Intracellular localization of catalase and of some oxidases in rat liver

In rat liver, the intracellular distribution of uricase has been found by several workers to resemble that of acid phosphatase, without, however, being entirely identical with it. The significance to be attached to these findings has been diversely appreciated. While HOGEBOOM *et al.*[1] have favoured the view that the two enzymes belong to the same particles, other authors like THOMSON AND MOSS[2] have preferred to leave the matter open. In this laboratory, acid phosphatase has been found to be associated, together with a number of other acid hydrolases, with a special group of cytoplasmic particles which have been called *lysosomes* (for a review see ref. 3). The general parallelism between uricase and the lysosomal enzymes has been confirmed, but attention has been drawn to the greater homogeneity and higher D_2O-binding capacity of the uricase-containing particles, as possible indications that they may be distinct from the lysosomes[3-5].

Conclusive evidence supporting the latter view has now been obtained by submitting mitochondrial fractions from rat liver to density equilibration in a gradient of glycogen, extending linearly from 0–0.23 g/ml in 0.5 *M* aq. sucrose. In this system, the uricase particles accumulate in the layers immediately below the sedimentation boundary of the glycogen and are almost completely separated from the lysosomes, which are concentrated at the bottom of the tube. The bulk of the mitochondria occupy an intermediate position. Like the lysosomes, the uricase-containing particles are associated with a very small proportion of the total cell nitrogen.

Two other enzymes, catalase and D-amino acid oxidase, have been found to share the main properties of uricase. As already indicated by the investigations of THOMSON AND KLIPFEL[6] on catalase and of PAIGEN[7] on D-amino acid oxidase, they tend to concentrate in the light mitochondrial fraction in fractionations performed according to DE DUVE *et al.*[4]. In addition, they show a higher equilibrium density than the mitochondria and even than the lysosomes in a sucrose–D_2O gradient prepared according to BEAUFAY *et al.*[5], and a lower one in the glycogen–0.5 *M* sucrose gradient described above. Their density–distribution curves do not, however, coincide perfectly with that of uricase in the same systems and it must therefore be concluded that the three enzymes either belong to separate particles showing very similar properties or that they are associated together, but in varying proportion, with a single group of particles. In the latter event, the particles concerned could be an important site of H_2O_2 metabolism, since they would contain two oxidases known to form this compound, in association with the most important enzyme destroying it. Another implication of these findings is that the protection of cellular constituents against H_2O_2, formed, for instance, under the influence of ionizing radiations, may be restricted to very small areas of the liver cell.

In contrast with the oxidases mentioned above, monoamine oxidase shows a true bimodal distribution. The mitochondrial component, which accounts for about 70% of the total activity, accompanies cytochrome oxidase in several types of gradients and must be considered truly mitochondrial. The microsomal component, on the other hand, can be largely separated from the bulk of the microsomal protein, phospholipid and RNA, as well as from several other enzymes present in this fraction (glucose-6-phosphatase, esterase, DPNH-cytochrome *c* reductase, β-glucuronidase,

Abbreviations: DPNH, reduced diphosphopyridine nucleotide; RNA, ribonucleic acid.

fumarase) by density equilibration in an aqueous sucrose gradient. In this system, monoamine oxidase shows a distinctly lower equilibrium density than the other components. Fumarase, on the other hand, shows an exceptionally high equilibrium density, which depends on the presence of intact RNA.

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The chromatographic behaviour and adenosine triphosphatase activities of the meromyosins

Previous investigations[1] have shown that when L-myosin is chromatographed on diethylaminoethylcellulose the ATPase activities of the eluted fractions vary in a characteristic way. Comparable results have also been obtained in preliminary studies with carboxymethylcellulose[2] and BRAHMS[3] has also reported a fractionation of the ATPase activity. To assess the significance of these indications of the enzymic heterogeneity of chromatographed myosin, L-meromyosin and H-meromyosin have been studied by similar techniques.

H-meromyosin, prepared by the method of SZENT-GYÖRGYI[4] and shown to sediment as a single peak in the ultracentrifuge, was consistently fractionated into two components when applied to a diethylaminoethylcellulose column equilibrated against 0.15 *M* KCl, 20 m*M* Tris–HCl, pH 7.6 or 8.2. Of the total eluted material estimated by its absorption at 280 mμ, 12–13 % passed through the column unheld, whereas the main component was eluted as a symmetrical peak at 0.19–0.20 *M* KCl,

Abbreviations: ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane.