THE ACTION OF PHENYLTHIOCARBAMIDE ON TYROSINASE

BY FREDERICK BERNHEIM AND MARY L. C. BERNHEIM

(From the Departments of Physiology and Pharmacology and of Biochemistry, Duke University School of Medicine, Durham, North Carolina)

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Richter and Clisby (1) have investigated the toxic effect of phenylthiocarbamide in rats. The acute toxic effect is characterized by an effusion of fluid into the thoracic cavity, but in chronic poisoning there is a lowered body temperature, which suggests a depression of metabolism, and compensatory changes in the thyroid. It was therefore of interest to test the effect of the drug in vitro on isolated enzyme systems.

Further, Richter and Clisby (2) observed graying of the hair in black rats after feeding phenylthiocarbamide to them for 2 or 3 months. This suggested an interference with melanin formation, and for this reason we investigated the effect of the drug on tyrosinase. Phenylthiocarbamide is a very effective inhibitor for tyrosinase, even in concentrations as low as $1 \times 10^{-4}$ M, and the characteristics of this inhibition are described. The experiments with animal enzyme preparations have yielded negative results.

EXPERIMENTAL

The tyrosinase of the common mushroom *Psalliota campestris* was used for most of the experiments, but similar results were also obtained with potato tyrosinase. The enzyme was used either as the crude water extract of the mushrooms or partially purified according to the method of Ludwig and Nelson (3). Catechol, p-cresol, dihydroxyphenylalanine, tyramine, adrenalin, and tyrosine were used as substrates. The oxidation was measured in the Warburg apparatus at 37°. 0.05 M phosphate buffer was used either at pH 6.7 or 7.8. Fig. 1 shows the effect of various concentrations of phenylthiocarbamide on the oxidation of equimolar concentrations of four compounds. In all cases the inhibition is complete at first and then suddenly disappears, and the subsequent rate of oxidation of the substrate is the same as that of the control. The length of the period of complete inhibition varies with the different substrates, and the more rapidly the compound is oxidized the shorter is the period of inhibition by a given concentration of phenylthiocarbamide. Thus catechol which is oxidized very rapidly is inhibited for about 25 minutes by 0.005 mg. of the drug, while tyramine, which under the same conditions is oxidized more slowly, is inhibited for more than 3 hours (see Fig. 1). This type of inhibition could be explained if the phenylthiocarbamide were destroyed by the
enzyme, or if the drug were displaced from the enzyme surface as the oxidation proceeds. However, shaking the phenylthiocarbamide with the purified enzyme for 4 hours before the addition of the substrates does not shorten the inhibition period, as would be expected if the drug were destroyed. To test the validity of the second hypothesis, an experiment was performed with p-cresol and is shown in Fig. 2. If the drug is added to the enzyme before the p-cresol, the usual inhibition of about 60 minutes is obtained. But if the p-cresol is added first and the oxidation is allowed to proceed for 5 minutes before the phenylthiocarbamide is added, very little, if any, inhibition occurs. If the oxidation is allowed to proceed to completion and at that time more p-cresol and the drug are added, inhibition again occurs, but for a shorter period. With the dihydroxy compounds, catechol, dihydroxyphenylalanine, and adrenalin, this effect can also be demonstrated. Evidently an intermediate product, and to a lesser extent the end-product, can displace the phenylthiocarbamide from the enzyme surface.

The length of the inhibition period varies with the concentration of the enzyme, the substrate, and the drug. The concentration of the enzyme used was standardized for comparative purposes so that an inhibition of 60 ± 10 minutes was obtained in the presence of 1.0 mg. of p-cresol and 3 × 10⁻⁸ M phenylthiocarbamide. The inhibition period with a given amount of phenylthiocarbamide is shorter as the concentration of the substrate is increased, indicating that the substrate or its end-product is competing with the drug for the enzyme surface. This is shown in Fig. 3.
The effect of pH on the inhibition period is shown in Fig. 4. Most of the experiments were carried out at pH 6.7 to prevent the autoxidation of the dihydroxyphenylalanine. The inhibition period for p-cresol and catechol is, however, longer at pH 7.8 but the rate of oxidation of these compounds is not appreciably affected by this change in hydrogen ion concentration. Phenylthiocarbamide has no effect on the autoxidation of dihydroxyphenylalanine at pH 7.8 nor does it affect the oxidation of cysteine catalyzed by inorganic copper ions.

![Fig. 2](image-url)

**Fig. 2.** Curve A, the oxidation of 1.0 mg. of p-cresol. Curve C, at the arrow more p-cresol (1.0 mg.) was added to the mixture. Curve B, the oxidation of 1.0 mg. of p-cresol; 0.01 mg. of phenylthiocarbamide was added to the mixture 5 minutes after the p-cresol. Curve D, at the arrow p-cresol (1.0 mg.) with 0.01 mg. of phenylthiocarbamide was added to the mixture. Curve E, 0.01 mg. of phenylthiocarbamide was added to the enzyme before 1.0 mg. of p-cresol. pH 6.7 and 37°.

![Fig. 3](image-url)

**Fig. 3.** The effect of 0.01 mg. of phenylthiocarbamide on the oxidation of different concentrations of catechol and p-cresol. pH 6.7 and 37°.

Keilin and Mann (4) state that sodium diethyldithiocarbamate inhibits laccase, but they give no details of the inhibition. Fig. 5 shows the effect of this compound on the oxidation of catechol. The type of inhibition is different from that of phenylthiocarbamide in that it increases with time. The lowest concentration of the diethyldithiocarbamate to give a significant inhibition with the standard amount of enzyme is considerably greater than that of phenylthiocarbamide. Moreover, the inhibition period with the latter compound is shorter for catechol than for p-cresol, whereas the former is a more effective inhibitor of the oxidation of catechol. The inhibition with thiourea, however, resembles that with phenylthiocarbamide but 10 to 20 times the concentration of thiourea are necessary to
obtain equivalent inhibitions. \( p \)-Aminobenzoic acid does not protect the enzyme against any of these compounds. Various urethanes even in large concentrations are without effect.

In an attempt to explain the action of phenylthiocarbamide in the rat, we investigated the effect of the drug on certain isolated enzyme systems.

![Figure 4](image)

**Fig. 4.** The effect of the hydrogen ion concentration on the inhibition of the oxidation of 1.0 mg. of \( p \)-cresol by 0.01 mg. of phenylthiocarbamide. \( 37^\circ \).

![Figure 5](image)

**Fig. 5.** A comparison of the effects of phenylthiocarbamide, thiourea, and sodium diethyldithiocarbamate on the oxidation of 1.0 mg. each of \( p \)-cresol and catechol. pH 6.7 and \( 37^\circ \).

Various rat tissues were prepared as slices or broken cell suspensions, but it was found that phenylthiocarbamide even in relatively large concentrations had no immediate effect on the oxygen uptake of such preparations, but caused a small depression after incubation with them for about 3 hours. The tissues used were liver, kidney, brain, and muscle. Investigation of
the effect of the drug on certain specific enzymes also yielded negative results, even after prolonged incubation of the drug with the enzyme. Thus the activity of the cytochrome, succinic acid, choline, l-proline, d-amino, amine, and sarcosine oxidases of liver and kidney was not affected by a concentration of 50 mg. per cent. The xanthine oxidase of liver was slightly inhibited but the oxidation of glucose, lactic acid, and pyruvic acid by brain was not.

We observed, however, that methemoglobin production, which occurs when broken cell suspensions of liver and kidney are shaken in air (5), is inhibited by phenylthiocarbamide without affecting the oxygen uptake of these tissues. The methemoglobin production has been shown to be caused by H₂O₂ formed when amines, d-amino acids, and purines are oxidized (5). The inhibition of the xanthine oxidase may thus partially account for the inhibition of methemoglobin production, but the possibility remains that methemoglobin is formed in part by a copper protein complex which Mann and Keilin (6) have shown to be present in liver and which may be inhibited by the phenylthiocarbamide.

DISCUSSION

Phenylthiocarbamide is a very effective inhibitor of tyrosinase, which is a copper protein enzyme. It has, however, no effect on oxidations catalyzed by inorganic copper. Since, with the possible exception of the dopa oxidase of skin which has not been isolated, copper protein enzymes have not been shown to play any important part in catalyzing oxidations in the mammalian body, the toxic effects of phenylthiocarbamide in the rat are not explained by its action on tyrosinase.

If the dopa oxidase of skin is a copper protein enzyme, it is possible to explain the graying of the hair on the basis of its inhibition by phenylthiocarbamide.

SUMMARY

1. Phenylthiocarbamide is a very effective inhibitor of tyrosinase.
2. The characteristics of the inhibition have been described. It differs from that produced by sodium diethyldithiocarbamate.
3. The significance of this inhibition for the effects produced by phenylthiocarbamide in the animal have been discussed.

BIBLIOGRAPHY

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Frederick Bernheim and Mary L. C. Bernheim


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