SPECIAL ARTICLES

AN ULTRACENTRIFUGAL STUDY OF CATALASE

THE enzyme catalase is interesting not only for its own sake but also because of its close relationship to hemoglobin. Like methemoglobin, and probably also peroxidase, it consists of *proto-ferriheme* in combination with a carrier protein.[1] The protein serves the double purpose of activating the catalytic properties of the prosthetic heme grouping and of rendering its action highly specific. Very probably different animals have slightly different catalases just as they have their characteristic hemoglobins. This is borne out by the fact that Sumner and Dounce's recent chemical method[2] for the crystallization of beef liver catalase does not yield crystalline horse liver catalase. The difference appears to be in the protein part rather than in the prosthetic group. We have prepared horse liver catalase in highly purified form with the help of an air-driven quantity ultracentrifuge.[3] The steps taken to achieve this purification have been determined by estimations of specific activity and hemin content combined with observations in the analytical ultracentrifuge. The latter have at the same time given definite information about the molecular composition of the various fractions; the sedimentation constants calculated from them provide a measure of the molecular weight of the enzyme.

The purification of horse liver catalase by adsorption and elution[4] ordinarily yields brown solutions which are free from hemoglobin, show an activity (k) of 1,000–6,000 and a purity (Kat.f.) of 4,000–9,000 [occasionally higher figures have been reported[4, 5]]. When such solutions are examined with the analytical ultracentrifuge they usually show evidence of containing three molecular species. The predominant species has a sedimentation constant of 11×10^{-13} cm sec^{-1} dynes^{-1}; this undoubtedly is the enzyme. There is also present some light "unsedimentable" matter and varying amounts of a heavier material with rather diffuse sedimenting boundaries corresponding to $s_{20°}$ = ca 65×10^{-13} cm sec^{-1} dynes^{-1}. This heavy substance has been purified by throwing it down as pellets in the quantity ultracentrifuge. It redissolves readily in water or saline to give colored solutions with a maximum of light absorption in the violet. Its specific catalytic activity is relatively low. We are continuing the study of the properties of this substance which, if it is a protein, probably has a molecular weight of the order of three to four millions.

We have purified the enzyme by successively concentrating it as a black-brown bottom layer in the centrifuge tube. The activity (k) then has ranged between 11,700 and 161,000 and the purity (Kat.f.) between 8,500 and 33,400. While the enzymatic hemin content of the original preparations varied between 10 and 35 milligrams per liter, the bottom solutions contained from 187 to 906 milligrams per liter. The total dry weight-to-hemin ratio has been decreased from 400–600 to 100–279. These products compare favorably in activity and iron content with the crystalline beef liver preparations of Sumner and Dounce.[2] The sedimentation constant, $s_{20°} = 11 \times 10^{-13}$, indicates that horse liver catalase molecules are considerably heavier than those of hemoglobin. Provided the enzyme has the same density as most other proteins and a more or less normal diffusion rate[6] its molecular weight should be in the neighborhood of 250,000–300,000.

Several analytical photographs have been made from a nearly pure catalase preparation from beef liver. The resulting sedimentation constant, $s_{20°} = 12 \times 10^{-13}$, is substantially the same as that for purified horse liver catalase.

A more detailed description of these experiments will shortly be published.

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THE ESSENTIALNESS OF MANGANESE FOR THE NORMAL DEVELOPMENT OF BONE

THE first report that perosis, a deformity of the tibio-metatarsal joint of young chickens, was due to a deficiency of manganese in the diet was made by Wilgus, Norris and Heuser[1] in 1936. Confirmation of these results has been reported by several laboratories.[2, 3, 4]

In recent experiments carried out with New Hampshire chicks fed a low-manganese diet (Mn, 10 p.p.m.) the development of perosis was studied by means of the x-ray.[5] X-ray pictures were taken of the leg bones and in some instances of the wing bones, at regular

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5 The x-ray pictures were taken by Dr. L. L. Barnes, Cornell University.