in the present study, but it was noted that venous blood samples withdrawn for CO analysis were consistently a bright red color in the high pressure experiments, in contrast to those drawn under other conditions, indicating a high degree of oxygen saturation of venous blood. In any case, it is apparent that the resting metabolic oxygen needs may be met almost completely by the dissolved oxygen when pure oxygen is breathed at 2.5 atm pressure. Thus, in addition to a significant acceleration in CO elimination rate, administration of oxygen at high pressure would also seem to relieve hypoxia immediately in victims of CO poisoning.

Clinical trial of high pressure oxygen as a means of therapeusis in CO poisoning therefore appears warranted, and is recommended where suitable pressure chamber facilities are available. There is no evident reason why its use cannot be coupled with a number of standard mechanical resuscitative devices, thereby extending its application to cases where respiration has stopped. In general, to go beyond CO poisoning, high pressure oxygen might be used profitably in many situations where the hemoglobin oxygen transport mechanism has been rendered inoperative, and may be regarded as a valuable adjunct to the already well-established technique of oxygen therapy.

References

1. BEAN, J. W. *Physiol. Rev.*, 1945, **25**, 1.
2. BERNARD, C. *Leçons sur les effets des substances toxiques et médicamenteuses.* Paris: J. B. Baillère et fils, 1857.
3. BRINK, F. In *Handbook of respiratory data in aviation*, OSRD cmr, Washington, D. C., 1944.
4. COURNAND, A. *et al. J. clin. Invest.*, 1945, **24**, 106.
5. DOUGLAS, C. G., HALDANE, J. S., and HALDANE, J. B. S. *J. Physiol.*, 1912, **44**, 275.
6. END, E. and LONG, C. W. *J. industr. Hyg. Tox.*, 1942, **24**, 302.
7. HALDANE, J. *J. Physiol.*, 1895, **18**, 201.
8. LINAS, A. J. and LIMOUSIN, S. *Bull. Mém. Soc. Thérap.*, 1868, **2**, 32.
9. PACE, N., STRAJMAN, E., and WALKER, E. L. *Fed. Proc.* 1948, **7**, 89.
10. ———. To be published.
11. SCHOLANDER, P. F. and ROUGHTON, F. J. W. *J. biol. Chem.*, 1943, **148**, 551.
12. SENDROY, J., JR., LIU, S. H., and VAN SLYKE, D. D. *Amer. J. Physiol.*, 1929, **90**, 511.
13. VAN SLYKE, D. D. and NEILL, J. M. *J. biol. Chem.*, 1924, **61**, 523.

Further Investigation of the Reducibility of Lyophilized Catalase

Alexander L. Dounce and Robert R. Schwalenberg

Department of Biochemistry, School of Medicine and Dentistry, University of Rochester, Rochester, New York

It was reported by Dounce and Howland (*1*) that crystalline beef liver catalase became completely reducible with sodium hydrosulfite after lyophilization, as shown by the change in the visible absorption spectrum, although activity of the material per dry weight was reduced only to about one-third of the value obtained before lyophilization. Keilin (*2*) subsequently denied that these experimental results had been interpreted correctly, stating that a mixture of denatured and undenatured catalase must have been obtained, in which only the undenatured catalase was active and only the denatured catalase was reducible. No experimental work was offered to support these statements, however.

Our previous work was handicapped by the lack of a modern spectrophotometer. We have recently repeated the experiments using the Beckman spectrophotometer, and have completely confirmed our previous results, as indicated by the accompanying figures.

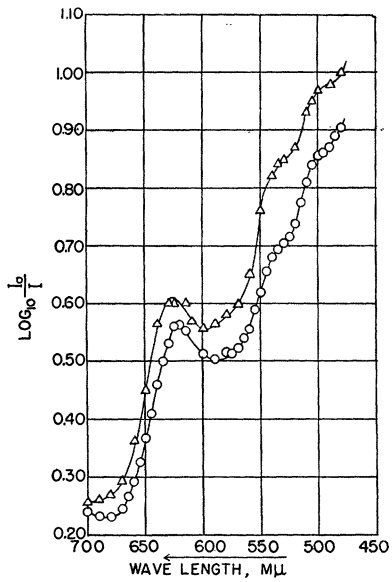

FIG. 1. Absorption spectra of lyophilized and nonlyophilized catalase. △ = 26.4 mg lyophilized catalase, Kat. f. = 14,000 in 4 ml of M/10 phosphate buffer, pH 8.0. ○ = 26.4 ml nonlyophilized catalase, Kat. f. = 25,000 in 4 ml of M/10 phosphate buffer, pH 8.0, containing 10% NaCl.

Fig. 1 shows the spectrum of a sample of recrystallized catalase which had not been lyophilized, with a Kat. f. value[1] of 25,000, together with the spectrum of a different sample of lyophilized material of Kat. f. about 14,000 which had been prepared from a sample of recrystallized catalase of Kat. f. about 23,000. The location of the three principal absorption bands is the same, but contrary to previous results (*1*) the spectra do not superimpose exactly. This might be partly ascribable to a difference in ionic strengths of the solutions, since M/10 phosphate buffer of pH 8.0 made up to contain 10% NaCl was used as the solvent for the material which

[1] The term *Kat. f.* is an abbreviation for *Katalase Fähigkeit.* It was introduced by von Euler and Josephson (*4*), and means

$$\frac{K \times \text{dilution}}{\text{g enzyme}}.$$

Here *K* is the so-called monomolecular reaction velocity constant extrapolated to zero time (*3*); *g enzyme* refers to the dry weight of enzyme per millimeter of the stock solution; and *dilution* means the number of times the stock solution is diluted before carrying out the determination.

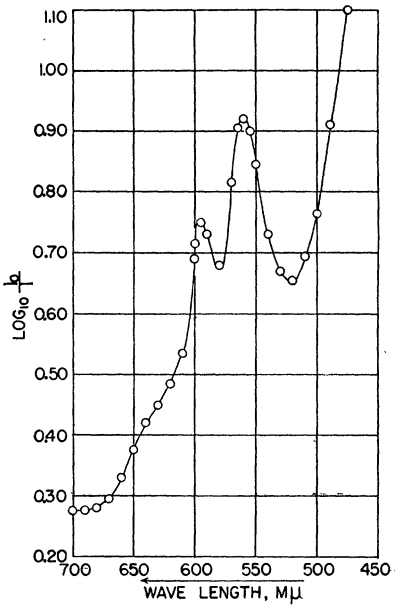

FIG. 2. Absorption spectrum of lyophilized catalase, Kat. f. = 14,000 (same sample as used for △, Fig. 1), after reduction with $Na_2S_2O_4$: 26.4 mg in 4 ml of M/10 phosphate buffer, pH 8.0.

had not been lyophilized, whereas M/10 phosphate buffer of pH 8.0 without NaCl was used as solvent for the lyophilized material. NaCl was omitted where it was not needed to dissolve the material, because it inevitably caused the gradual formation of a precipitate in the solution. Moreover, the curves were not adjusted so as to coincide at any particular point, as they were in the earlier work.

Fig. 2 shows the absorption spectrum of the lyophilized catalase sample which had been reduced with $Na_2S_2O_4$. In this case, also, the solvent was M/10 phosphate buffer of pH 8.0, without NaCl. A two-banded spectrum is obtained which is characteristic of ferroheme compounds. It seems certain that the catalase has really been reduced, since treatment of lyophilized catalase with sodium boron hydride, $NaBH_4$, gradually causes the production of a spectrum with bands at the same location as those observed with hydrosulfite reduction. Moreover, if carbon monoxide is passed into a solution of lyophilized catalase which has been reduced with hydrosulfite, the bands are shifted from 595 mμ and 560 mμ to approximately 576 mμ and 544 mμ.

It can be seen from Fig. 2 that the amount of residual unaltered catalase left over after reduction with hydrosulfite must be very low, as judged by the smallness of the bulge in the curve at about 640 mμ. In spite of this, the Kat. f. of the lyophilized material was approximately 14,000 as compared with a Kat. f. of about 23,000 before lyophilization. Thus a major portion at least of the lyophilized catalase must be active, and no more than 5%, or at most 10% of the lyophilized material can be in the form of undenatured catalase. The only way to escape the conclusion that the lyophilized, reducible catalase is active would be to assume a great enhancement in activity of the small portion of catalase that may have escaped change during lyophilization. Even if the Kat. f. of this material were doubled, the activity of the lyophilized sample could not be explained in this manner. We feel quite safe, therefore, in reiterating our original conclusion (*1*) that lyophilized beef liver catalase is changed in some way so as to render it reducible. As stated previously (*1*), completely heat-denatured catalase behaves in quite a different manner.

The most likely explanation of the behavior of lyophilized catalase reported here and previously (*1*) is that a mild sort of denaturation has loosened the attachment of the iron atom to the protein part of the molecule so that the iron becomes reducible but still remains active catalytically. A detailed investigation of this phenomenon would be worth while to someone working on the mechanism of catalase action or on the phenomenon of protein denaturation. For the benefit of any such investigator, it should be noted that the flask in which the enzyme is being lyophilized must be kept in the air during evaporation of the water and not in a cooling bath. If a cooling bath of ice and water is used, the enzyme can be lyophilized without any apparent change in its behavior.

The Kat. f. values for our recrystallized catalase samples are somewhat lower than usual but this fact does not alter the arguments presented.

References

1. DOUNCE, A. L. and HOWLAND, J. W. *Science*, 1943, **97**, 21.
2. KEILIN, D. and HARTREE, E. F. *Biochem. J.*, 1945, **39**, 148.
3. SUMNER, J. B. *Adv. Enzymol.*, 1941, **1**, 163.
4. VON EULER, H. and JOSEPHSON, K. *Liebig's Ann. der Chem.*, 1927, **452**, 158.

Methods of Preparing Bone and Tooth Samples for Viewing in the Electron Microscope[1]

R. A. Robinson and F. W. Bishop[2]

***Division of Orthopaedic Surgery* and *Department of Radiation Biology*,**
***School of Medicine and Dentistry*,**
University of Rochester, New York

One of the main characteristics of bone is its resistance to bending, torsion, compression, and tensile stresses. This resistance is offered by a combination of

[1] This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

[2] Grateful acknowledgment is made for technical assistance to Mrs. Bessie L. Jacobson, research associate, The University of Rochester Atomic Energy Project, in making the electron micrographs; and to Miss B. Kindig, of the Research Laboratories of the Eastman Kodak Company, for the ultrasonic dispersion of the various preparations.