# 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 $11 \times 10^{-13}$ 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 $65 \times 10^{-13}$ 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

1 H. S. Wilgus, Jr., L. C. Norris and G. F. Heuser, SCIENCE, 84: 252, 1936.
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3 V. G. Heller and R. Penquite, *Poultry Sci.*, 16: 243–246, 1937.
4 Personal communications to the authors.
5 The x-ray pictures were taken by Dr. L. L. Barnes, Cornell University.

intervals from the time of hatching until the chicks were seven weeks old. Likewise, pictures were taken of control chicks that received the same basal diet with the manganese content adjusted to 100 p.p.m. Although calcification appeared normal in both groups, the bones of the chicks on the low-manganese diet were observed to be perceptibly shorter and thicker at the twenty-fourth day of age than those of the controls. Bone measurements were made from the x-ray films of the chicks on the low-manganese diet showing by gross observations no characteristic deformities of perosis and compared to those of the controls of the same sex and age and of approximately the same weight. The smaller gains in weight and high incidence of deformities among the chicks that received the low-manganese diet limited the number of comparisons possible. However, in practically all cases, the measurements confirmed these observations.

Measurements were then made of the leg bones of a strain of chicks which had proven very susceptible to perosis when placed on a manganese-deficient diet. These chicks together with suitable controls receiving adequate amounts of manganese were sacrificed at four and five weeks of age. At four weeks of age, the tibiae and metatarsi of the chicks on the low-manganese diet were approximately 7 per cent. shorter than the corresponding bones of their controls of the same age and of about the same weight ($\pm$ 10 grams). The results were obtained from fourteen comparisons in which the bone length of the control chick exceeded in all but one instance that of the chick on the low-manganese diet. Twelve such comparisons made with chicks five weeks old showed similar differences in eight instances, and no appreciable difference in four instances. Seven comparisons of length of tibiae made at six weeks of age on a rapidly growing strain of New Hampshire chicks gave differences which again favored the control chicks in every instance with an average difference of 8 per cent. Studies now in progress with White Leghorn chicks which are not very susceptible to perosis indicate that the bone development of this breed of chicks is also affected by manganese deficiency in the same manner as that of the New Hampshire chicks.

These facts agree with a concurrent observation that a large percentage of chicks hatched from eggs of low manganese content[6] have short leg bones and with a report by Lyons and Insko[7] that chondrodystrophic embryos characterized by shortened and thickened legs, short wings and other deformities were produced from the eggs of hens fed manganese-deficient diets. According to these workers the deformities could be prevented by manganese supplements in the diet of the hen, thereby increasing the manganese content of the egg, and by direct injection of manganese into the egg previous to incubation. Other investigators[8] have noted that "the shafts of the bones often appeared to become shortened and thickened" in chicks on diets that produced perosis.

The wide distribution of manganese in biological material and its possible function in metabolism has been commented upon by VonOettinger.[9] In a study of manganese distribution in animal tissues Lund, Shaw and Drinker[10] reported that the bones of rabbits contained 0.101 mg of manganese per 100 gm of fresh bone. Reiman and Minot[11] found 0.047 mg of manganese per 100 gm of bone marrow in dogs that had been fed manganese ores. Skinner, Peterson and Steenbock[12] found 0.038 and 0.034 mg of manganese per 100 gm in the shaft and head, respectively, of fresh bone. Only traces were found in the marrow. By feeding a diet of high manganese content, the latter investigators were able to increase the manganese content of the bones of rats from 0.079 mg per 100 gm of dry material to 0.221 mg. In recent studies[13] of manganese metabolism during early growth the minimum amount of manganese found in the leg bones of chicks that received a manganese-deficient diet was 0.060 mg per 100 gm of dry material. A reasonably constant value of 0.200 mg was found in the bones of chicks that received adequate manganese. Partial depletion of the bones in manganese resulted in deformities at the joints and at the ends of the bones.

It appears from these results that manganese in small amounts is a necessary constituent of bone in the chick and that it is essential for normal bone development in addition to preventing other deformities resulting from or coincident with perosis. The presence of manganese in the bones of other species, particularly in the shaft and head, indicates that it may be essential for bone development in general. The fact that gross symptoms of perosis have not been recognized in animals other than the fowl may be related to differences in anatomical structure of the skeleton and in manganese requirements.

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