarboxylation, ethanolamine to serine conversion, serine synthesis from glycine and serine deamination, have been followed with cell fractions obtained by differential centrifugation.

Serine decarboxylation, ethanolamine to serine conversion and glycine to serine conversion are associated largely with the mitochondria, while serine deamination is maximum in the nuclear fraction.

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