of the count variation within each group. More homogeneous groups could thus be expected to present smaller standard errors and greater intergroup differences.

The interpretation of these findings will have to await further morphological and biochemical studies.

References and Notes

1. P. Constantinides, *Science* **117**, 505 (1953).
2. This study was supported by the National Research Council of Canada. The cooperation of the pathology department of the University of British Columbia in providing human tissues is gratefully acknowledged.
3. G. Asboe-Hansen, *Scand. J. Clin. Lab. Invest.* **2**, 271 (1950).
4. C. Cavallero and C. Braccini, *Proc. Soc. Exptl. Biol. and Med.* **78**, 141 (1951).
5. P. Constantinides and J. Rutherdale, *Proc. 67th Ann. Meeting Am. Assoc. Anat.*, Apr. 1954, in press.

10 March 1954.

Sulfide Inhibition of Catalase

Roland F. Beers, Jr.,* and Irwin W. Sizer

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Current investigations on the mechanism of inhibition of catalase by anions (*1*) have revealed several phenomena peculiar to sodium sulfide inhibition of catalase. In view of the recent report by Boeri and Bonnischen (*2*) of the oxidation of thiol groups by catalase, we are presenting these preliminary findings in advance of the general work on anion inhibition (*3*). Sulfide exhibits two distinct kinds of inhibition. The first, typical of monovalent anions such as acetate and chloride, appears to occur only in the presence of the substrate; the second occurs in its absence and is actually reversed by the substrate.

In these studies, the rate of destruction of hydrogen peroxide (Merck), initial concentration approximately $10^{-2}M$, by crystallized beef liver catalase (Worthington) was followed spectrophotometrically by the method previously described (*4*). The reaction mixture was maintained at *p*H 7.0 with 0.01*M* phosphate buffer at 25°C. The concentration of the uninhibited enzyme at any time t was determined graphically from the tangents of the semi-log slope of the optical density of H_2O_2 versus time curve (*5*).

If sodium sulfide is added simultaneously with the substrate to the catalase solution, the initial inhibition is slight or absent, but during catalysis the enzyme is rapidly inhibited in a manner identical to that found with acetate and chloride (Fig. 1, upper curve). In contrast, if sodium sulfide is added to the catalase solution before the substrate, the initial inhibition is appreciable, and its magnitude increases slowly with the incubation time (5 to 15 min) allowed before the substrate is added. This inhibition appears to occur at a much slower rate than that observed when peroxide is present and is proportional to the hydrogen ion

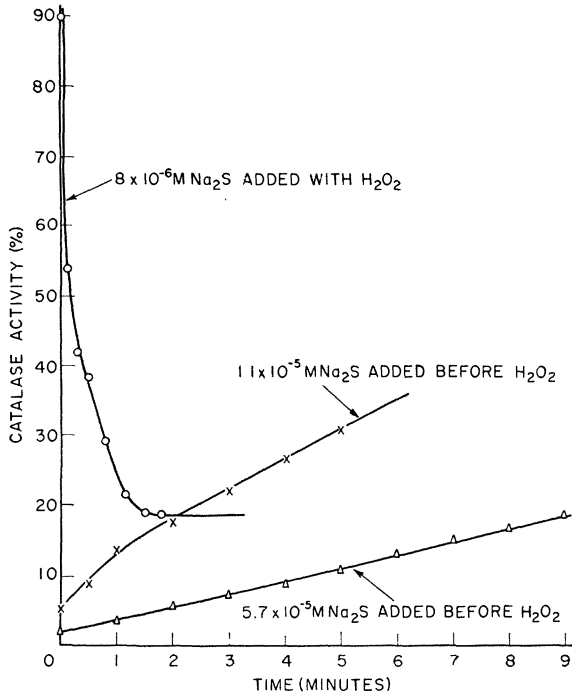

Fig. 1. Change in activity of catalase during the catalysis of hydrogen peroxide (initial concentration $10^{-2}M$). (Upper curve) Sulfide and peroxide added simultaneously to the catalase solution. (Lower curves) Sulfide and catalase incubated together for 10 min before the addition of peroxide.

and sulfide concentrations. However, this inhibition is partially reversed during the catalysis of hydrogen peroxide (Fig. 1, middle and lower curves). It is also reversed to a small degree by bubbling nitrogen and to a greater degree by bubbling oxygen through the catalase and sodium sulfide solution. This represents a true reactivation of the enzyme and not a destruction of the substrate through a stoichiometric reaction with the sulfide, because (i) the initial rate, when the sulfide concentration is the highest, is the slowest rate, and (ii) the concentration of the hydrogen peroxide is approximately 10^4 times greater than that of the sulfide (*6*). By choosing the proper concentration of sulfide and time of contact with the enzyme before adding the substrate, it is possible to obtain inhibition curves intermediate between the extremes shown in the figure.

During the course of these inhibition studies, we have observed that the oxidation of sodium sulfide to colloidal sulfur (*p*H 7.0) is accelerated by catalase in the presence or absence of peroxide. This oxidation is manifest by the appearance at the surface of the reaction mixture of a cloudy area of colloidal sulfur, which rapidly extends downward as oxygen mixes with the system. Since, in all probability, the enzyme is refractory to hydrogen peroxide under these conditions, it is very probable that the mechanism for oxidation of sulfide is a nonspecific phenomenon asso-

* Present address, Division of Physical Biochemistry, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.

ciated with the iron of the heme groups or, as suggested by Boeri and Bonnischen for the oxidation of thiol group (*2*), may be a new property of the catalase molecule unrelated to the catalytic properties heretofore ascribed to it. A few preliminary studies of the absorption curve of the protein moiety at the end of the reaction show a rather marked increase at 2800 A. As yet we have not been able to rule out an artifact as a possible cause of this increase.

It appears that sulfide may inhibit catalase in two ways. First, it may form an inactive compound with the primary catalase-peroxide complex in a manner similar to that postulated for monovalent anions (*1, 7*). Second, it may react directly with either the protein moiety or with the heme. Both processes are reversible to a degree depending on the experimental conditions.

References and Notes

1. R. F. Beers, Jr., and I. W. Sizer, In preparation.
2. E. Boeri and R. K. Bonnischen, *Acta Chem. Scand.* **6**, 968 (1952).
3. This work was done under an American Cancer Society Fellowship recommended by the Committee on Growth of the National Research Council.
4. R. F. Beers, Jr., and I. W. Sizer, *J. Biol. Chem.* **195**, 133 (1952).
5. ———, *Science* **117**, 710 (1953).
6. Controls indicate that under these conditions peroxide is not broken down by the sodium sulfide.
7. R. F. Beers, Jr., Ph.D. thesis, Massachusetts Institute of Technology (1951).

10 February 1954.

The Role of Magnesium in Photosynthesis

Leonard S. Levitt

Department of Chemistry,
Stevens Institute of Technology, Hoboken, New Jersey

The obvious connection between the presence of an Fe atom and the oxidation-reduction properties of certain biologically important porphyrin compounds, such as the heme pigments, catalase, and the cytochromes, has long been recognized, whereas, in the important phenomenon of photosynthesis, which also is an oxidation-reduction process, the presence of the Mg atom and its role in the reaction has been overlooked, if not studiously ignored.

From the simple fact that the Mg-containing molecules of chlorophyll *a* and *b* are effective in photosynthesis, whereas the Mg-free pheophytins are without effect, it is apparent that the Mg atom plays an important, and indeed, an indispensable role in the process. It might be thought that the presence of Mg merely endows the chlorophyll molecule with the particular absorption spectrum which is the peculiar requisite for the energy requirements of photosynthesis, and that other than this, the Mg atom takes no direct part in the reaction. That such is not the case may be seen from the fact that pheophytin has an absorption spectrum quite similar in many respects to that of the chlorophylls, and yet is inert in photosynthesis (*1*). It may be concluded that the Mg atom is inexorably bound up with the entire phenomenon, as well as with the chlorophyll molecule.

According to the photoelectric theory of photosynthesis (*2*), in which it is postulated that light-activated chlorophyll transfers electrons to an oxidant (presumably, the disulfide group of pyruvic oxidase (*3*), which will be abbreviated $\overline{\text{S—R—S}}$) and abstracts electrons from water, it is believed that the chlorophyll might be converted intermediately to a positively charged ionic species, representing an oxidized state. Direct evidence in support of this thesis will now be presented; and in addition, it will be shown that the specific micro-site of the positive charge is at the Mg atom.

In the ground state the chlorophyll molecule contains Mg covalently linked to any two of the four pyrrole nitrogen atoms. From the standpoint of resonance the Mg atom may be thought of as linked simultaneously by one-half a covalent bond to each of the four N atoms. Extreme forms in which the Mg and N atoms have, respectively, one or more positive and negative formal charges and vice-versa, also contribute, undoubtedly, to the resonance hybrid; but on the whole the Mg atom may be considered neutral. It is here postulated that in photosynthesis it is this essentially neutral Mg atom which absorbs the photon with resultant activation of one of its 3*s* electrons to a higher energy state:

$$\text{Chl–Mg} + h\nu \rightarrow \text{Chl–Mg}^{*} \qquad (1a)$$

Subsequent loss of the excited electron to the weak oxidant results in formation of oxidized chlorophyll, characterized by unipositive Mg:

$$\text{Chl–Mg}^{*} + \overline{\text{S—R—S}} \rightarrow \text{Chl–Mg}^{+} + \cdot\,\text{S—R—S}:^{-} \qquad (1b)$$

Alternatively, it would appear that absorption of a quantum of sufficiently high energy could result in the immediate ejection of a photoelectron and its direct capture by the oxidant:

$$\text{Chl–Mg} + h\nu \rightarrow \text{Chl–Mg}^{+} + e^{-}, \qquad (2a)$$

$$\overline{\text{S—R—S}} + e^{-} \rightarrow \cdot\,\text{S—R—S}:^{-} \qquad (2b)$$

In either case (Eqs. 1 and 2), positively ionized chlorophyll should result, and it may be supposed that during active photosynthesis in ordinary sunlight, the bombardment of chlorophyll by photons is of such intensity as to produce and maintain a considerable concentration of the unipositive oxidized form. If an oxidized chlorophyll molecule should fail to recapture an electron from a water molecule before being struck by a second photon, its remaining 3*s* electron may, presumably, absorb the energy of the incident photon and be excited to a higher quantum level, and then be removed by the oxidant (*4*). Such a sequence may be represented by

$$\text{Chl–Mg}^{+} + h\nu \rightarrow \text{Chl–Mg}^{+*}, \qquad (3a)$$

$$\text{Chl–Mg}^{+*} + \cdot\,\text{S—R—S}:^{-} \rightarrow \text{Chl–Mg}^{++} + :\text{-S—R—S}:^{-} \qquad (3b)$$

which would be followed immediately by

$$\text{Chl–Mg}^{++} + \text{H}_2\text{O} \rightarrow \text{Chl–Mg} + 2\text{H}^{+} + \tfrac{1}{2}\,\text{O}_2. \qquad (3c)$$

Only extremely small amounts of the dipositive oxi-