EFFECT OF MONOBENZYL HYDROQUINONE ON OXIDASE SYSTEMS IN VIVO AND IN VITRO

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It was observed by Oettel (1) in 1936 that the peroral administration of hydroquinone to black haired cats turned the hair gray. Discontinuation of the drug resulted in repigmentation of the hair. In an investigation of occupational leukoderma by Oliver, Schwartz and Warren (2, 3), it was found that the monobenzyl ether of hydroquinone, contained as an antioxidant in the rubber gloves of the workers, was responsible for the depigmentation. Their experiments as well as our own demonstrated that the depigmentation was due to the action of monobenzyl hydroquinone on the system “dopa”-oxidase dihydroxyphenyl alanine (“dopa”).

EXPERIMENTAL PART

In a number of human subjects, both white and colored, the monobenzyl compound was applied in the form of a 50 per cent ointment or as a 50 per cent ethereal suspension. In a few instances the concentrated powder was used. Leukoderma was produced after incubation periods varying from weeks to months.

Histologic examination of the areas of depigmentation revealed a negative “dopa” reaction and an almost complete disappearance of the melanin. The microscopic picture could not be differentiated from a viteligo in many instances since the depigmentation was seen to occur without preceding inflammatory reaction.

A number of guinea pigs were fed approximately 12 grams of monobenzyl ether of hydroquinone over a period of 5 months. This was well tolerated but no pigmentary changes were observed.

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Local application over a period of months, just as in the human subjects, caused depigmentation. The disappearance of the pigment was accompanied by a negative “dopa” reaction in the affected areas. This observation only applied to the previously pigmented epidermis whereas the hair bulbs were not affected. This was probably due to a lack of penetration of the monobenzyl compound to the melanoblasts in the hair matrix.

When blood smears are exposed to a 0.1 per cent “dopa” solution according to the method of Bloch and Peck (4), a striking effect of polyphenolase on “dopa” is observed which is manifested by the formation of intense black granules in the leukocytes. The blood smears are first fixed in formalin vapor before immersion in the “dopa” solution. When such a fixed preparation is exposed to ether or ethanol for 1 hour prior to immersion in “dopa” solution, the oxidase reaction is markedly weakened or in some instances even completely abolished. However, if the formalin fixed blood smears are immersed in an ethereal solution of monobenzyl ether of hydroquinone in concentrations of 1 to 5 per cent for a period of 1 hour the action of the oxidase on the “dopa” solution is increased. Moreover, blood films in which the oxidase action has apparently been abolished by previous exposure to ether for a period of several hours can be brought back to full activity by the monobenzyl compound and the enhanced effect described above may then be seen. When monomethyl ether of hydroquinone and dibenzylether were substituted for the monobenzyl compound the enhanced effect was not observed.

The action of hydroquinone monobenzyl ether and other so-called anti-oxidants on oxidase systems in vitro was also studied. Tryosinase was prepared from potatoes (5). This acts upon “dopa”, tyrosine and non-nitrogenous polyphenols. The oxidation of tyrosine by this “potato tyrosinase” first leads to dihydroxyphenyl alanine; the latter reacts faster than tyrosine and according to Raper and Wormall (6) the further reaction takes place in three steps. The first step yields a red substance. The second step, which may only be observed under special precautions leads to a colorless phase from which melanin then forms as the end product.
When 15 to 50 mg. of potato preparation are added to 5 ml. of a phosphate buffered solution of 0.5 to 1.0 mg. of tyrosine or "dopa," the pink stage appears within a few minutes, persists for two hours and then gradually changes to melanin. When 0.2 ml. of a 1 per cent solution of Agerite alba\(^1\) or of monobenzyl ether of hydroquinone in alcohol is added to the preceding mixture, development of the pink color lags behind the control and the pink shades of the control are usually reached a few hours later. However, instead of continuing to the melanin stage, the pink product accumulates without being further converted to melanin even on prolonged standing over 12 hours. There results a deep purple solution which continues to be transparent in spite of the depth of the color. This is in marked contrast to the relatively opaque colloidal melanin suspensions in the controls. In control experiments with 0.2 ml. of alcohol without antioxidant, normal melanin formation took place. When the monobenzyl ether of hydroquinone is incubated without the potato enzyme, it does not form colored oxidation products in spite of its monophenolic character.

A crude oxidase was prepared from mealworms. The monophenolase in this preparation is stronger and more stable than potato enzyme and acts on the monobenzyl ether itself yielding a red end product. When the monomethyl ether of hydroquinone was used, instead of the monobenzyl ether or Agerite alba, similar results were obtained but the color was not as pure.

The dibenzyl ether and a number of agerite antioxidants designated "Agerite resin, AR powder, AR white" which consist of aldol-\(\alpha\)-naphthylamine, phenyl-\(\beta\)-naphthylamine, and sym. di-\(\beta\)-naphthyl-\(p\)-phenylene diamine, have no effect on melanin formation.

In a parallel series of experiments the potato enzyme was used with and without Agerite alba on the various diphenols. Catechol in a solution of 1 mg. in 5 ml. plus potato enzyme in the same concentrations as given above passed through a greenish-yellow

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\(^1\) "Agerite alba" is a trade name for an antioxidant which is reputed to be monobenzyl ether of hydroquinone containing less than 1 per cent unchanged hydroquinone.
stage but turned pink after 2 hours and on prolonged standing melanin was formed. Test tubes which in addition contained antioxidant stayed pink. The substrate alone and a solution containing substrate and Agerite alba but no enzyme remained practically colorless under the same conditions.

When resorcinol and hydroquinone plus the oxidase were used with Agerite alba there was a definite increase in the oxidase reaction. The resorcinol-containing solution first became yellow and then passed into a stage showing strong green fluorescence. Solutions containing hydroquinone first became pink and then yellow. A solution without Agerite alba remained slightly pink.

When hydroquinone acetic acid (homogentisic acid) was used in such a system, it turned pink, then yellow and on longer standing, a deep orange-yellow. Control solutions without Agerite alba stayed slightly yellow.

It appears from these observations that mono ethers of hydroquinone inhibit the melanin formation by “dopa” oxidase in the skin and by potato tyrosinase in vitro with “dopa” as well as with tyrosine. They do not prevent the primary enzymatic reaction leading to the pink orthoquinoid compound. As this compound is in molecular solution it is too diffuse for microscopic observation; hence skin sections exposed to monobenzyl hydroquinone appear less black than the controls.

SUMMARY AND CONCLUSIONS

1. The monobenzyl ether of hydroquinone specifically modifies the action of “dopa” oxidase on the propigment in the melano blasts so that the reaction does not proceed to the melanin stage.

2. In contrast to the above the monobenzyl ether of hydroquinone enhances the effect of leukocyte polyphenolase on dihydroxyphenyl alanine.

3. The action of potato oxidase on tyrosine and dihydroxyphenyl alanine in vitro is interrupted by monobenzyl ether of hydroquinone at the red stage.

\[\text{This substance was prepared for us by Prof. Mario Volterra from the urine of an \textit{sic}kaptonuric.}\]
BIBLIOGRAPHY


