THE ACTION OF PHENYLMERCURIC NITRATE

III. INABILITY OF SULFHYDRYL COMPOUNDS TO REVERSE THE DEPRESSION OF CYTOCHROME OXIDASE AND YEAST RESPIRATION CAUSED BY BASIC PHENYLMERCURIC NITRATE*

BY ELTON S. COOK AND GLADYS PERISUTTI

(From the Laboratories of the Institutum Divi Thomae, Cincinnati)

(Received for publication, October 12, 1946)

In Papers I and II (1, 2) it was shown that basic phenylmercuric nitrate depressed a number of enzymes (cytochrome oxidase, succinoxidase, catalase, and several dehydrogenases), that it reacted with sulfhydryl groups, and that the depression of yeast respiration by the basic nitrate could be prevented by the presence of sulfhydryl-containing compounds, such as cysteine or homocysteine. These findings suggested that the depression might involve interaction of the mercurial with essential —SH groups in the enzymes. However, it has been reported that certain of the above enzymes, namely cytochrome oxidase and catalase, do not depend upon —SH groups for their activity (3). If the basic phenylmercuric nitrate behaves like certain of the simple RHgX compounds which react specifically and reversibly with —SH groups, it should be possible to reverse the depression by adding a relatively large proportion of competing —SH groups, as noted by Barron and Singer (3). The present paper demonstrates that the basic phenylmercuric nitrate depression of cytochrome oxidase, like that of yeast respiration, can be prevented by the prior addition of sulfhydryl-containing compounds, but the depression of neither cytochrome oxidase nor yeast respiration can be reversed by subsequent addition of sulfhydryl-containing compounds. A series of amino acids containing various reactive groups did not protect either cytochrome oxidase or yeast respiration against the action of phenylmercuric nitrate with the exception of an effect of histidine upon the depression of cytochrome oxidase.

EXPERIMENTAL

Methods and Materials—The cytochrome oxidase system, with ascorbic acid as the substrate, was studied at 37.5° by the method of Schneider and Potter (4) as described in Paper I (1). The center well of the respirometer flask contained 0.2 ml. of 2 \( \times \) sodium hydroxide. The main chamber contained 1 ml. of \( 2.4 \times 10^{-4} \) \( \mu \) cytochrome c (referred to a molecular weight of 16,400), 0.3 ml. of a freshly prepared solution of 0.114 \( \mu \) ascorbic acid as the substrate, was studied at 37.5° by the method of Schneider and Potter (4) as described in Paper I (1). The center well of the respirometer flask contained 0.2 ml. of 2 \( \times \) sodium hydroxide. The main chamber contained 1 ml. of \( 2.4 \times 10^{-4} \) \( \mu \) cytochrome c (referred to a molecular weight of 16,400), 0.3 ml. of a freshly prepared solution of 0.114 \( \mu \) ascorbic acid as the substrate, was studied at 37.5° by the method of Schneider and Potter (4) as described in Paper I (1). The center well of the respirometer flask contained 0.2 ml. of 2 \( \times \) sodium hydroxide. The main chamber contained 1 ml. of \( 2.4 \times 10^{-4} \) \( \mu \) cytochrome c (referred to a molecular weight of 16,400), 0.3 ml. of a freshly prepared solution of 0.114 \( \mu \) ascorbic

* Presented before the Division of Biological Chemistry at the meeting of the American Chemical Society at Chicago, September 12, 1946.
acid in phosphate buffer, pH 7.4, 0.3 ml. of $4 \times 10^{-3}$ aluminum chloride, 
0.4 ml. of a cytochrome oxidase-containing tissue extract, and 1.0 ml. 
of phosphate buffer, pH 7.4. In the experimental flasks 0.5 ml. of the buffer 
was replaced by basic phenylmercuric nitrate of proper concentration 
dissolved in buffer. For protection experiments, a further 0.5 ml. of the 
buffer was replaced by a solution of the desired sulfhydryl-containing 
compound in buffer. For reversal experiments, the 0.5 ml. of sulfhydryl-
containing compound was placed in the side arm and tipped in at the 
desired time; a similar amount of buffer was tipped in at the same time in 
the control vessels. Both types of experiments were further controlled 
by flasks containing the sulfhydryl compound, but with omission of the 
phenylmercuric nitrate, and by flasks containing cytochrome c, ascorbic 
acid, and aluminum chloride. In these cases, the balance of the flask 
contents was made up with buffer.

The respiration of a pure culture of Saccharomyces cerevisiae was measured 
as described previously (2) in ~$/15$ KH$_2$PO$_4$ containing 0.02 per cent 
glucose, pH 4.5, at $30^\circ$ in Warburg respirometers. In each experiment 
duplicate flasks were used for each of the following: yeast control, sulf-
hydryl-containing compound, basic phenylmercuric nitrate, and sulf-
hydryl compound plus phenylmercuric nitrate. The control flasks held 
0.2 ml. of N KOH in the center well and, in the other chamber, 2 ml. of 
phosphate-glucose containing 4 mg., dry weight, of yeast; the side arm 
contained 1 ml. of phosphate-glucose which was tipped in at the desired 
time. In the appropriate flasks 1 ml. of the phosphate-glucose was re-
placed by a solution of the sulfhydryl (or other) compound in phosphate 
which was tipped in from the side arm.

Cytochrome $c$ was prepared from beef hearts by the method of Keilin 
and Hartree (5) with the exception that it was dialyzed against distilled 
water instead of 1 per cent sodium chloride. Rat heart extract served as a 
source of cytochrome oxidase.

Basic phenylmercuric nitrate (C$_8$H$_4$HgNO$_3$·C$_8$H$_4$HgOH) was obtained 
from The Hamilton Laboratories, Inc. l(+)—Cysteine hydrochloride, l(−)
cystine, and dl-methionine were obtained from General Biochemicals, Inc. 
l(−)—Tyrosine, dl serine, l(−)—proline, l(+)—arginine monohydrochloride, 
dl-lysine dihydrochloride, dl-phenylalanine, dl-aspartic acid, l(−)—trypto-
phane, and l(−)—histidine monohydrochloride were Eastman Kodak 
products. Glutathione was obtained from the Schwarz Laboratories, Inc. 
All chemicals were dissolved in the appropriate buffer with adjustment of 
pH if necessary.

Results

Cytochrome Oxidase—Preliminary experiments confirmed the previously 
observed depression of cytochrome oxidase by basic phenylmercuric
nitrate. The concentrations tested ranged from $1.6 \times 10^{-5}$ M to $8.4 \times 10^{-5}$ M, the latter concentration depressing the oxygen uptake to that of the cytochrome c control. Preliminary experiments also showed that concentrations of cysteine or glutathione of $10^{-3}$ M or less were without significant effect upon cytochrome oxidase activity, while $10^{-2}$ M was inhibitory under our conditions.

Fig. 1. The effect of glutathione in protecting cytochrome oxidase against basic phenylmercuric nitrate. Curve I, cytochrome oxidase control; Curve II, glutathione, $10^{-3}$ M, present from the start; Curve III, glutathione, $10^{-3}$ M, and basic phenylmercuric nitrate, $4.2 \times 10^{-5}$ M, present from the start; Curve IV, basic phenylmercuric nitrate, $4.2 \times 10^{-6}$ M, present from the start; Curve V, cytochrome c control (oxidase omitted).

Fig. 2. The ineffectiveness of glutathione in reversing the depression of cytochrome oxidase by basic phenylmercuric nitrate. Curve I, cytochrome oxidase control; Curve II, glutathione, $10^{-3}$ M, added at 15 minutes; Curve III, basic phenylmercuric nitrate, $4.2 \times 10^{-6}$ M, present from the start; Curve IV, basic phenylmercuric nitrate, $4.2 \times 10^{-4}$ M, present from the start; glutathione, $10^{-3}$ M, added at 15 minutes; Curve V, cytochrome c control (oxidase omitted).

Both glutathione and cysteine at $10^{-3}$ M, when placed in the flask with $4.2 \times 10^{-5}$ M phenylmercuric nitrate at the beginning of the experiment, protected the oxidase from the depressing effects of the mercurial. Concentrations of the sulfhydryl compounds of $10^{-4}$ M also protected, but less effectively, and in general, glutathione, mole for mole, was somewhat superior to cysteine. Fig. 1 shows the results of a typical experiment with glutathione; the protection amounted to approximately 65 per cent.

In contrast with their protecting ability, neither glutathione nor cysteine, $10^{-3}$ M, was effective in reversing the depression caused by $4.2 \times 10^{-5}$ M
basic phenylmercuric nitrate when the sulphydryl compound was added at intervals of 15, 30, or 60 minutes after the mercurial. Results of a typical experiment are shown in Fig. 2.

Protection against depression of cytochrome oxidase by $4.2 \times 10^{-5}$ M basic phenylmercuric nitrate was not afforded by concentrations up to $10^{-3}$ M of $l$-cystine, $dl$-methionine, $l$-tyrosine, $dl$-serine, $l$-arginine, $l$-proline, $dl$-lysine, $dl$-phenylalanine, $dl$-aspartic acid, or $l$-tryptophane. $l$-Histidine did afford approximately 55 per cent protection; this observation will receive further study. The $10^{-3}$ M concentrations of these amino acids in themselves caused 10 to 30 per cent depression of cytochrome oxidase activity, which was additive to the mercurial depression.

**Fig. 3.** The ineffectiveness of glutathione in reversing the depression of yeast respiration by basic phenylmercuric nitrate. Curve I, yeast control; Curve II, glutathione, $10^{-4}$ M, added at 15 minutes; Curve III, basic phenylmercuric nitrate, $1.5 \times 10^{-4}$ M, present from the start; glutathione, $10^{-4}$ M, added at 15 minutes; Curve IV, basic phenylmercuric nitrate, $1.5 \times 10^{-5}$ M, present from the start.

**Yeast Respiration**—The previously reported experiments (2) showed that $10^{-4}$ M cysteine or homocysteine (the highest concentrations that did not depress oxygen consumption) would protect yeast respiration against depression caused by adding $1.5 \times 10^{-5}$ M basic phenylmercuric nitrate after 30 minutes. In preliminary experiments these findings were confirmed and it was found, in addition, that glutathione could be used in concentrations up to $10^{-3}$ M. Both concentrations ($10^{-3}$ M and $10^{-4}$ M) of glutathione protected yeast against depression of respiration by $1.5 \times 10^{-5}$ M basic phenylmercuric nitrate. Similar results have been obtained with rat skin in place of yeast.

Cysteine, $10^{-4}$ M, added at 15 or 30 minutes after the phenylmercuric nitrate, did not reverse the depressant action. Similarly, glutathione, $10^{-3}$ M or $10^{-4}$ M, added 15 or 30 minutes after the mercurial, failed to reverse the depression. Results of a typical experiment with $10^{-4}$ M
glutathione are shown in Fig. 3; $10^{-3}$ m glutathione gave almost identical results.

As with cytochrome oxidase, L-tyrosine, DL-serine, L-arginine, L-proline, DL-lysine, DL-phenylalanine, DL-aspartic acid, and L-tryptophane in $10^{-4}$ m concentrations failed to protect yeast respiration against $1.5 \times 10^{-5}$ m basic phenylmercuric nitrate. In addition, L-histidine, was also ineffective. L-Cystine and DL-methionine had previously been shown to be ineffective in this respect (2). The amino acids in this concentration did not affect yeast respiration, thus confirming earlier findings (6) under somewhat different conditions.

**DISCUSSION**

These experiments and those reported earlier (1, 2) show that the basic phenylmercuric nitrate depression of cytochrome oxidase and of yeast respiration may be prevented by combination of the mercurial with a sulfhydryl-containing compound. After 15 minutes, phenylmercuric nitrate inhibition of cytochrome oxidase could not be reversed by adding as high as 24 moles (12 equivalents) of cysteine or glutathione and the inhibition of yeast respiration could not be reversed by addition of as much as 66 moles (33 equivalents) of glutathione. Experiments, to be published elsewhere with G. W. Thomas, also show that the inhibitory activity of basic phenylmercuric nitrate on *Escherichia coli*, *Eberthella typhosa*, and *Staphylococcus aureus* can be prevented by cysteine, homocysteine, or glutathione, but not by cystine or methionine; to date, efforts to reverse this inhibitory activity by means of the sulfhydryl compounds have been unsuccessful.

The experimental evidence at present available does not afford an adequate explanation of the findings. As a tentative working hypothesis it might be suggested that, while basic phenylmercuric nitrate may react with —SH groups in enzymes and depress those enzymes for which these groups are essential, thus accounting for the previously observed behavior with succinic dehydrogenase (1), it may not be specific for such groups but may also react with other groups present in the enzyme protein. This would explain the depression of cytochrome oxidase which does not require —SH groups for its activity according to Barron and Singer (3). The alternative explanation would require that the basic phenylmercuric nitrate react irreversibly with essential —SH groups not accessible to the variety of reagents employed by Barron and Singer. This seems unlikely. Aside from the possibility offered by the behavior of histidine on cytochrome oxidase depression, which requires further study, the attempted use of the other amino acids as protecting agents throws no positive light upon the nature of other groups with which phenylmercuric nitrate might react.
SUMMARY

The depression of cytochrome oxidase activity and of yeast respiration by basic phenylmercuric nitrate can be prevented by the sulfhydryl-containing compounds, cysteine and glutathione. This depression cannot be reversed by adding the sulfhydryl compounds in great excess (12 to 33 equivalents). Cystine, methionine, tyrosine, serine, arginine, proline, lysine, phenylalanine, aspartic acid, and tryptophane, containing additional reactive groups, were ineffective in protecting cytochrome oxidase and yeast respiration from depression by basic phenylmercuric nitrate. Histidine was likewise ineffective in protecting yeast respiration but did exert a protective effect upon cytochrome oxidase.

BIBLIOGRAPHY

THE ACTION OF PHENYLmercuric NITRATE: III. INABILITY OF SULFHYDRYL COMPOUNDS TO REVERSE THE DEPRESSION OF CYTOCHROME OXIDASE AND YEAST RESPIRATION CAUSED BY BASIC PHENYLmercuric NITRATE

Elton S. Cook and Gladys Perisutti


Access the most updated version of this article at [http://www.jbc.org/content/167/3/827.citation](http://www.jbc.org/content/167/3/827.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/167/3/827.citation.full.html#ref-list-1](http://www.jbc.org/content/167/3/827.citation.full.html#ref-list-1)