

Activation of guanylate cyclase by superoxide dismutase and hydroxyl radical: A physiological regulator of guanosine 3',5'-monophosphate formation

(superoxide ion/catalase/hydrogen peroxide/nitrate reductase/free radicals)

CHANDRA K. MITTAL AND FERID MURAD

Division of Clinical Pharmacology, Departments of Internal Medicine and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22903

Communicated by Edwin G. Krebs, August 5, 1977

ABSTRACT Partially purified soluble rat liver guanylate cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] was activated by superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1). This activation was prevented with KCN or glutathione, inhibitors of superoxide dismutase. Guanylate cyclase preparations formed superoxide ion. Activation by superoxide dismutase was further enhanced by the addition of nitrate reductase. Although guanylate cyclase activity was much greater with Mn^{2+} than with Mg^{2+} as sole cation cofactor, activation with superoxide dismutase was not observed when Mn^{2+} was included in incubations. Catalase also decreased the activation induced with superoxide dismutase. Thus, activation required the formation of both superoxide ion and H_2O_2 in incubations. Activation of guanylate cyclase could not be achieved by the addition of H_2O_2 alone. Scavengers of hydroxyl radicals prevented the activation.

It is proposed that superoxide ion and hydrogen peroxide can lead to the formation of hydroxyl radicals that activate guanylate cyclase. This mechanism of activation can explain numerous observations of altered guanylate cyclase activity and cyclic GMP accumulation in tissues with oxidizing and reducing agents. This mechanism will also permit physiological regulation of guanylate cyclase and cyclic GMP formation when there is altered redox or free radical formation in tissues in response to hormones, other agents, and processes.

Guanylate cyclase [GTP pyrophosphate-lyase(cyclizing), EC 4.6.1.2] catalyzes the formation of cyclic GMP from GTP (1-3). Many laboratories have suggested regulatory roles for this nucleotide because some hormones, neurohormones, and other agents can increase cyclic GMP accumulation in tissues (4, 5). Although a few reports have described effects of hormones on guanylate cyclase in cell-free systems (6-10), most of these reports have not been confirmed. In some instances these effects can be attributed to bile salt contaminants in hormone preparations (11), nonenzymatic formation of cyclic GMP (12), and perhaps other nonspecific mechanisms.

We have described the activation of guanylate cyclase from numerous tissues with sodium azide, hydroxylamine, nitrite ion, nitroglycerin, nitroprusside, or nitric oxide (13-16). Sodium azide activation of guanylate cyclase requires a protein activator factor such as catalase and oxygen and is altered with oxidizing and reducing agents (13-17). All of these agents under the appropriate conditions can lead to nitroso groups or nitric oxide which are capable of activating guanylate cyclase (14, 16, 18). Recently, nitrosoamines (19) and nitrosoureas (20) have also been reported to activate guanylate cyclase preparations. Azide and hydroxylamine are known to inhibit catalase by formation of a catalase-nitric oxide complex in the presence of H_2O_2 (21,

22). Hydrogen peroxide in tissues can be derived from superoxide ion with superoxide dismutase (23). The availability of superoxide ion and H_2O_2 in incubations could regulate the activation of guanylate cyclase by azide and other agents.

In this report we describe the activation of partially purified soluble rat liver guanylate cyclase by superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1). Because these preparations did not contain catalase or another protein activator factor (15, 16), they were not activated by azide. Activation with superoxide dismutase was enhanced by nitrate reductase and inhibited by catalase, Mn^{2+} , CN^- , glutathione, and hydroxyl radical scavengers. These observations indicate that the mechanisms of superoxide dismutase and azide activation are different. We propose that superoxide ion and H_2O_2 formed in incubations lead to the formation of hydroxyl radicals which can activate guanylate cyclase. This mechanism of guanylate cyclase activation can explain numerous reports in the literature and may be implicated in the physiological regulation of guanylate cyclase and cyclic GMP synthesis under conditions in which factors alter free radicals and the redox state in tissues.

METHODS AND MATERIALS

Male Sprague-Dawley rats weighing 150-250 g were sacrificed by cervical dislocation. Livers were removed quickly and placed in cold 0.25 M sucrose containing 10 mM Tris-HCl buffer, pH 8.0/1 mM EDTA/1 mM dithiothreitol. Livers were homogenized in 8 volumes of this medium with a glass homogenizer and Teflon pestle at 4°. Homogenates were centrifuged at $105,000 \times g$ for 60 min, and supernatant fractions were used for purification of guanylate cyclase as described (13, 15, 16). HCl (1 M) was added to the supernatant fraction to yield pH 5.0. The precipitate was collected by centrifugation at $12,000 \times g$ for 15 min, suspended in 50 mM Tris-HCl, pH 7.6/1 mM EDTA/1 mM dithiothreitol, and recentrifuged. Solid ammonium sulfate was added to the resulting supernatant fraction to obtain 20% saturation. The precipitate was removed by centrifugation at $12,000 \times g$ for 15 min and discarded. Ammonium sulfate was added to the supernatant fraction to achieve 45% saturation. The resulting precipitate was dissolved in 10 mM Tris-HCl, pH 7.6/1 mM EDTA/1 mM dithiothreitol. The sample was desalted on a Sephadex G-25 column and chromatographed on DEAE-cellulose (15, 16). Guanylate cyclase was eluted by using a NaCl gradient (0-0.5 M). Fractions containing guanylate cyclase were pooled and used as a source of partially purified enzyme. Fresh preparations or those stored at -70° for more than 2 years were qualitatively similar in these studies.

Guanylate cyclase activity was determined in 100- μ l incu-

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

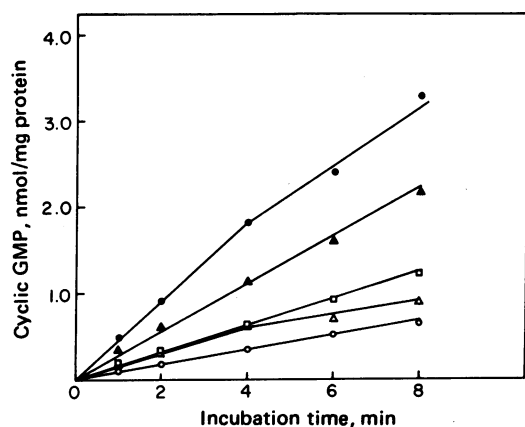


FIG. 1. Activation of guanylate cyclase with superoxide dismutase. Superoxide dismutase (0.5 μ g) was added to incubations of partially purified liver guanylate cyclase (6.5 μ g of protein) as indicated. Some reaction mixtures were preincubated 2 (\blacktriangle), 5 (\square), or 10 (\triangle) min with superoxide dismutase at 37°, as indicated, before the addition of 1 mM GTP and 4 mM $MgCl_2$. \bullet , No preincubation; \circ , control.

bations as described with some modification (11–16). Reaction mixtures contained 50 mM Tris-HCl buffer (pH 7.6), 10 mM theophylline, 1 mM GTP, and 4 mM $MgCl_2$. Concentrations of EDTA and dithiothreitol in incubations were 0.1 mM or less and were due to their introduction with the enzyme preparations. Reactions were initiated by the addition of GTP and $MgCl_2$, incubated for 10 min at 37°, terminated by the addition of 0.5 ml of cold 50 mM sodium acetate buffer (pH 4.0), and heated for 3 min at 90°. Cyclic GMP formed was determined by the radioimmunoassay of Steiner *et al.* (24) with minor modification (25). Cyclic GMP formation was linear with respect to time of incubation and protein concentration used. Values presented are means of duplicate or triplicate incubations from representative experiments.

Superoxide ion formed in incubations was determined by a cytochrome *c* reduction assay in the presence of catalase as described by McCord and Fridovich (26) using conditions of the guanylate cyclase assay. Protein was determined by the procedure of Lowry *et al.* (27).

Bovine erythrocyte superoxide dismutase (2900 units/mg), beef liver catalase (3400 units/mg), *Escherichia coli* nitrate reductase (12.6 units/g), *l*-norepinephrine, *l*-epinephrine, butylated hydroxyanisole, and hydroquinone were obtained from Sigma Chemical Company. Other agents were obtained as described (11–16, 25).

RESULTS

Addition of superoxide dismutase to partially purified rat liver guanylate cyclase preparations caused a marked activation (Fig. 1). The effect was diminished when superoxide dismutase was preincubated with guanylate cyclase prior to initiating cyclase incubations with GTP and Mg^{2+} . Decreased effects of superoxide dismutase were also observed when guanylate cyclase preparations were preincubated prior to the addition of superoxide dismutase (not shown). Under either set of conditions, further addition of superoxide dismutase was not effective. Two known inhibitors of superoxide dismutase, KCN and glutathione, were also tested without and with superoxide dismutase (Table 1). These agents had little or no effect alone, as reported previously (13, 14, 16), but markedly decreased the activation due to superoxide dismutase.

With some preparations of partially purified guanylate cy-

Table 1. Effect of superoxide dismutase and other agents on liver guanylate cyclase activity

Addition (mM)	Cyclic GMP, pmol/mg protein per min		Ratio: with/without
	Without SDase	With SDase	
Exp. 1			
None	55	232	4.2
KCN (0.2)	62	236	3.8
KCN (0.5)	65	182	2.8
KCN (1.0)	58	110	1.9
Glutathione (0.1)	53	186	3.5
Glutathione (0.5)	50	77	1.5
Glutathione (1.0)	48	67	1.4
Exp. 2			
None	62	463	7.5
$MnCl_2$ (1)	309	284	0.9
$MnCl_2$ (2)	765	765	1.0
$MnCl_2$ (4)	528	480	0.9

Partially purified liver guanylate cyclase (6.5 μ g of protein) was incubated without and with 0.5 μ g of superoxide dismutase (SDase) in Exp. 1 and 2 μ g in Exp. 2. Some incubations also contained KCN or glutathione at the concentrations indicated. Mn^{2+} at the concentrations indicated was used as sole cation instead of 4 mM Mg^{2+} in some incubations.

clase, superoxide dismutase activation was much less (Fig. 2). Addition of *E. coli* nitrate reductase, a metalloflavoprotein that donates electrons to oxygen as well as nitrate (28), had little or no effect on guanylate cyclase activity. However, it markedly increased the effect of superoxide dismutase with all preparations examined (Figs. 2 and 3). Heated preparations of superoxide dismutase or nitrate reductase were ineffective (not shown). Activation of guanylate cyclase by superoxide dismutase was concentration dependent as was the enhancement with nitrate reductase (Figs. 2 and 3).

Under the conditions of the guanylate cyclase assay we observed the formation of superoxide ion by partially purified preparations of liver guanylate cyclase (not shown). The formation of superoxide ion did not appear to correlate with the degree of guanylate cyclase activation by superoxide dismutase. Basal guanylate cyclase activity was greater with Mn^{2+} than

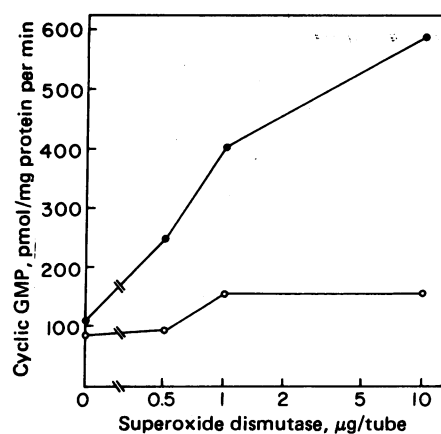


FIG. 2. Effects of superoxide dismutase and nitrate reductase on guanylate cyclase activity. A partially purified preparation of rat liver guanylate cyclase (6.6 μ g of protein) with a relatively small effect of superoxide dismutase was incubated with the amount of superoxide dismutase indicated without (\circ) and with (\bullet) 10 μ g of nitrate reductase.

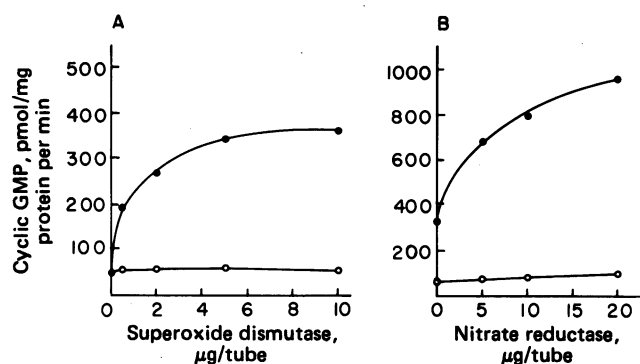
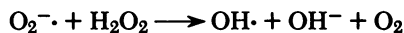


FIG. 3. Effects of superoxide dismutase, catalase, and nitrate reductase on guanylate cyclase activity. (A) Partially purified rat liver guanylate cyclase (6 µg of protein) was incubated with superoxide dismutase at the concentrations indicated, without (●) and with (○) 5 µg of beef liver catalase. (B) Partially purified rat liver guanylate cyclase (13 µg of protein) was incubated with the amount of nitrate reductase indicated in the presence (●) and absence (○) of 10 µg of superoxide dismutase.

with Mg^{2+} as sole cation as reported (1–3, 13, 16, 29). Activation of guanylate cyclase with superoxide dismutase was observed when Mg^{2+} but not when Mn^{2+} was used as the sole cation in incubations (Table 1). This could be due to the inhibition of superoxide ion formation by Mn^{2+} as reported (30). Addition of beef liver catalase totally abolished the activation due to superoxide dismutase (Fig. 3). Addition of either catalase or KCN after 2 min of incubation decreased the rate of cyclic GMP formation with superoxide dismutase (not shown). These experiments indicate that activation is reversible.

Because the formation of both superoxide ion and H_2O_2 was required and because catalase inhibited the superoxide dismutase effect, the formation of another material involving both superoxide ion and H_2O_2 was examined. This was thought to be the hydroxyl radical which is produced by interaction of superoxide ion and H_2O_2 (31):



Our efforts to mimic the activation of superoxide dismutase by the addition of H_2O_2 were unsuccessful. No activation with H_2O_2 was observed with several guanylate cyclase preparations that demonstrated different degrees of superoxide dismutase activation in the presence or absence of added nitrate reductase (not shown). However, White *et al.* (32) have reported H_2O_2 activation of lung guanylate cyclase preparations. Evidence for the formation of hydroxyl radicals was obtained by using hydroxyl radical scavengers (33). Activation of guanylate cyclase with superoxide dismutase was markedly decreased or totally prevented with butylated hydroxyanisole, hydroquinone, epinephrine, norepinephrine, and hemoglobin (Table 2). Some of these agents also decreased basal activity, which suggests that part of the basal activity may be partially activated enzyme. Other scavengers such as ethanol, mannitol, and benzoate had little or no effect on basal activity or superoxide dismutase activation. Thus, inhibition correlated with the ability of these agents to scavenge hydroxyl radicals (33).

DISCUSSION

Guanylate cyclase is found in virtually all tissues and is present in both soluble and particulate fractions (34). The properties of the soluble and particulate forms are different (29, 35, 36). Although numerous agents may increase cyclic GMP accumulation in intact tissues, relatively few agents can increase

Table 2. Effect of hydroxyl radical scavengers on guanylate cyclase activation by superoxide dismutase

Addition (mM)	Cyclic GMP, pmol/mg protein per min		Ratio: with/without
	Without SDase	With SDase	
Exp. 1			
None	62 (100)	463	7.5
Norepinephrine (1)	58 (94)	454	7.8
Norepinephrine (5)	32 (52)	212	6.6
Norepinephrine (10)	21 (34)	81	3.9
Exp. 2			
None	127 (100)	454	3.6
Mannitol (1)	127 (100)	379	3.0
Mannitol (5)	148 (117)	404	2.7
Mannitol (10)	145 (114)	382	2.6
Ethanol (650)	82 (65)	246	3.0
Ethanol + BHA* (1)	85 (67)	106	1.2
Ethanol + BHA (2)	87 (69)	50	0.6
Ethanol + BHA (5)	82 (65)	54	0.7
Hydroquinone (1)	67 (53)	42	0.6
Hydroquinone (2)	69 (54)	44	0.6
Hydroquinone (5)	52 (41)	36	0.7
Epinephrine (1.3)	87 (69)	219	2.5
Epinephrine (2.5)	98 (77)	178	1.8
Epinephrine (6.3)	109 (86)	172	1.6
Hemoglobin (0.01 µM)	102 (80)	97	1.0

Partially purified soluble rat liver guanylate cyclase preparations (6.5 µg of protein in Exp. 1; 6.7 µg of protein in Exp. 2) were incubated with various hydroxyl radical scavengers at the concentrations indicated. Some incubations contained 2 µg (Exp. 1) of superoxide dismutase (SDase) or 10 µg of superoxide dismutase and 10 µg of nitrate reductase (Exp. 2).

* BHA, butylated hydroxyanisole.

guanylate cyclase activity in cell-free systems (13–20, 32, 37). Many of these agents, such as azide, hydroxylamine, nitroglycerin, nitroprusside, and others, probably lead to the formation of nitric oxide which markedly activates most preparations (14, 18, 38). Because oxidizing and reducing agents can alter the formation of nitric oxide or the activation induced by nitric oxide and other agents (14, 16, 18), models could be constructed to implicate this mechanism as a physiological regulator of guanylate cyclase and cyclic GMP formation. Concentrations of nitrite, nitrate, and various amines are sufficiently high in liver and other tissues to permit nitric oxide formation and activation in physiological settings. Obviously, additional studies are required.

We have shown in this study that superoxide dismutase can markedly increase guanylate cyclase activity. This effect is increased with the addition of nitrate reductase. With some guanylate cyclase preparations, superoxide dismutase was less effective and the addition of nitrate reductase to increase superoxide ion formation enhanced the activation due to superoxide dismutase. Superoxide ion was formed under the conditions of the guanylate assay. However, the formation of superoxide ion was variable with different guanylate cyclase preparations and did not appear to correlate with the ability of superoxide dismutase to activate. These observations suggest that the content of flavoproteins in our guanylate cyclase preparations was variable. The effect of superoxide dismutase was diminished with inhibitors of the enzyme, KCN and glutathione, and when Mn^{2+} was included in incubations. The absence of an effect of superoxide dismutase with Mn^{2+} could be due to decreased superoxide ion formation (30) or the high guanylate cyclase activity observed in the presence of this

cation. We propose that superoxide dismutase catalyzes the formation of H_2O_2 which reacts with superoxide ion to yield hydroxyl radicals that activate guanylate cyclase. Nitrate reductase as a metalloflavoprotein might also enhance the formation of hydroxyl radical via the Haber-Weiss reaction because a chelated metal is required (31). Our hypotheses are strengthened by the observations that the addition of either catalase (to diminish H_2O_2 concentration) or hydroxyl radical scavengers markedly inhibited the activation of guanylate cyclase with superoxide dismutase. It seems quite unlikely that the effects of the high concentrations of catecholamines that were required as hydroxyl radical scavengers can relate to the pharmacological effects of these agents.

The diminished effect of superoxide dismutase with enzyme preincubation or after a second addition cannot be explained at present. These observations may relate to the very short half-lives of superoxide ion and hydroxyl radical due to their reactivity (39). Because activated enzyme was inhibited by subsequent additions of CN^- or catalase, activation is reversible.

Obviously, an important question is whether or not activation with nitric oxide (14, 16, 18, 38) and hydroxyl radical is accomplished through similar mechanisms. Some of the requirements for activation with azide, nitro compounds, and nitric oxide (13-18, 38, 40, 41) are different from those for the activation induced with superoxide dismutase. Activation of guanylate cyclase with azide or hydroxylamine requires a protein factor such as catalase or peroxidase (16) whereas catalase inhibits activation with superoxide dismutase. Hemoglobin can inhibit activation by either class of agents (Table 2) (42). Additional studies are required to determine whether or not activation of guanylate cyclase with hydroxyl radical and nitric oxide is through a common mechanism.

The formation of superoxide ion, hydrogen peroxide, and hydroxyl radical is likely to be the mechanism for physiological and hormonal regulation of guanylate cyclase and cyclic GMP formation. In some cell-free systems, guanylate cyclase is activated by H_2O_2 (32), arachidonic acid, prostaglandin endoperoxide, and other fatty acids (37, 43, 44). Oxygen tension and ascorbic acid also influence cyclic GMP levels in tissues (45). The formation or utilization of superoxide ion is involved in thromboxane and prostaglandin formation (46), peroxidation of membranes (30, 47), reduction of oxygen by flavin enzymes (48), autooxidation of hemoglobin (49), phagocytosis and lysosomal enzyme release from leukocytes (50), and other processes. Some of these processes can influence cyclic GMP levels in tissues.

Additional studies are required to test our hypotheses. Clearly, guanylate cyclase appears to be a unique enzyme that is activated by hydroxyl radical or nitric oxide, because these materials are inhibitory in all other systems in which they have effects.

The authors thank Bing Chang, Joanne Holmes, Robert Aldred, Robert Pistner, and Thomas Mickler for their technical assistance. These studies were supported by U.S. Public Health Service Research Grants HL 18260 and AM 15316 and Diabetes Endocrinology Research Center Grant AM 17042. F.M. is the recipient of U.S. Public Health Service Research Career Development Award AM 70456.

1. Hardman, J. G. & Sutherland, E. W. (1969) *J. Biol. Chem.* **244**, 6363-6370.
2. White, A. A. & Aurbach, G. D. (1969) *Biochim. Biophys. Acta* **191**, 686-687.
3. Schultz, G., Böhme, E. & Munki, K. (1969) *Life Sci.* **8**, 1323-1332.
4. George, W. J., Polson, J. B., O'Toole, A. G. & Goldberg, N. D.

- (1970) *Proc. Natl. Acad. Sci. USA* **66**, 398-403.
5. Goldberg, N. D., Haddox, M. K., Hartle, D. K. & Hadden, J. W. (1973) *Proceedings of the Vth International Congress of Pharmacology, San Francisco 1972* (Karger, Basel) pp. 146-169.
6. Thompson, W. J., Williams, R. H. & Little, S. A. (1973) *Biochim. Biophys. Acta* **302**, 329-337.
7. Howell, S. L. & Montagne, W. (1974) *Biochem. J.* **142**, 379-384.
8. George, W. J., Ignarro, L. J. & White, L. E. (1975) *Recent Adv. Stud. Card. Struct. Metab.* **7**, 381-390.
9. Amer, M. S. & McKinney, G. (1973) *Pharmacologist* **15**, 157.
10. Rudland, P. S., Gospodarowicz, D. & Seifert, W. (1974) *Nature* **250**, 741-742, 773-774.
11. Ichihara, K., Larner, J., Kimura, H. & Murad, F. (1977) *Biochim. Biophys. Acta* **481**, 734-740.
12. Kimura, H. & Murad, F. (1974) *J. Biol. Chem.* **249**, 329-331.
13. Kimura, H., Mittal, C. K. & Murad, F. (1975) *J. Biol. Chem.* **250**, 8016-8022.
14. Katsuki, S., Arnold, W. P., Mittal, C. K. & Murad, F. (1977) *J. Cyclic Nucleotide Res.* **3**, 23-35.
15. Mittal, C. K., Kimura, H. & Murad, F. (1975) *J. Cyclic Nucleotide Res.* **1**, 261-269.
16. Mittal, C. K., Kimura, H. & Murad, F. (1977) *J. Biol. Chem.* **252**, 4384-4390.
17. Miki, N., Nagano, M. & Kuriyama, K. (1976) *Biochem. Biophys. Res. Commun.* **72**, 952-959.
18. Arnold, W. P., Mittal, C. K., Katsuki, S. & Murad, F. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 3203-3207.
19. DeRubertis, F. R. & Craven, P. A. (1976) *Science* **193**, 897-899.
20. Veseley, D. C., Rovere, L. E. & Levey, G. S. (1977) *Cancer Res.* **37**, 28-31.
21. Theorell, H. & Ehrenberg, A. (1952) *Arch. Biochem. Biophys.* **41**, 462-474.
22. Keilin, D. & Hartree, E. F. (1954) *Nature* **173**, 720-723.
23. Singer, T. P. & Edmondson, D. E. (1974) in *Molecular Oxygen and Biology: Topics in molecular oxygen research*, ed. Hayaishi, O. (North Holland Pub. Co., Amsterdam), pp. 315-337.
24. Steiner, A. L., Parker, C. W. & Kipnis, D. M. (1972) *J. Biol. Chem.* **247**, 1106-1113.
25. Kimura, H., Thomas, E. & Murad, F. (1974) *Biochim. Biophys. Acta*, **343**, 519-528.
26. McCord, J. M. & Fridovich, I. (1969) *J. Biol. Chem.* **244**, 6049-6055.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275.
28. Fewson, C. A. & Nicholas, D. J. D. (1961) *Biochim. Biophys. Acta* **49**, 335-349.
29. Kimura, H. & Murad, F. (1974) *J. Biol. Chem.* **249**, 6910-6916.
30. Fong, K., McCay, P. B., Poyer, J. L., Keele, B. B. & Misra, H. P. (1973) *J. Biol. Chem.* **248**, 7792-7797.
31. Haber, F. & Weiss, J. (1934) *Proc. R. Soc. Edinburgh Sect. A* **147**, 332-351.
32. White, A. A., Crawford, K. M., Pratt, C. S. & Lad, P. J. (1976) *J. Biol. Chem.* **251**, 7304-7312.
33. Cohen, G. & Heikkila, R. E. (1974) *J. Biol. Chem.* **249**, 2447-2452.
34. Kimura, H. & Murad, F. (1975) *Life Sci.* **17**, 837-844.
35. Kimura, H. & Murad, F. (1975) *Metabolism* **24**, 439-445.
36. Chrisman, T. D., Garbers, D. L., Parks, M. A. & Hardman, J. G. (1975) *J. Biol. Chem.* **250**, 374-381.
37. Wallach, D. & Pastan, I. (1976) *J. Biol. Chem.* **251**, 5802-5809.
38. Mittal, C. K. & Murad, F. (1977) *J. Biol. Chem.* **252**, 3136-3140.
39. Schwartz, H. A. (1964) *Radiat. Res. Suppl.* **4**, 89-113.
40. Kimura, H., Mittal, C. K. & Murad, F. (1976) *J. Biol. Chem.* **251**, 7769-7773.
41. Kimura, H., Mittal, C. K. & Murad, F. (1975) *Nature* **257**, 700-702.

42. Murad, F., Mittal, C. K., Arnold, W. P., Katsuki, S. & Kimura, H. (1977) *Adv. Cyclic Nucleotide Res.* **9**, in press.
43. Glass, D. B., Frey, W., Carr, D. W. & Goldberg, N. D. (1977) *J. Biol. Chem.* **252**, 1275-1285.
44. Glass, D. B., Gerrard, J. M., Townsend, D., Carr, D. W., White, J. G. & Goldberg, N. D. (1977) *J. Cyclic Nucleotide Res.* **3**, 37-44.
45. Clyman, R. I., Blacksin, A. S., Manganiello, V. C. & Vaughan, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3883-3887.
46. Goldstein, I. M., Malmsten, C. L., Kaplan, H. B., Jindahl, H., Samuelsson, B. & Weissmann, G. (1977) *Clin. Res.* **25**, 518a.
47. Pederson, T. C. & Aust, S. D. (1973) *Biochem. Biophys. Res. Commun.* **52**, 1071-1078.
48. Massey, V., Strickland, S., Mayhew, S. G., Howell, L. G., Engel, P. C., Matthews, R. C., Schuman, M. & Sullivan, P. A. (1969) *Biochem. Biophys. Res. Commun.* **36**, 891-897.
49. Misra, H. P. & Fridovich, I. (1972) *J. Biol. Chem.* **247**, 6060-6062.
50. Babior, B. M., Kipnes, R. S. & Curnutte, J. T. (1973) *J. Clin. Invest.* **52**, 741-744.