Crystalline erythrocyte catalase may now be considered a readily available enzyme.

It is interesting to calculate the catalase content of the human erythrocyte. This is obtained from the *Kat.f.* of the pure enzyme (63,000) and of washed red cells, which in two different preparations was found to be 104 and 166. Hence catalase accounts for about 0.16–0.26 % of the total dry matter of the human red-blood corpuscle.

SUMMARY

1. The isolation of crystalline catalase from human red cells is described. The method is simple and the

yield of crystalline enzyme is about 25 % of that present in the red cell.

2. The crystalline enzyme had a *Kat.f.* of 63,000 and a haematin content of 1.15 %. The sedimentation constant was 11.26×10^{-13} . The molecular weight was calculated to be 220,000 from the haematin content. There are four haematin groups/molecule; no verdohaematin is present.

Our thanks are due to Miss M. Mackay of the Medical Research Council's Blood Products Research Group, for kindly supplying us with large amounts of human red cells.

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Examination of Crystalline Catalases in the Ultracentrifuge

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Three samples of crystalline catalase were supplied by Dr Denis Herbert (Herbert & Pinsent, 1948 *a*, *b*). They were examined in a Svedberg oil-turbine ultracentrifuge by the

hydrogen phosphate 0.05M, sodium dihydrogen phosphate 0.05M.

The speed was approximately 1015 rev./sec., and the wave length of light used 620–660 m μ . For the

Fig. 1.

Fig. 2.

Fig. 3a.

Fig. 3b.

Fig. 1. Sample AI (bacterial catalase) approx. 25 min. after reaching full speed.

Fig. 2. Sample AII (bacterial catalase) approx. 25 min. after reaching full speed.

Fig. 3a. Erythrocyte catalase immediately on reaching full speed, showing heavier component.

Fig. 3b. Erythrocyte catalase approx. 25 min. after reaching full speed.

Note. The different appearance of the meniscus in Figs. 1 and 2 was due to the use of a layer of paraffin in these two runs.

method of Philpot (1938). The solutions all contained approximately 0.5 % catalase in a buffer of composition sodium chloride 0.2M, disodium

calculation of the sedimentation constants, the partial specific volume was taken as 0.73 ml./g. (Sumner & Gralén, 1938),

Bacterial catalase (from Micrococcus lysodeikticus)

Sample A I. This had an activity corresponding to a *Kat.f.* of 99,000 (Herbert, private communication). It gave a single homogeneous component with some heavier and lighter impurities (Fig. 1). The S_{20} (corr.) was 11.0×10^{-13} , and integration of the boundary gave 85% of the initial refracting material.

Sample A II. This was another preparation of slightly lower activity, *Kat.f.* = 90,000 (Herbert, private communication). It also gave a single homogeneous component with heavier and lighter impurities (Fig. 2), but integration gave only 70% of the initial refracting material. The S_{20} (corr.) was again 11.0×10^{-13} .

Human erythrocyte catalase

A single sample was examined. The *Kat.f.* when first prepared was 63,000, but during keeping it dropped to 44,300 (Herbert, private communication), suggesting that some denaturation had taken place. The sedimentation diagram was similar to that of the two previous samples except for a

larger proportion of the heavier impurity, possibly denatured catalase. This impurity can be seen clearly in an early photograph (Fig. 3a), but it dispersed as the run proceeded (Fig. 3b) suggesting heterogeneity. Some of the pigment was seen to be associated with it. The S_{20} (corr.) of the main component was 11.2×10^{-13} .

CONCLUSION AND SUMMARY

1. The ultracentrifugal data suggest that, while none of the preparations is fully homogeneous, there is in each case a main component which is associated with most of the pigment.

2. The sedimentation constants of this component in preparations from *Micrococcus lysodeikticus* and from human blood are similar (11.0 and 11.2×10^{-13} respectively), though the absolute difference is probably outside the experimental error.

3. The value of 11.2×10^{-13} for the sedimentation constant of human-blood catalase agrees with Agner's (1938) value of 11.2×10^{-13} for horse-liver catalase and with Sumner & Gralén's (1938) value of 11.3×10^{-13} for cow-liver catalase.

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Studies in Detoxication

17. THE FATE OF CATECHOL IN THE RABBIT AND THE CHARACTERIZATION OF CATECHOL MONOGLUCURONIDE

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The main object of this investigation was to find out if conjugation of catechol occurs *in vivo* on one or both of its hydroxyl groups. The study of the fate of catechol was also necessary because it had a bearing on other problems being studied in this laboratory, namely, the metabolism of adrenaline (cf. Dodgson, Garton & Williams, 1947) and of benzene (cf. Porteous, Smith & Williams, 1947).

Despite the importance of catechol compounds in biochemistry, no significant study of the fate of catechol in the intact animal has been made other than that of Baumann (1876) who showed that

administration of catechol to dogs caused an increased output of ethereal sulphate. However, numerous studies have been carried out *in vitro* of the formation and oxidation of catechol by enzymes of the tyrosinase type (see review by Nelson & Dawson, 1944).

From a consideration of the structure of catechol and of the results of enzyme studies, it is clear that it may form in the body (1) an ethereal mono- or disulphate or both, (2) a mono- or di-glucuronide or both, (3) *o*-benzoquinone by oxidation, (4) hydroxy-quinol (1:2:4-trihydroxybenzene) and its conju-