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On the tissue and subcellular distribution of multiple forms of catalase in the rat

Although mammalian catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) is one of the most intensively investigated of all enzymes, the extent and nature of its multiplicity remain ill defined. In 1954, PRICE AND GREENFIELD[1] provided suggestive evidence for the existence of multiple forms, and several other indications have been reported since[2–5]. NISHIMURA *et al.*[2] have reported that purified rat liver catalase can be separated by chromatography into two peaks with different immunoelectrophoretic patterns. In 1965, HOLMES AND MASTERS[3] provided the first clear evidence of an extensive multiplicity of catalase activity in rat liver by elution electrophoresis and continuous gradient chromatography. More recently, the interrelationships of catalase heteromorphs from other tissue sources have been investigated by CANTZ *et al.*[6] and HEIDRICH[7] and HEIDRIG AND HANNIG[8].

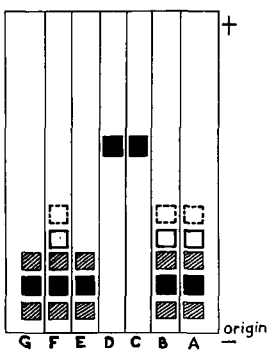
In the present communication, some recent findings on the cellular and subcellular distribution of catalase multiple forms, which appear to necessitate a reappraisal of current views on the biological characteristics of this enzyme, are presented.

For the study of this enzyme, fresh 10% (w/v) homogenates of the appropriate tissues were prepared in Potter–Elvehjem homogenizers in 0.25 M sucrose made 0.01 M with respect to potassium phosphate buffer (pH 7.0). Fractionation of the homogenates was carried out subsequently by the differential centrifugation procedure of HOGEBOOM[9]. Zone electrophoresis of tissue extracts was performed in vertical starch gels (14%). Tris–citrate buffers (pH 7.6; I = 0.017 in gels and 0.03 in electrode buffer) were used, and 400-V potential difference was applied for 24 h. Gels were sliced, stained for catalase activity by the method of SCANDALIOS[10], and photographed. The results shown in this paper are diagrammatic illustrations of the negatives (*i.e.* black and white transposed). Because of the massive differences between the respective turnover numbers, this staining procedure enables the effective exclusion of interference by artefactual catalatic activity (*e.g.* haem pigments or per oxidase.)

By means of this gel electrophoretic procedure, multiple forms of catalase activity were separated from rat liver and kidney preparations, and these results are illustrated in Figs. 1 and 2, respectively. The supernatant fraction of the liver homogenate showed three clearly defined regions of activity, with two further bands which were not always clearly distinguishable. The 'mitochondrial'* fraction when extracted with 0.05% Triton X-100 (following a previous aqueous extraction), gave a similar pattern and neither of these bandings were altered by treatment with Cleland's reagent (dithiothreitol). By contrast, when the 'mitochondrial' pellet was extracted with water, a single band of catalase activity resulted which possessed a distinctive mobility and was not altered by treatment with Cleland's reagent.

The situation in rat kidney (Fig. 2) was quite different from that in liver. The supernatant from the sucrose homogenate and the Triton X-100 extract of aqueous-

* Catalase activity is not necessarily associated with the mitochondria *per se* but rather with microbodies which sediment in the mitochondrial fraction[11].



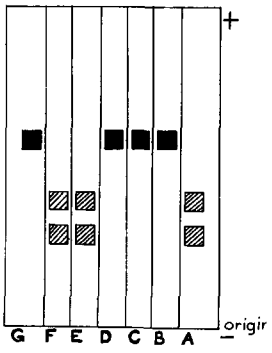


Fig. 1. Zymograms of rat liver catalase. (A) Supernatant from 0.25 M sucrose homogenate; (B) (A) treated with Cleland's reagent; (C) aqueous extract of mitochondrial pellet; (D) (C) treated with Cleland's reagent; (E) Triton X-100 extract of aqueous extracted mitochondrial pellet; (F) same as (A); (G) (E) treated with Cleland's reagent.

Fig. 2. Zymograms of rat kidney catalase. (A) Supernatant from 0.25 M sucrose homogenate; (B) (A) treated with Cleland's reagent; (C) aqueous extract of mitochondrial pellet; (D) (C) treated with Cleland's reagent; (E) Triton X-100 extract of aqueous-extracted mitochondrial pellet; (F) same as (A); (G) (E) treated with Cleland's reagent.

extracted 'mitochondria' resolves into two clear bands of different mobility from the corresponding liver extracts. A distinctive band is again present in the water extract of mitochondria, but Cleland's reagent results in all three tissue extracts (B, D, G) being present in a single form of high electrophoretic mobility.

These results may be considered in relation to recent investigations on catalase by other groups. CANTZ *et al.*[6] have shown that some changes in the electrophoretic mobility of catalase are associated with oxidation, and have postulated that such conversions are caused by the formation of disulphide bridges or conformers. HEIDRICH[7] and HEIDRICH AND HANNIG[8] have provided an important extension to these observations, and have demonstrated the interconversions of five catalase forms with oxidation or reduction. In addition, they have solubilized catalase from the light mitochondrial fraction of beef liver with Triton X-100 and shown that this 'native' form of the enzyme was a single 'reduced' form. From this fact, they deduce that the catalase activity of beef liver exists only as this single heteromorph *in vivo*, and consider that a similar situation may pertain in respect of catalase activity in other mammalian sources. We would like to suggest that this finding in a single particle type of one organ of one species may not be representative enough to justify broad conclusions.

The zymograms in the present communication clearly show that a very different situation is evident in rat tissues. Rat liver catalase, for example, not only differs in properties from the enzyme in rat kidney, but also from the reported properties of beef liver catalase[7,8]. The multiple forms of rat liver catalase do not appear to be interconverted by reducing sulphydryl reagents, whereas the kidney enzyme not only exhibits different mobility characteristics from the liver enzyme, but also undergoes alteration in the presence of Cleland's reagent. Additionally, these zymograms provide further evidence for the existence of two pools of catalase activity in the 'mitochondrial' fraction of rat liver and kidney homogenates. One of these is particu-

late and the other water soluble in nature, but it is not yet clear whether this behaviour signifies a difference in particle type origin or separate genetic definition. These aspects are under investigation at present.

In summary then, evidence has been presented for a differential distribution of catalase multiple forms in rat tissues. Oxidoreductive interconversions evidently do not explain the existence of all these observable forms of the enzyme.

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