120 M^{-1}). These data indicate that anions do not substitute for the bridging histidine in native SOD.

The coordination geometry of the anion adducts is similar for the two enzyme derivatives and the major effect of zinc removal is probably that of providing a more tetragonal chromophore.

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Diphenoloxidase from Human Erythrocytes

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The oxidation of *o*-diphenols such as epinephrine and dihydroxyphenylalanine (DOPA) has been observed in human platelets [1], red blood cells both in the cytosol and membranes [2, 3] as well as in most animal tissues [4].

The diphenoloxidase (DPO) from red blood cells (1,2-benzenediol:oxygen oxidoreductase, EC 1.10.3.1) has been recently purified [5], but as yet insufficiently characterized; it is a protein of 150,000 molecular weight arranged in two identical subunits [5]. It oxidizes L-epinephrine and L-DOPA to their respective quinones adrenoquinone and dopaquinone, which in turn give rise spontaneously to adrenochrome (ϵ_{mol}, 485 nm = 4.35 X 10^3) and dopachrome (ϵ_{mol}, 475 nm = 3.72 X 10^3). The latter compound is known as a precursor of melanins.

This DPO may play a role in the *in vivo* oxidation of catecholamines [6], but also in some redox reactions of red blood cells involving reduced glutathione.

We have purified the diphenoloxidase according to Tuil and Demos [5] with minor modifications. Polyacrylamide gel electrophoresis (PAGE) (Fig. 1) of the pure enzyme under both native and denaturing conditions shows virtually only one band in both gels stained for proteins; when gels with native enzyme are stained for activity (incubation with L-DOPA), a black spot of melanin appears at the same position of the protein band. This indicates that the enzyme separated on PAGE is a DPO. Our pure preparation is active on L-epinephrine, L- and D-DOPA, while L-tyrosine is not accepted as substrate.

Fig. 1. Polyacrylamide gel electrophoresis of DPO. Protein 20 μg; gel A: native enzyme stained with L-DOPA 2 X 10^{-3} *M* at 25 °C; gel B: native enzyme stained for proteins with Coomassie G-250; gel C: sodium dodecylsulphate (SDS) denatured enzyme stained with Coomassie R-250.

TABLE I. Effect of Some Cations on the Oxidation of L-epinephrine by Diphenoloxidase from Human Erythrocytes. The reaction mixture contained 0.375 *M* Tris-HCl, pH 9.55, L-epinephrine 2 X 10^{-3} *M*, 40 μg enzyme protein and cations 5 X 10^{-7} *M*. Reaction rates were recorded at 485 nm for 2 minutes at 30 °C.

Cation added	Specific activity (nmol adrenochrome per min per mg protein)	% Activation
None	84	–
Mn^{2+}	126	50
Fe^{3+}	164	95
Co^{2+}	122	45
Ni^{2+}	170	102
Cu^{2+}	108	28
Zn^{2+}	116	40

We have also checked the effect of some cations on catalytic activity. Results reported in Table I show that Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and Fe^{3+} all en-

hance the catalytic activity of DPO as assayed with L-epinephrine. In some experiments we obtained no stimulation, or even inhibition, of the enzymatic activity at a cation concentration ten times higher than that used for Table I.

There are no marked differences when L- or D-DOPA are used as substrates (results not shown).

Studies are in progress in order to better characterize the DPO enzyme.

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Reaction of Copper–Zinc Superoxide Dismutase with Hydrogen Peroxide: a Possible Source of Heterogeneity?

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The reaction of hydrogen peroxide with copper–zinc bovine superoxide dismutase at ratios of 0.2 to 4.0 per active site at pH 10.0 results in the formation of distinct electrophoretic forms of the enzyme. The mobilities of these forms are identical to the mobilities of the forms present in heterogeneous, untreated native samples in two different electrophoretic systems, pH 6.96 (TRIS-acetic acid) and pH 8.38 (TRIS-glycine) (Fig. 1). Increasing ratios of hydrogen peroxide result in an apparent increase in this heterogeneity in addition to progressive inactivation (Fig. 2). Inactivation of the dismutase and loss of histidine was reported by Hodgson and Fridovich [1]. Furthermore this reaction results in the loss of copper from the dismutase at all ratios of added peroxide and the loss of zinc at the higher ratios (Table I). The loss of copper parallels the increase in the faster moving species suggesting the possibility that this species is copper-deficient. However, in the pH 6.96 electrophoretic system, the mobility of this faster form does not coincide with the mobility of a copper-deficient form prepared by reconstituting the apoenzyme with equimolar copper and zinc [2]. If it is copper-deficient, the faster electrophoretic form must be modified by the reaction with peroxide. It should be noted that this form is not capable of giving rise to native enzyme upon the addition of copper and zinc. In addition the reaction with peroxide forms distinct electrophoretic forms of the enzyme at the more physiological pH 7.0. Analysis of several purified preparations of superoxide dismutase have indicated that a

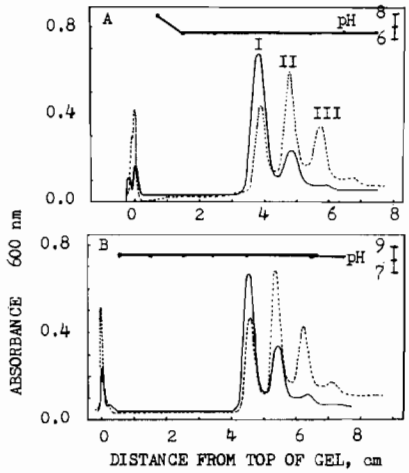

Fig. 1. Scans of polyacrylamide gels of native enzyme (——) and peroxide-treated enzyme (- - - -) (2.2 per site). (A) pH 6.96 ^ 022 *M* TRIS–0.02 *M* acetic acid; (B) pH 8.38 0.05 *M* TRIS–0.38 *M* glycine.

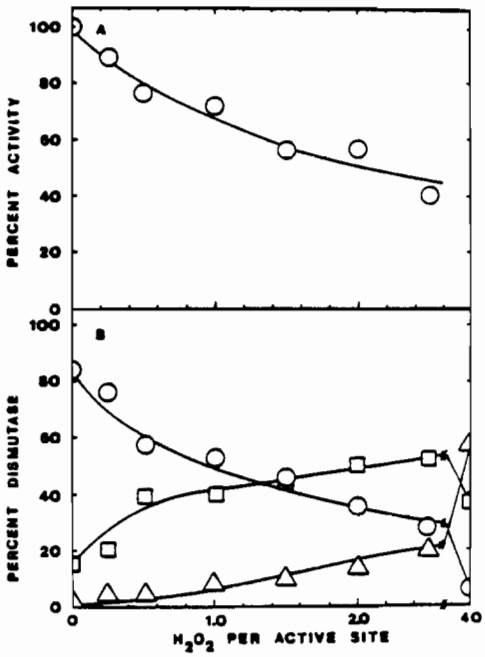

Fig. 2. (A) Percent dismutase activity remaining in the reaction with hydrogen peroxide at various ratios; (B) Percent dismutase species as a function of added peroxide: –○–, I; –□–, II; and –△–, III.

lower copper content is correlated with an increased heterogeneity (Table II). For all samples listed but one, this heterogeneity was not altered by the addition of copper and zinc.

In view of these observations we would like to suggest that the reaction with hydrogen peroxide *in vivo* may be the source of the heterogeneity so often seen in purified preparations of the copper–zinc enzyme. Hydrogen peroxide is the product of the enzyme-catalyzed dismutation reaction of superoxide anion and its reaction with the dismutase, though slow, could lead to the accumulation of altered proteins