The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase*

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SUMMARY

The rate of autoxidation of epinephrine and the sensitivity of this autoxidation to inhibition by superoxide dismutase were both augmented, as the pH was raised from 7.8 to 10.2. O$_2^-$, generated by the xanthine oxidase reaction, caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O$_2$ introduced, increased with increasing pH in the range 7.8 to 10.2 and also increased with increasing concentration of epinephrine. These results, in conjunction with complexities in the kinetics of adrenochrome accumulation, lead to the proposal that the autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving O$_2^-$ and hence inhibitable by superoxide dismutase. This chain reaction accounted for a progressively greater fraction of the total oxidation as the pH was raised. The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for this enzyme.

EXPERIMENTAL PROCEDURE

Superoxide dismutase was prepared from bovine erythrocytes and was assayed as previously described (11). Catalase, DL-epinephrine, and adrenochrome (Grade II) were obtained from the Sigma Chemical Co. Spectrophotometric assays were performed in a Gilford model 2000 absorbance indicator. Unless stated otherwise, all reactions were at 30°C. Reactions under controlled atmospheres were accomplished in cuvettes which allowed purging of the reaction volume with the desired gas (15). The oxidation of epinephrine was followed in terms of the production of adrenochrome, which exhibits an absorption maximum at 480 nm whose extinction coefficient has been given as 4020 M$^{-1}$ cm$^{-1}$ (16). Adrenochrome is unstable at pH 10.2 in air. Its further oxidation was accompanied by a loss of its 480-nm absorption band and an increase in absorbance at 500 nm with an isosbestic point at 415 nm. The oxidation of adrenochrome was followed in the aerobic oxidation of epinephrine, although observations suggest that the generation of "active oxygen" was proposed as the basis of the stimulation of epinephrine oxidation by p-hydroquinone (7). Oxygen radicals, generated by the interaction of Fe$^{3+}$ and H$_2$O$_2$, were proposed as the species responsible for the oxidation of epinephrine to adrenochrome in Fenton's reagent (8). The co-oxidation of epinephrine, by xanthine oxidase acting on xanthine, which was observed by Valerino and McCormack (9, 10), was explained by McCord and Fridovich (11) on the basis of the oxidation of epinephrine by superoxide anions, which were generated by the xanthine oxidase reaction. The availability of superoxide dismutase, an enzyme capable of removing superoxide radical from reaction mixtures, by catalyzing its dismutation to O$_2$ plus H$_2$O$_2$ (11-14), presented the opportunity of testing for the involvement of O$_2^-$ in the spontaneous, as well as in the enzymatic, oxidation of epinephrine. The following report describes and interprets experiments which indicate that O$_2^-$ is an intermediate in the autoxidation of epinephrine which occurs at elevated pH. The observation that superoxide dismutase acted as a potent inhibitor of the spontaneous oxidation of epinephrine at elevated pH also provided a convenient and sensitive assay for this enzyme.

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In all cases, reactions were started by adding an aliquot of a stable, acidic (pH 2.0) stock solution of epinephrine to the buffered reaction mixture. The pH of the reaction mixtures was not detectably changed by this addition of epinephrine.

RESULTS AND DISCUSSION

Autoxidation of Epinephrine—Epinephrine was quite stable in acid solutions but oxidized with increasing facility as the pH was raised. At pH 10.2 and 30° the accumulation of adrenochrome and of other substances contributing to the absorbance at 480 nm, exhibited a lag of approximately 1 min, during which the rate accelerated to a limiting rate; which was then maintained for many minutes. This lag was eliminated by adding $1 \times 10^{-3}$ M adrenochrome to the reaction mixtures. Adrenochrome also had the effect of moderately stimulating the linear rate which was achieved. All of these effects have already been thoroughly documented and discussed (17). It has been proposed (17) that the autoxidation of epinephrine is initiated by traces of heavy metals present as contaminants of the reagents used; that the gradual augmentation of rate is due to catalysis by the accumulating adrenochrome and that the linear rate then achieved is due to a balance between the catalytic effect of adrenochrome and the retarding effects of later oxidation products. There can be no doubt that the reaction mechanism is exceedingly complex. For our limited purposes, the linear rate of accumulation of adrenochrome was routinely recorded as the rate of the reaction.

At pH 10.2 the linear rate of accumulation of adrenochrome, in solutions of epinephrine, was a complex function of the concentration of epinephrine. EDTA inhibited this reaction at any given concentration of epinephrine and also modified the response to changing the concentration of epinephrine. These effects are shown in Fig. 1. No satisfactory explanation for these results can be presented now. However, their complexity suggests free radical mechanisms and multiple reaction pathways. Although the inhibition by EDTA indicates a role for trace metal contaminants; one must consider the possibility that aspects of the chemistry of EDTA, unrelated to its chelating ability, may also be involved.

Inhibition by Superoxide Dismutase—The autoxidation of epinephrine at pH 10.2 was strongly inhibited by superoxide dismutase. This is illustrated in Fig. 2. It is apparent that the maximum inhibition actually achieved was 91%, whereas the maximum inhibition arrived at, by extrapolation of the data, was 100%. This difference between actual and extrapolated maximum inhibition, by superoxide dismutase, may be related to the availability of alternate and competing oxidative pathways. Thus, in the absence of a perturbing influence, virtually all of the autoxidation at this pH proceeded by an O$_2^-$-dependent and superoxide dismutase-inhibitable pathway. As this pathway was made progressively unavailable, by the addition of superoxide dismutase, intermediates accumulated to an extent which activated the alternate pathways.

At pH 9.0, the ability of superoxide dismutase to inhibit epinephrine autoxidation was less than at pH 10.2. Thus, as shown in Fig. 3 the maximum inhibition by superoxide dismutase, both actual and extrapolated, was only 70%. EDTA, which inhibited epinephrine oxidation, increased the sensitivity to superoxide dismutase, while having no effect on the maximum degree of inhibition which could be achieved.

The effect of pH on the sensitivity of epinephrine autoxidation toward superoxide dismutase was further explored. Because the spontaneous rate of oxidation of epinephrine decreased with pH and became inconveniently slow below pH 10.2, ferrous sulfate was added, as a catalyst, in amounts sufficient to cause increases in absorbance at 480 nm of 0.025 per min. The effect of superoxide dismutase was then explored at each pH. The results of these manipulations are summarized in Fig. 4, which gives the inhibition at each pH which was caused by 9.5 pg per ml of superoxide dismutase. This level of the dismutase was saturating and higher concentrations did not cause further inhibition. It was nevertheless considered useful to establish that iron salts do not inhibit superoxide dismutase. This was done by using the xanthine oxidase reaction as a source of O$_2^-$ and the reduction of cytochrome c as the detector of O$_2^-$. The conditions were those of the standard superoxide dismutase assay (11) except that EDTA was omitted. Ferric sulfate at $9 \times 10^{-4}$ M exhibited superoxide dismutase activity, nor inhibited the activity of superoxide dismutase. It is clear, from the results in Fig. 4, that the sensitivity of the oxidation of epinephrine toward inhibition by superoxide dismutase, decreased with pH and became zero at pH 8.5 and below.

It follows that the mechanism of epinephrine oxidation changes with pH, such that the involvement of O$_2^-$ diminishes with decreasing pH and becomes undetectable below pH 8.5. Since the rate of spontaneous oxidation of epinephrine also decreased with pH; we proposed that chain mechanisms involving O$_2^-$, as a chain-propagating species, are quantitatively important at high pH and become progressively less important as the pH is lowered. Thus, if RH$_2^-$ represents epinephrine and R represents adrenochrome, we write the following reactions to represent the chain reaction as it might occur at high pH.

$$
RH_2^- + Me^- \rightarrow RH_1^- + Me^- 
$$

(a)
Superoxide Dismutase, pg/ml

Superoxide Dismutase, µg/ml

FIG. 2. Inhibition of the autoxidation of epinephrine at pH 10.2, by superoxide dismutase. Superoxide dismutase at the indicated concentrations, was added to epinephrine, $1 \times 10^{-4} \text{ M EDTA}$, and $0.05 \text{ M sodium carbonate}$ at pH 10.2 and the effect on the rate of adrenochrome formation was recorded. In the absence of superoxide dismutase the rate of increase of absorbance at 480 nm was 0.025 per min. $\bullet$, concentration of superoxide dismutase expressed as micrograms per ml at $3 \times 10^{-4} \text{ M epinephrine}$; $\Delta$, concentration of superoxide dismutase expressed as micrograms per ml at $4 \times 10^{-4} \text{ M epinephrine}$; $\otimes$, concentration of superoxide dismutase expressed as nanograms per ml at $3 \times 10^{-4} \text{ M epinephrine}$; $\triangle$, concentration of superoxide dismutase expressed as nanograms per ml at $4 \times 10^{-4} \text{ M epinephrine}$. The observed percentage of inhibition is here presented, on both linear and on reciprocal coordinates, as a function of the concentration of superoxide dismutase, which is expressed both as micrograms per ml and as nanograms per ml, to allow presentation of data collected over a wide range of superoxide dismutase concentration.

In this way, one initiating event, here shown as the univalent oxidation of an epinephrine anion by a metal cation (Reaction a) or by a superoxide anion (Reaction c), starts a chain reaction in which $O_2^-$ is a propagating species. It is clear that superoxide dismutase should strongly inhibit this mechanism. At lower pH, the organic radical generated by the initiating event could lead to adrenochrome formation by a series of dismutation reactions such as:

- $\text{RH}_2^- + O_2 \rightarrow \text{RH}_3 + O_2^- + H^+$  
- $\text{RH}_2 + O_2^- + H^+ \rightarrow \text{RH}^- + \text{H}_2\text{O}_2$  
- $\text{RH}^- + O_2^- \rightarrow R + O_2^- + H^+$  
- $\text{RH}_3^- + O_2^- + 2H^+ \rightarrow \text{RH}_2 + \text{H}_2\text{O}_2$  

In this way, one initiating event, here shown as the univalent oxidation of an epinephrine anion by a metal cation (Reaction a) or by a superoxide anion (Reaction c), starts a chain reaction in which $O_2^-$ is a propagating species. It is clear that superoxide dismutase should strongly inhibit this mechanism. At lower pH, the organic radical generated by the initiating event could lead to adrenochrome formation by a series of dismutation reactions such as:

- $\text{RH}_2^- + \text{RH}_2 \rightarrow \text{RH}_3 + \text{RH}_4$  
- $\text{RH}_3^- + \text{RH}_4 \rightarrow \text{RH}^- + \text{RH}_2$  
- $\text{RH}^- + \text{RH}^- \rightarrow R + \text{RH}_2$  

In this case superoxide dismutase could not inhibit adrenochrome formation. The reduced metal generated in Reaction a would, in any case, be reoxidized by reaction with oxygen.

$\text{Mo}^{3+} + O_2 \rightarrow \text{Mo}^{4+} + O_2^-$

The $O_2^-$ generated by Reaction i could either dismute or react with epinephrine as in Reaction e.

**Effect of Epinephrine on Sensitivity of Autoxidation, toward Superoxide Dismutase**—If the conversion of epinephrine to adrenochrome is inhibited by superoxide dismutase, then lower concentrations of epinephrine should inhibit adrenochrome formation in the presence of superoxide dismutase. In the absence of superoxide dismutase, at lower pH, it is likely that epinephrine will form adrenochrome by a series of dismutation reactions such as:

- $\text{RH}_2^- + \text{RH}_2 \rightarrow \text{RH}_3 + \text{RH}_4$  
- $\text{RH}_3^- + \text{RH}_4 \rightarrow \text{RH}^- + \text{RH}_2$  
- $\text{RH}^- + \text{RH}^- \rightarrow R + \text{RH}_2$  

In this case superoxide dismutase could not inhibit adrenochrome formation. The reduced metal generated in Reaction a would, in any case, be reoxidized by reaction with oxygen.

$\text{Mo}^{3+} + O_2 \rightarrow \text{Mo}^{4+} + O_2^-$

The $O_2^-$ generated by Reaction i could either dismute or react with epinephrine as in Reaction e.

**Effect of pH on the Superoxide Dismutase Sensitivity of Epinephrine Oxidation**—The rate of oxidation of epinephrine to adrenochrome was measured at 25°C in the presence and absence of 9.5 μg per ml of superoxide dismutase. The conditions of buffering and the amounts of ferrous sulfate were:

<table>
<thead>
<tr>
<th>pH</th>
<th>$P_{4O_2}$</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.8</td>
<td>$8.8 \times 10^{-4} \text{ M}$</td>
<td>0.05 M potassium phosphate</td>
</tr>
<tr>
<td>8.5</td>
<td>$1.32 \times 10^{-4} \text{ M}$</td>
<td>0.05 M Tris chloride</td>
</tr>
<tr>
<td>9.0</td>
<td>$1.00 \times 10^{-4} \text{ M}$</td>
<td>0.05 M Tris chloride or sodium carbonate</td>
</tr>
<tr>
<td>9.5</td>
<td>$0.66 \times 10^{-4} \text{ M}$</td>
<td>0.05 M Tris chloride or sodium carbonate</td>
</tr>
<tr>
<td>9.75</td>
<td>$0.33 \times 10^{-4} \text{ M}$</td>
<td>0.05 M sodium carbonate</td>
</tr>
<tr>
<td>10.2</td>
<td>None</td>
<td>0.05 M sodium carbonate</td>
</tr>
</tbody>
</table>

In all cases the rate of increase of absorbance at 480 nm in the absence of superoxide dismutase was 0.025 per min.
nochrome can proceed by two reaction pathways, only one of which is a chain process and sensitive to superoxide dismutase, then we should expect that the sensitivity of the over-all process to superoxide dismutase would depend upon the relative quantitative importance of the chain process and the nonchain process. Reaction chains can be shortened by decreasing the concentration of reactants. It might therefore be anticipated that decreasing the concentration of epinephrine would decrease the proportion of adrenochrome produced by the chain reaction and would therefore decrease the superoxide dismutase sensitivity of the over-all process. Table I, which summarizes data obtained at pH 10.2, shows that this was the case. The maximum inhibition caused by superoxide dismutase did not exceed 91% even when the concentration of epinephrine was raised to 6 × 10⁻⁴ M. The relative proportions of chain to nonchain pathways did thus not appear to be affected by increasing the epinephrine above 2.6 × 10⁻⁴ M. This correlates with the data in Fig. 1, which indicated that the variation of rate with epinephrine concentration was complex below 2.6 × 10⁻⁴ M but was first order at higher concentrations.

**Effect of Epinephrine on O₂⁻-induced Reaction Chains**—The chain mechanism, symbolized by Reactions a → e above, can be initiated by O₂⁻ as in Reaction c. Since known amounts of O₂⁻ can be generated in reaction mixtures, by the action of milk xanthine oxidase (11, 12); the effective chain length could be estimated in terms of the molecules of adrenochrome produced per O₂⁻ introduced. Such measurements were performed by using saturating levels of ferricytochrome c to trap and thus to estimate the flux of O₂⁻; while in parallel experiments, in which epinephrine replaced cytochrome c, the rate of adrenochrome accumulation was measured. Table II presents the results of such measurements, performed at pH 10.2, as a function of the concentration of epinephrine. These results show that chain length did increase with increasing concentration of epinephrine. Because the conversion of epinephrine to adrenochrome is a 4-electron oxidation, the ratio, adrenochrome produced per O₂⁻ introduced, would be only 0.25 in the absence of any chain propagation. The value observed at pH 10.2 and at 3.7 × 10⁻⁴ M epinephrine, was 10 times greater than this minimal value.

The ratio, adrenochrome produced per O₂⁻ introduced, also increases with increasing pH. Thus at pH 6.8 the ratio was 0.57 and at pH 7.8 it was 0.72 (19) and we now see that it is much higher at pH 10.2. This increasing chain length correlates with the increase, with pH, in the sensitivity of epinephrine oxidation to inhibition by superoxide dismutase.

Doubts have been expressed (10) that the O₂⁻, generated by xanthine oxidase, accounts for the co-oxidation of epinephrine at

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**TABLE I**

**Effect of epinephrine on maximum inhibition by superoxide dismutase**

Reaction mixtures contained the indicated concentrations of epinephrine in 0.05 M sodium carbonate buffer at pH 10.2. Initial rates of adrenochrome production were measured as a function of superoxide dismutase and the plateau of inhibition by superoxide dismutase was then calculated.

<table>
<thead>
<tr>
<th>Epinephrine (M)</th>
<th>Maximum inhibition by superoxide dismutase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 × 10⁻⁴</td>
<td>91</td>
</tr>
<tr>
<td>2.6 × 10⁻⁴</td>
<td>91</td>
</tr>
<tr>
<td>1.25 × 10⁻⁴</td>
<td>67</td>
</tr>
<tr>
<td>0.25 × 10⁻⁴</td>
<td>53</td>
</tr>
</tbody>
</table>

**TABLE II**

**Effect of epinephrine on length of O₂⁻-initiated reaction chains**

Reaction mixtures contained 5 × 10⁻³ M xanthine, 1 × 10⁻⁴ M EDTA, 0.05 M sodium carbonate, pH 10.2, and, where indicated, 3.7 × 10⁻⁴ M xanthine oxidase. When adrenochrome production was measured, in terms of ΔA 480 nm per min, epinephrine was present at the indicated concentrations: whereas when cytochrome c reduction was measured at 550 nm, cytochrome c was present at 1 × 10⁻⁴ M. The rates of absorbance change were corrected by dividing them by the molar extinction coefficients, before the ratio shown in the last column was computed.

<table>
<thead>
<tr>
<th>Epinephrine (M)</th>
<th>Xanthine oxidase</th>
<th>ΔA480 per min</th>
<th>ΔA550 per min</th>
<th>Ratio of adrenochrome to cytochrome c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 × 10⁻⁴</td>
<td>−</td>
<td>0.0010</td>
<td>0.008</td>
<td>0.62</td>
</tr>
<tr>
<td>0.25 × 10⁻⁴</td>
<td>+</td>
<td>0.0020</td>
<td>0.008</td>
<td>1.60</td>
</tr>
<tr>
<td>1.25 × 10⁻⁴</td>
<td>−</td>
<td>0.0045</td>
<td>0.008</td>
<td>1.50</td>
</tr>
<tr>
<td>2.6 × 10⁻⁴</td>
<td>+</td>
<td>0.0070</td>
<td>0.008</td>
<td>2.0</td>
</tr>
<tr>
<td>2.6 × 10⁻⁴</td>
<td>−</td>
<td>0.0152</td>
<td>0.008</td>
<td>2.0</td>
</tr>
<tr>
<td>3.7 × 10⁻⁴</td>
<td>+</td>
<td>0.0184</td>
<td>0.008</td>
<td>2.5</td>
</tr>
<tr>
<td>3.7 × 10⁻⁴</td>
<td>−</td>
<td>0.0220</td>
<td>0.008</td>
<td>2.5</td>
</tr>
</tbody>
</table>

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*Fig. 5.* The co-oxidation of epinephrine by xanthine oxidase. Xanthine oxidase, at the indicated concentrations, was added to 1.5 × 10⁻⁴ M epinephrine and 1 × 10⁻⁴ M EDTA at pH 7.8 and 10.2, and its effect on the rate of adrenochrome accumulation was recorded. □, 0.05 M sodium carbonate buffer, pH 10.2; ■, 0.05 M potassium phosphate buffer, pH 7.8. Superoxide dismutase at 2 μg per ml was added to the reaction mixture at pH 10.2 (Q) and pH 7.8 (□) at the indicated concentrations of xanthine oxidase.
TABLE III

Effects of EDTA and of superoxide dismutase on epinephrine oxidation at pH 10.2

Reaction mixtures contained 4.7 × 10⁻⁴ M epinephrine plus the indicated concentrations of EDTA and superoxide dismutase in 0.05 M sodium carbonate at pH 10.2. Oxygen consumption was measured polarographically with a Clark electrode.

<table>
<thead>
<tr>
<th>EDTA</th>
<th>Superoxide dismutase</th>
<th>Initial rate of oxygen consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>µg/ml</td>
<td>(10⁻⁹)/min</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>7.47</td>
</tr>
<tr>
<td>1 × 10⁻⁴</td>
<td>1.60</td>
<td>9</td>
</tr>
<tr>
<td>5.6 × 10⁻⁴</td>
<td>0.67</td>
<td>14</td>
</tr>
<tr>
<td>1 × 10⁻⁵</td>
<td>2.67</td>
<td>35.7</td>
</tr>
<tr>
<td>8.3 × 10⁻⁵</td>
<td>5.53</td>
<td>7.1</td>
</tr>
</tbody>
</table>

pH 7.8; although this has been shown to be the case at pH 10.1 (11). The data in Fig. 5 clearly indicate that the co-oxidation of epinephrine, by xanthine oxidase at pH 7.8 as well as at pH 10.2, is susceptible to inhibition by superoxide dismutase. The base-line rate of epinephrine autoxidation was, of course, higher at pH 10.2 than at 7.8. In addition O₂⁻, generated electrolytically, has been shown to cause the oxidation of epinephrine to adrenochrome at pH 7.8 (20). It is clear that the co-oxidation of epinephrine by xanthine oxidase is due to the O₂⁻ generated by that enzyme whether at pH 7.8 or 10.2.

Effects of EDTA—If the autoxidation of epinephrine is initiated by traces of transition metals, as in Reaction a, and the metal is then restored to its initial valence state by reaction with oxygen, we may anticipate that EDTA might have two distinct effects. The first of these is an inhibition of the rate of epinephrine oxidation, based upon a decrease in the reactivity of the metal with epinephrine (Reaction a). The second effect would be an enhancement of the role of O₂⁻ in epinephrine oxidation, with a concomitant increase in sensitivity toward superoxide dismutase. This would occur because the EDTA complex of a metal cation is likely to react with oxygen by an outer sphere mechanism which generates O₂⁻, whereas the corresponding incompletely sequestered metal will probably react by an inner sphere mechanism which cannot liberate O₂⁻ into the solution. This difference depends upon electrostatic considerations which have been discussed in another context (18). The oxidation of epinephrine, at pH 10.2 and 30°, was monitored in terms of oxygen consumption. As shown in Table III, EDTA did both inhibit epinephrine oxidation and markedly enhance the sensitivity toward superoxide dismutase. EDTA has no effect on the activity of superoxide dismutase in other assay systems. It follows that O₂⁻, generated by reoxidation of the reduced complexed metal (Reaction 0), contributes significantly to the rate of autoxidation of epinephrine at pH 10.2. In all cases, addition of catalase caused a liberation of half of the oxygen which had been consumed, this indicates that H₂O₂ was the stable product of oxygen reduction.

Role of O₂⁻ in Adrenochrome Oxidation—Adrenochrome is not very stable at pH 10.2 and is itself subject to further oxidation (17). Thus, when 4 × 10⁻⁴ m adrenochrome was incubated at 30°, in the presence of 0.05 m sodium carbonate, 1 × 10⁻⁴ m EDTA, pH 10.2, the absorbance at 480 nm decreased by 0.017 per min. Superoxide dismutase at 3 µg per ml inhibited 25% and 20 µg per ml, inhibited 30%. It is clear that O₂⁻ does play a role in adrenochrome oxidation at this pH but to a lesser extent than it does in the oxidation of epinephrine to adrenochrome.

Assays for Superoxide Dismutase—The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 (Fig. 2) suggested this reaction as the basis of a simple assay for this dismutase. The production of adrenochrome in reaction mixtures containing 3 × 10⁻⁴ m epinephrine, 1 × 10⁻⁴ M EDTA, and 0.05 m sodium carbonate at pH 10.2 and 30° was followed at 480 nm. The absorbance change was 0.025 per min and 50% inhibition was achieved by 46 µg per ml of bovine superoxide dismutase. This assay was applicable to crude extracts of yeast and Escherichia coli. Purified bovine superoxide dismutase, when added to these crude extracts, as an internal standard, exerted its full inhibitory effect. Boiled bovine superoxide dismutase or boiled extracts of yeast or E. coli were without ability to inhibit epinephrine oxidation. The inhibition caused by a given amount of superoxide dismutase was the same whether it was added to reaction mixtures before the epinephrine or after epinephrine oxidation had started. Since the bovine superoxide dismutase is a cupro-protein, another copper-containing protein (coenzyme) was tested and found to be devoid of superoxide dismutase activity in this assay. Copper sulfate, at levels below that of the EDTA, was without effect but did augment epinephrine oxidation when added in excess of the EDTA. Apo-superoxide dismutase was devoid of activity in this assay. Superoxide dismutase can thus be assayed, very simply, in terms of its ability to inhibit epinephrine autoxidation under specified conditions.

In comparison with other assay methods recently described (21) this one has the advantage of simplicity and thus has found favor in our laboratory for situations in which multiple assays must be performed, as in monitoring column eluates for superoxide dismutase activity. In addition, this assay is twice as sensitive as the assay originally described; which depended upon detecting O₂⁻ in terms of the reduction of cytochrome c (11).

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