14690

# Inhibitory Action of Thiouracil, Thiocarbamide and Other Compounds on Melanin Formation by Tyrosinase.

K. E. PASCHKIS,* A. CANTAROW, W. M. HART, AND A. E. RAKOFF.

*From the Jefferson Medical College and Hospital, Philadelphia, Pa.*

Sulfonamides, thiocarbamide, and a number of other compounds containing —NH·CS·NH— or $NH_2 \cdot C_6H_4$— groups have been found to suppress formation of thyroid hormone.[1,2] Because of indications that this action depends upon interference with an oxidative enzymatic process,[3] it was thought to be of interest to study the action of these and similar agents on enzyme systems *in vitro*. The present report deals with the influence of thiouracil† and thiocarbamide on the tyrosinase-tyrosine system as compared to that of glutathione, cysteine, sulfonamides, *p*-aminobenzoic acid and ascorbic acid.

*Methods.* The technic of Baker *et al.*[4] was employed, the substrate being a 0.001 M solution of tyrosine in phosphate buffer pH 7.0. The agents studied were dissolved in this substrate in 0.001 M concentration. A crude tyrosinase solution was prepared by grinding potatoes and centrifuging the mash. The volumes of the solutions employed are indicated in the tables. The solutions were observed for 2 hours, then hourly for 7 hours, and again after 24 hours.

*Results and Comment.* The pertinent data are presented in the tables. Inhibition of melanin formation by *p*-aminobenzoic acid and sulfanilamide has been reported by Martin *et al.*[5] A similar effect is produced by sodium sulfathiazole and sulfadiazine. The inhibiting action of glutathione has been described by Figge[6] and that of phenylthiocarbamide and thiocarbamide by Bernheim and Bernheim[7]. We found thiouracil to be the most potent in this regard of the compounds investigated by us. It is also a more potent anti-thyroid agent than thiocarbamide, *p*-aminobenzoic acid and sulfonamides.[8] Ascorbic acid exerts no anti-thyroid effect;[8] the influence of glutathione and cysteine in this connection has not been studied. The absence of strict parallelism between *in vitro* tyrosinase-inhibiting effect and *in vivo* thyroid-inhibiting effect is indicated by the fact that phenylthiourea is 10-20 times as potent as thiourea in inhibiting tyrosinase[7] whereas phenylthiourea has no effect and thiourea a strong anti-thyroid effect in the rat.[8]

The influence of glutathione and cysteine is of interest in view of the demonstration by György[9] that sulfhydryl compounds as well as thiocarbamide and thiouracil inhibit autoxidation (rancidity) of fat. He offers the hypothesis that this antioxygenic action of thiocarbamide is due to the sulfhydryl radicle in its tautomeric form (isothiocarbamide), and considers the possibility that the action of these agents as antioxidants may explain their anti-thyroid activity. Our findings do not support this hypothesis when applied to inhibition of tyrosinase activity. As indicated in Table I, melanin formation was completely inhibited by thiouracil in 0.0005 M concentration and only partially inhibited by

* J. Ewing Mears Fellow in Physiology and Medicine.

1 Mackenzie, C. G., and Mackenzie, J. B., *Endocrinol.*, 1943, **32**, 185.

2 Astwood, E. B., Sullivan, J., Bissell, A. B., and Tyslowitz, R., *Endocrinol.*, 1943, **32**, 210.

3 Dempsey, E. W., *Endocrinol.*, 1944, **34**, 27.

† Thiouracil used in this study was supplied by the Lederle Laboratories through the courtesy of Dr. B. Carey and Dr. S. M. Hardy.

4 Baker, A. K., Kline, B. E., and Rush, H. P., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 361.

5 Martin, G. J., Wisansky, W. A., and Ansbacher, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 26.

6 Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 269.

7 Bernheim, F., and Bernheim, M. L. C., *J. Biol. Chem.*, 1942, **145**, 213.

8 Astwood, E. B., *J. Pharm. Exp. Therap.*, 1943, **78**, 79.

9 György, P., Stiller, E. T., and Williamson, M. B., *Science*, 1943, **98**, 518.

TABLE I.
Relative Potency of Various Inhibitors.
Melanin Formation. 7 Hours.

| | 0.001 M | 0.00075 M | 0.0005 M | 0.00025 M |
|---|---|---|---|---|
| Thiouracil | 0 | 0 | 0 | + |
| Thiocarbamide | ± | ++ | +++ | +++ |
| *p*-Aminobenzoic acid | 0 | + | ++ | +++ |
| Sod. Sulfathiazol | 0 | + | ++ | ++ |
| Sulfadiazine | 0 | + | ++ | ++ |
| Glutathione | 0 | | ++ | |

Substrate: 20 cc 0.001 M tyrosine solution in phosphate buffer pH 7.0. Inhibitors dissolved in tyrosine solution. Enzyme solution: 0.5 cc.

TABLE II.
Inhibition of Melanin Formation in 0.001 M Tyrosine Solution.

| | Inhibitors | Volume, cc | Molarity, M | Activator | Pink (Hallachrome?) | Melanin 7 | Melanin 24 hr |
|---|---|---|---|---|---|---|---|
| 1. | 0 | | | 0 | 10 min | +++ | ++++ |
| 2. | Thiouracil | | | 0 | 0 | 0 | 0 |
| | 2.5 mg per | 20 | 0.001 | Iodoacetic acid | trace 60 min | 0 | 0 |
| | 0.5 cc enzyme | | | $CuSO_4$ | | 0 | 0 |
| 3. | 1.2 mg per | 10 | 0.001 | 0 | " 30 " | 0 | 0 |
| | 0.5 cc enzyme | | | Iodoacetic acid | " 30 " | + | +++ |
| | | | | $CuSO_4$ | | ++ | ++++ |
| 4. | Thiocarbamide | | | 0 | 10 min | + | + |
| | 1.6 mg per | 20 | 0.001 | Iodoacetic acid | 10 " | + | + |
| | 0.5 cc enzyme | | | $CuSO_4$ | | ++ | +++ |
| 5. | 0.8 mg per | 20 | 0.001 | 0 | 10 " | + | + |
| | 0.5 cc enzyme | | | Iodoacetic acid | 10 " | + | +++ |
| | | | | $CuSO_4$ | | + | ++++ |
| 6. | Glutathione | | | 0 | 0 | 0 | 0 |
| | 6.1 mg per | 20 | 0.001 | Iodoacetic acid | | 0 | + |
| | 0.5 cc enzyme | | | $CuSO_4$ | | ++ | ++ |
| 7. | 3.0 mg per | 10 | 0.001 | 0 | | 0 | ++++ |
| | 0.5 cc enzyme | | | Iodoacetic acid | | + | ++++ |
| | | | | $CuSO_4$ | | + | ++++ |
| 8. | 1.5 mg per | 10 | 0.0005 | 0 | | ++ | ++++ |
| | 0.5 cc enzyme | | | Iodoacetic acid | | ++ | ++++ |
| | | | | $CuSO_4$ | | ++ | ++++ |
| 9. | Cysteine HCl | | | 0 | 30 " | ++ | +++ |
| | 1.2 mg per | 10 | 0.001 | Iodoacetic acid | 30 " | +++ | ++++ |
| | 0.5 cc enzyme | | | $CuSO_4$ | | ++ | ++++ |
| 10. | Ascorbic acid | | | 0 | 0 | 0 | 0 |
| | 3.5 mg per | 20 | 0.001 | Iodoacetic acid | 0 | 0 | 0 |
| | 0.5 cc enzyme | | | $CuSO_4$ | 0 | 0 | 0 |

glutathione in the same concentration. If it be assumed that 50% of the thiouracil is present in its tautomeric form, the glutathione solution is a weaker inhibitor than the thiouracil solution containing one-fourth the number of —SH groups.

Ascorbic acid was included in this study because of the contradictory results obtained by other investigators. Evans and Raper[10] found that ascorbic acid inhibited melanin

formation, whereas Figge[11] observed increased pigment formation in the presence of this agent. We have found that ascorbic acid in 0.001 M solution (column 10, Table II), inhibits melanin formation as completely as thiouracil in equimolecular concentration (column 2, Table II).

In an attempt to gain more information regarding the mechanism of inhibition of melanin production by these substances, the possible anti-inhibiting influence of certain agents was investigated. Tyrosinase is known to be a copper proteinate[12] and it is conceivable that the inhibitors may combine with the prosthetic copper group. Addition of $CuSO_4$ to a tyrosinase-tyrosine solution neither hastened nor intensified melanin formation, but the inhibitory effect of glutathione, thiocarbamide, and thiouracil was to some extent overcome by copper. Data presented in Table II indicate that at higher inhibitor/enzyme ratios the inhibitory effect of glutathione and thiocarbamide was still overcome somewhat by copper, but that of thiouracil and ascorbic acid was not affected, demonstrating the higher inhibiting potency of the latter compounds.

In view of György's suggestion[9] that the antioxygenic action of thiocarbamide may be due to its tautomeric form isothiocarbamide, the effect of iodoacetic acid which inactivates —SH, was investigated. The results obtained were identical with those obtained with copper (Table II).

Raper[13] has shown that tyrosinase is necessary only for the initial phases of melanin formation, *i. e.*, for oxidation of tyrosine to dopa and hallachrome, the subsequent stages proceeding in the absence of the enzyme. We have observed a trace of pink color, probably due to hallachrome, in some of the experiments in which inhibition of melanin formation was complete. It was therefore thought possible that this inhibition might be due to interference with the later, non-enzymatic phases of melanin formation rather than to an anti-enzyme action. However, this seems unlikely, because the melanin precursor derived from hallachrome is tan-colored[11] and should have been detectable in our observations. A tan color developed with the sulfonamides and *p*-aminobenzoic acid, but not with thiouracil, thiocarbamide, glutathione or ascorbic acid. The possibility that the reaction might be arrested at the stage of a colorless compound cannot be excluded.

*Summary and Conclusions.* 1. Melanin formation in a tyrosinase-tyrosine system was inhibited by thiouracil, thiocarbamide, glutathione, cysteine, ascorbic acid, *p*-aminobenzoic acid, and sodium sulfathiazole and sulfadiazine. 2. This inhibition is overcome by copper and by iodoacetic acid. 3. Thiouracil and ascorbic acid were more potent inhibitors than the other compounds studied. 4. Available data do not permit a definite statement regarding the nature of the inhibitory mechanism, but they suggest a direct anti-enzyme action.

[10] Evans, W. C., and Raper, H. S., *Biochem. J.*, 1937, **31**, 2162.

[11] Figge, F. H. J., *J. Cell. Comp. Physiol.*, 1940, **15**, 233.

[12] Kubowitz, F., *Biochem. Z.*, 1938, **296**, 443.

[13] Raper, H. S., *Erg. Enzymforsch.*, 1932, **1**, 720.