# INHIBITION OF MELANIN FORMATION BY CHEMICAL AGENTS

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Physicians and laity alike have long been interested in depigmentation of the skin by chemical agents. It has been hoped that these substances might be useful in the treatment of patients having various forms of disfiguring hyperpigmentation.

Ointments of mercury compounds probably have been used longer and more frequently than other substances to produce depigmentation. Their use as "freckle creams" has been persistent despite the inconclusive results produced. Vitamin C in large oral doses decreases the pigmentation of some patients with Addison's disease (1, 2, 3). Recently Schuppli (4) reported depigmenting scars, chloasma, and chloasma-like dermatoses with the use of iontophoretic application of ascorbic acid. During the past 15 years several reports have appeared showing that hydroquinone and two other p-hydroxyphenyl derivatives inhibit melanin formation in mammals. Other chemicals which inhibit pigment formation have been discussed in detail recently (5). When the different inhibitors of melanin formation are compared with each other, it is apparent that at the present time the p-hydroxyphenyl derivatives probably offer the best means of effecting depigmentation of the skin. These chemicals shown in Fig. 1 are hydroquinone, monobenzylether of hydroquinone and p-hydroxypropiophenone. Because of this similarity in structure, it was believed worthwhile to compare these three compounds experimentally. In this report the inhibition of pigmentation by hydroquinone, monobenzylether of hydroquinone and p-hydroxypropiophenone will be reviewed; and recent studies with these compounds will be presented.

*Hydroquinone.* Oettel (6) reported in 1936 that when hydroquinone was fed to black haired cats in doses of 30 mg. per kilogram daily over a period of six to eight weeks, the hair turned gray. In a similar period after stopping the drug the hair become repigmented. Martin and Ansbacher (7) confirmed Oettel's finding and reported in addition that young mice given hydroquinone in their feedings developed achromatrichia within a period of from four to twenty weeks.

*Monobenzylether of hydroquinone.* Oliver, Schwartz, and Warren (8) first reported in 1939 that an antioxidant known by the trade name of Agerite Alba* in rubber gloves was responsible for depigmentation of the skin of workers wearing the gloves. This preliminary report was followed in 1940 with publications by the same authors (9, 10) giving a detailed study of the clinical and pathological characteristics of the effect of monobenzylether of hydroquinone on skin. Spencer (11, 12) has reported leukoderma occurring also from rubber used in covering wire dish trays, adhesive tape, hat bands, and from washing contraceptive

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* Agerite Alba is monobenzylether of hydroquinone with dibenzylether of hydroquinone and benzyl chloride as impurities.

diaphragms. Botvinick (13) reported a case of dermatitis and secondary leukoderma due to fabric-lined rubber gloves. Bernstein and Sachs (14) observed cases of leukoderma arising from contact during the manufacturing process of rubber dolls and the synthetic rubber, neoprene. All of the above materials were found to contain or assumed to contain monobenzylether of hydroquinone.

Peck and Sobotka (15) fed a number of guinea pigs approximately 12 grams of monobenzylether of hydroquinone over a period of five months with no pigmentary changes being noted. However, the local application of the compound over a period of months caused depigmentation of the epidermis. The hair bulbs were not affected.

*Para-hydroxypropiophenone.* In 1949, Perrault and co-workers (16) noted variable success in the treatment of hot flashes with the oral use of p-hydroxypropiophenone (H-365, Frénateur Hypophysaire de Synthèse). These workers then tried the drug in a case of

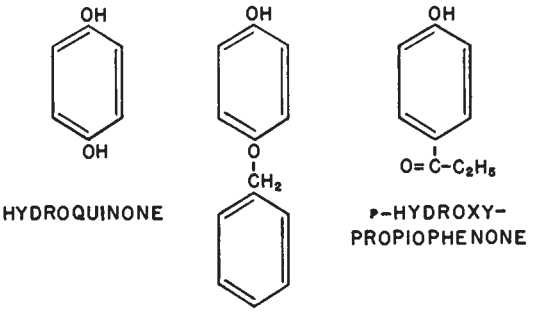


FIG. 1.

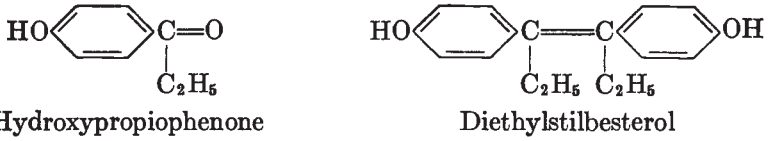


FIG. 2

malignant chorioepithelioma with pulmonary metastases. Six months later the patient was apparently cured as indicated by clinical, biological, and radiological studies. In addition, there was no recurrence eight months following termination of therapy. The compound was successfully employed in the treatment of toxic diffuse goiter and malignant exophthalmus, especially in those cases with a strong hypophyseal influence. The drug was reported to be effective in cases of ovarian and hypophyseal-ovarian dysfunction. In 1950, Perrault (16) summarized the studies regarding p-hydroxypropiophenone and indicated that the compound had no direct cellular effect and that its action was probably through hypophyseal inhibition.

Grupper, Plas, and Boudin (17) cited evidence indicating that Riehl's melanosis might be due to pituitary dysfunction. This belief was further borne out by reference to the favorable influence on Riehl's melanosis by x-ray therapy directed to the pituitary. With these concepts in mind they treated five cases of Riehl's melanosis with p-hydroxypropiophenone in dosage of 1–2 Gm. daily for four to six weeks with success. In a subsequent report Grupper and Plas (18) reported the successful treatment of a total of eight cases of Riehl's melanosis,

two cases of chloasma, a case of cervico-facial pigmentation, and two cases of erythrose peribuccale pigmentaire de Brocq. Perrault (16) cited two cases of Riehl's melanosis treated with p-hydroxypropiophenone in which he obtained success in one and failure in the other, possibly on the basis of insufficient dosage.

Para-hydroxypropiophenone was studied during a search for synthetic estrogen-like compounds. It has a chemical structure much like diethylstilbesterol (Fig. 2) but seems to lack its estrogenic and melanin influencing properties according to Perrault (16).

## EXPERIMENTAL STUDIES

The experiments were divided into three groups as follows: 1) *In vitro* studies to determine the effect of p-hydroxyphenyl derivatives on the enzymatic formation of melanin from tyrosine and dopa. 2) Studies on the effect of oral and parenteral administration of these compounds to pigmented guinea pigs and mice. 3) Studies on the effect of local application of these agents to human skin.*

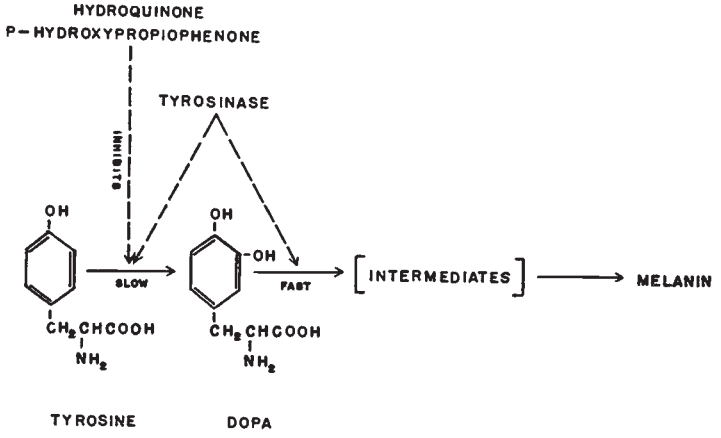


FIG. 3.

*In vitro studies.* The effect of p-hydroxyphenyl derivatives on the enzymatic oxidation of tyrosine and dopa to melanin was studied using tyrosinase from the Harding-Passey mouse melanoma (19). Detailed reports of these investigations will be published elsewhere. Hydroquinone, monobenzylether of hydroquinone and p-hydroxypropiophenone were added to tyrosine-tyrosinase and dopa-tyrosinase reaction mixtures. Hydroquinone in 12 times the molar concentration of tyrosine completely inhibited melanin formation as determined by measurements of oxygen uptake and quantitative analyses of unreacted tyrosine at the end of the reaction. No inhibition occurred when dopa was used as substrate. These findings show that hydroquinone inhibits the first step in melanogenesis, that is, the conversion of tyrosine to dopa, Fig. 3.

Para-hydroxypropiophenone in 9.7 times the concentration of tyrosine prolonged the induction period of tyrosine oxidation. There was no effect on dopa

* Reagent grade Eastman hydroquinone and p-hydroxypropiophenone were used. Monobenzylether of hydroquinone was prepared by recrystallizing the commercial grade chemical with 40 per cent methanol.

oxidation. Here, as in the case of hydroquinone, p-hydroxypropiophenone inhibits only the conversion of tyrosine to dopa.

Monobenzylether of hydroquinone in 3.6 times the concentration of tyrosine stimulated the reaction. The induction period in tyrosine oxidation was reduced, and monobenzylether of hydroquinone was oxidized. That is, the oxygen uptake of the tyrosine-tyrosinase monobenzylether of hydroquinone reaction mixtures was much greater than that of the control tyrosine-tyrosinase preparation. Monobenzylether of hydroquinone also increased the oxygen uptake of the dopa-tyrosinase reaction. Since tyrosinase does not catalyze the oxidation of monobenzylether of hydroquinone, this compound must be oxidized by chemicals formed when dopa is oxidized to melanin. It cannot be determined from these studies how monobenzylether of hydroquinone inhibits melanin formation. It is possible that in the skin this compound is converted to hydroquinone which then

TABLE I

*Effect on pigmentation of male guinea pigs after daily oral administration of p-hydroxyphenyl derivatives for 76 days*

Five guinea pigs (average weight 564 g./animal) were used in each group.

| COMPOUND | DAILY DOSE mg./kg./day | TOTAL DOSE (grams) | NUMBER OF ANIMALS SHOWING DEPIGMENTATION OF HAIR |
|---|---|---|---|
| Hydroquinone | 22 → 88 | 2.38 | 1? |
| Monobenzylether of hydroquinone | 40 → 160 | 4.37 | 3 |
| p-Hydroxypropiophenone | 30 → 120 | 3.23 | 2 |
| None | | | 0 |

inhibits the reaction. It is also possible that the compound produces depigmentation through its action as a reducing agent so that melanin is reduced to a light-colored substance. A third hypothesis is that monobenzylether of hydroquinone acts as an anti-oxidant to prevent oxidation of sulfhydryl groups, thereby making more sulfhydryl groups available for tyrosinase inhibition. However, this hypothesis is not supported by our experimental work (20).

*Animal tests.* Three groups of five adult male guinea pigs were fed capsules of increasing amounts of hydroquinone, monobenzylether of hydroquinone, and p-hydroxypropiophenone daily for 76 days. A fourth group was used as a control. Since we had no information on the toxicity of these compounds, the animals were given an arbitrary initial dose as shown in Table I. The chemicals did not produce any toxic reaction, and the dose was rapidly increased to the level indicated in Table I. Again no toxic effects were noted, and it is believed that the highest dose finally used could have been employed from the onset of the experiment. None of the control group exhibited depigmentation. Depigmentation, when occurring, was first evident after four weeks of treatment; and it became more marked from then on throughout the experimental period of 76 days.

One of the hydroquinone-treated animals showed questionable depigmentation, three of the monobenzylether of hydroquinone group (Figs. 4 & 5) became

lighter, and two of the p-hydroxypropiophenone group (Figs. 6 & 7) turned lighter. In general it appeared that very light colored or black guinea pigs showed the greatest change in color. The dark brown guinea pigs showed no change.

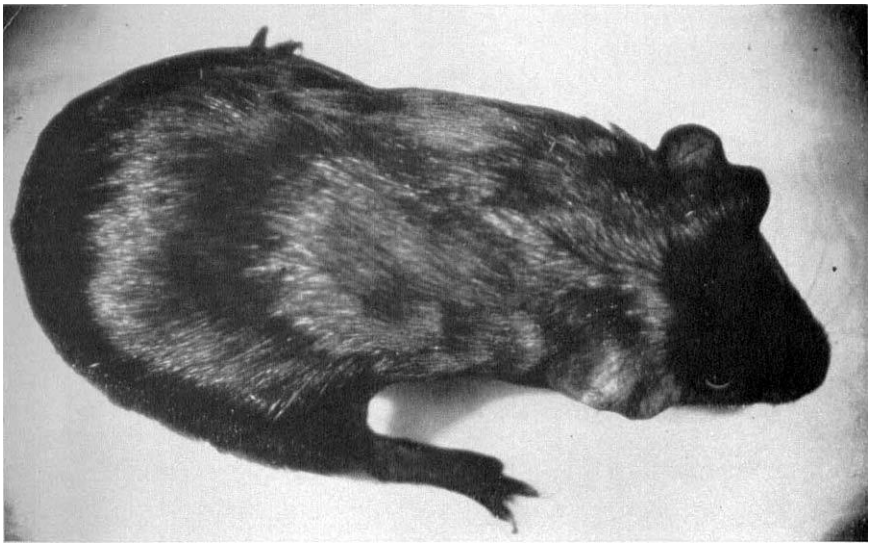

FIG. 4. Guinea pig prior to oral administration of monobenzylether of hydroquinone.

FIG. 5. Guinea pig following 76 days of oral administration of monobenzylether of hydroquinone.

In the case of the guinea pigs receiving monobenzylether of hydroquinone the results varied from those of Peck and Sobotka (15), who found no pigmentary

change in guinea pigs fed a similar daily dose over a five-month period. This might be accounted for by the use of different strains of guinea pigs. In addition it is possible that our final doses per kilogram per day may have been higher than those used by Peck and Sobotka.

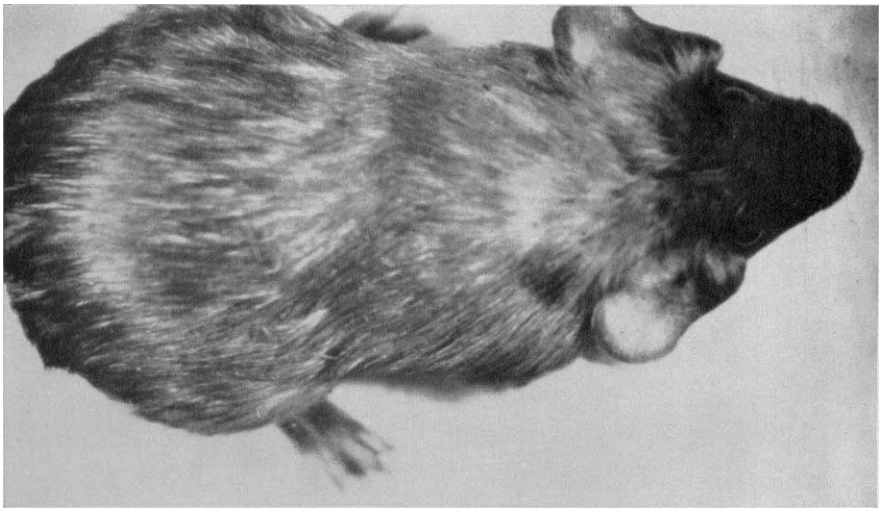

FIG. 6. Guinea pig prior to oral administration of para-hydroxypropiophenone.

FIG. 7. Guinea pig following 76 days of oral administration of para-hydroxypropiophenone.

Another experiment was conducted using four groups of four adult black C-57 male mice. These animals were observed over a 76-day period for pigmentary change in their hair. One group was given daily 0.05 ml. subcutaneous

injections of hydroquinone in water in doses of 22 mg. per kilogram. Another group was given similar injections of p-hydroxypropiophenone in propylene glycol in doses of 30 mg. per kilogram—a dose comparable to that used by Grupper and coworkers (17) (1–2 Gm. per day in adults). The fourth group was given hydroquinone in their drinking water in increasing doses from 37 to 262 mg. per kilogram per day. As noted in Table II the hydroquinone group showed marked depigmentation at the site of injection (Figs. 8 & 9). The p-hydroxypropiophenone group showed no local depigmentation, but there was noted a diffuse increase in the number of grey hairs throughout the body hair. The group receiving hydroquinone in the drinking water showed only questionable pigmentary change compared to the control group over the period of observation. When the study was repeated using 7-week old male C-57 black mice, it was found that 2 out of 4 mice showed some general depigmentation. The results of

TABLE II

*Effect on pigmentation of black C-57 male mice after daily administration of p-hydroxyphenyl derivatives for 76 days*

Four mice 7 months old of approximately 20 g. average weight were used in each group.

| COMPOUND | ADMINISTRATION | DAILY DOSE mg./kg./day | TOTAL DOSE (mg.) | DEPIGMENTATION OF HAIR |
|---|---|---|---|---|
| Hydroquinone in water. | 0.05 ml. subcut. | 22 | 33.3 | At site of injection |
| p-Hydroxypropiophenone in propylene glycol | 0.05 ml. subcut. | 30 | 45.6 | Generally over body |
| Propylene glycol control | 0.05 ml. subcut. | | | None |
| Hydroquinone | Orally in drinking water | 37 → 262 | 247 | None |

oral administration of hydroquinone to black mice seem to depend on some unknown factor associated with the age of the mice. However, injections of hydroquinone consistently produced depigmentation at the site of injection—probably as a result of the great concentration of hydroquinone in the injection area. As in the case of the guinea pigs, depigmentation, when it occurred, was first observed after about 4 weeks and became more marked as the experiment progressed. These results were in general agreement with the finding of Martin and Ansbacher (7) that young mice given hydroquinone orally developed achromotrichia in an interval of from four to twenty weeks.

Two of the 4 adult mice and all 4 young mice receiving hydroquinone in the drinking water developed alopecia on the back of the neck during the course of the experiments.

*Clinical studies.* After it was shown that monobenzylether of hydroquinone (Agerite Alba) would produce depigmentation, it was only natural that the material should be suggested for clinical trial in cases of hyperpigmentation (8, 9, 21). Oliver and coworkers (8) first pointed out that in the clinical use of

this chemical, one should take into account the facts that it requires a long time for depigmentation to occur, that there is a possibility of depigmenting a larger area than desired, and that sensitization to the compound may occur. Although

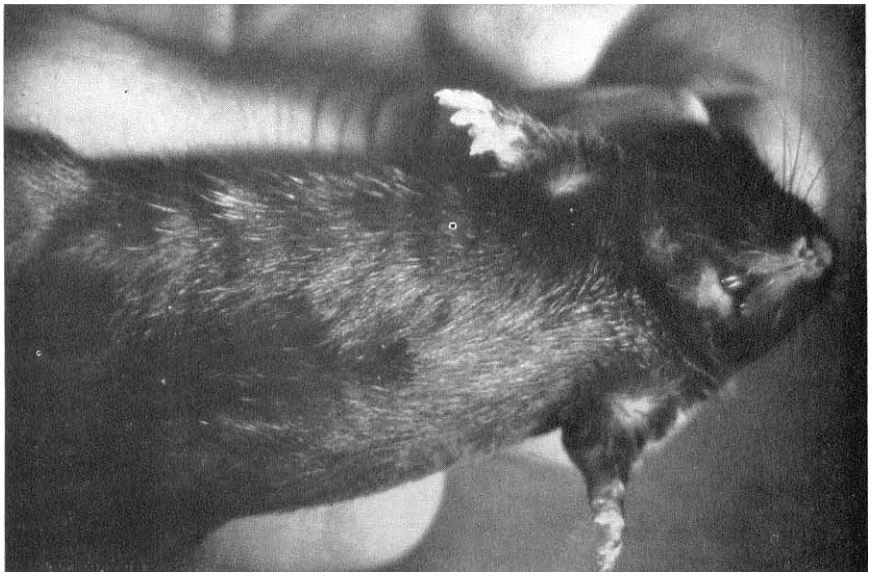

FIG. 8. Black male C-57 control mouse.

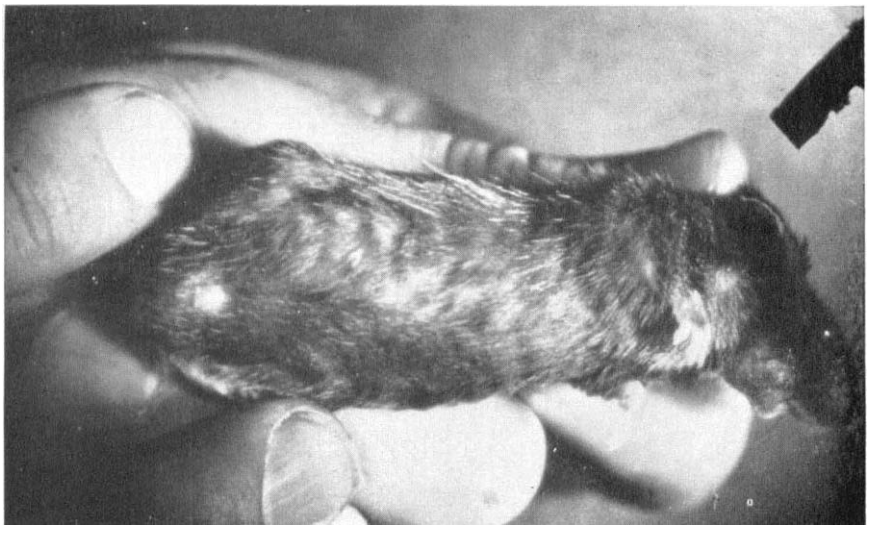

FIG. 9. Black male C-57 mouse showing depigmentation of hair at site of subcutaneous injection of hydroquinone.

these precautions must be kept in mind, they should not prevent the careful clinical use of monobenzylether of hydroquinone. This view is supported by the following studies on clinical cases.

*Normal subjects.* Continuous patch tests of ten and thirty per cent hydroquinone, monobenzylether of hydroquinone, and p-hydroxypropiophenone in yellow petrolatum were applied to the backs of seven young adults for thirty days. Four of the subjects were Negro males, one was a Negro female, and two were white males with freckles. As noted in Table II two of the Negro subjects developed depigmentation from hydroquinone and monobenzylether of hydro-

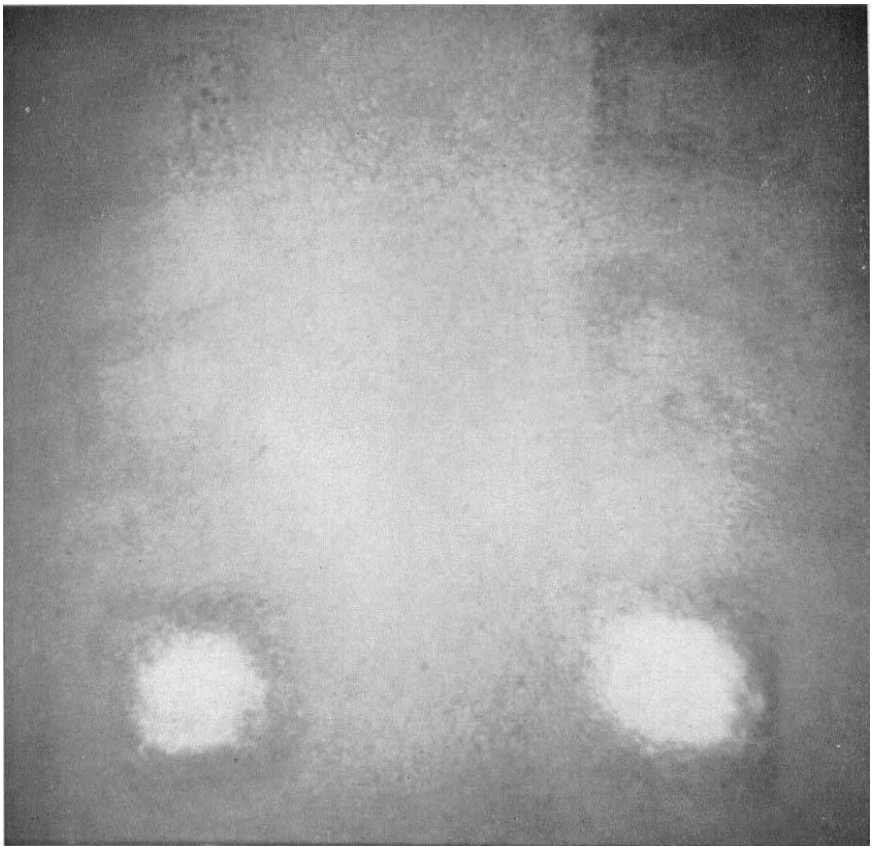

FIG. 10. Sites of continuous patch tests on back of a Negro male volunteer. From above downwards, the patches are para-hydroxypropiophenone, hydroquinone, and monobenzylether of hydroquinone. Ointments containing 10 per cent of the chemicals are on the left, and those containing 30 per cent are on the right.

quinone. The higher concentration of these substances gave increased depigmentation (Fig. 10). Following the termination of the experiment, pigmentation gradually returned about the hair follicles in all areas of depigmentation after one month. These findings in the case of monobenzylether of hydroquinone paralleled those of Oliver and coworkers (8, 9, 10). In two of the Negro volunteers there was dermatitis due to the hydroquinone. There was no reaction from monobenzylether of hydroquinone. There was neither dermatitis nor evidence of depigmentation from the patches of p-hydroxypropiophenone during the

TABLE III

*Results of continuous application on seven young adults with 10 and 30 per cent hydroquinone, monobenzylether of hydroquinone, and p-hydroxypropiophenone in yellow petrolatum for a period of one month*

| SUBJECT | HYDROQUINONE | | MONOBENZYLETHER OF HYDROQUINONE | | p-HYDROXYPROPIOPHENONE | |
|---|---|---|---|---|---|---|
| | 10% | 30% | 10% | 30% | 10% | 30% |
| J. K., colored male | No change | No change | No change | No change | No change | No change |
| B. B., colored male | Dermatitis with secondary pigmentation | Dermatitis with secondary pigmentation | No change | No change | No change | No change |
| L. C., colored male | No change | No change | No change | No change | No change | No change |
| B. A., colored male | Dermatitis at end of 2 weeks and patch removed. Slight depigmentation noted at 4 weeks. | Dermatitis at end of 2 weeks and patch removed. Slight depigmentation noted at 4 weeks. | Moderate depigmentation without dermatitis. | Marked depigmentation without dermatitis. | No change | No change |
| J. H., colored female | Slight depigmentation | Moderate depigmentation | Moderate depigmentation without dermatitis | Marked depigmentation without dermatitis | No change | No change |
| A. L., white male with freckles | No change | No change | No change | No change | No change | No change |
| R. L., white male with freckles | No change | No change | No change | No change | No change | No change |

period of observation. It is possible that if the ointments had been applied for a longer period of time more cases would have shown depigmentation.

Twenty per cent monobenzylether of hydroquinone in a special vanishing cream base* was applied to the forearms of 70 normal individuals as a patch test for 48 hours. No case of sensitivity was noted over the test period. These results indicate that the preparation is satisfactory for general clinical use.

### *Pigmentary Disorders*

Case 1. D. P., a white male age 24, with generalized lentigenes and psychoneurosis, developed pigmented cutaneous macules on the exposed and unexposed surfaces of the body at 2-3 years of age. These lesions were more prominent in the summer. At the age of 15, the lesions became much darker and more numerous. It was at this time that the patient had mumps followed by atrophy of the right testicle. There was no familial history of pigmentary disturbances. The patient developed a psychoneurosis in addition to having pigmentary changes.

General physical examination was essentially negative except for atrophy of the right testicle. Over the skin surface including the lips there were many hyperpigmented macules varying in diameter from 0.5 mm. to 3 mm. being most abundant on the exposed surfaces of the body. In some areas these lesions coalesced to form macules which were 3 to 7 cm. in diameter.

Laboratory reports were within normal limits. X-ray examination of the gastrointestinal tract for polyposis was negative. The skin biopsy of one of the lesions was characteristic of lentigo on microscopic examination.

In May 1950, when this patient presented himself to the Mayo Clinic for the treatment of his pigmented lesions, it was suggested by one of us (T. B. F.) that he be treated with monobenzylether of hydroquinone. Since then the patient has been consistently applying 33 per cent monobenzylether of hydroquinone in petrolatum and more recently in an alcohol-acetone lotion to the face, neck, and one hand one or two times daily. He has shown marked improvement of the areas treated (Figs. 11 & 12). This is also evident when the treated hand is compared with the untreated hand. There has been no dermatitis and the patient is very pleased with the result.

Case 2. B. R., a white male age 24, with generalized lentigenes and psychoneurosis. This patient's early history is obscure; but apparently at the age of 3–4 years, he developed swelling of the face and neck which lasted for some time. This disturbance was followed by pigmented lesions on his skin, the initial site being unknown. At the age of 8, the patient became aware of the lesions and he felt that many more lesions appeared over his body at that age. Since then the lesions apparently have not increased either in number or in size. The lesions become darker on exposure to sunlight. There was no history of ingestion of heavy metals. The family history was of interest in that the patient's mother died of a tumor of the eye with metastases to the liver at age 55; two full brothers have epilepsy, one being a mental patient; one maternal half-brother is described as nervous; and one paternal half-sister has a nervous tic. The patient has a psychoneurosis.

General physical examination was essentially normal. Over the patient's skin surface were many macular brown and black lesions varying in size from 0.1–1.5 cm. in diameter. The mucous membranes were clear and the eye grounds showed no abnormal pigmentation.

Laboratory reports were within normal limits. X-ray examination of the gastrointestinal tract for polyposis was negative. The skin biopsy of one of the lesions was characteristic of lentigo on microscopic examination.

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* This material was supplied through the courtesy of Paul B. Elder Company, Bryan, Ohio.

This patient applied 33 per cent monobenzylether of hydroquinone in yellow petrolatum and in alcohol-acetone lotion to the face, neck, and hands intermittently for a period of six months. He cooperated poorly with the treatment and there was only slight decrease in pigmentation. There was no dermatitis.

Case 3. L. E., white female age 32 with chloasma. The patient developed transitory hyperpigmentation of the face 13 years ago during her first pregnancy. Two years later with her second pregnancy she again developed hyperpigmentation over the face. This darkening has increased in severity since that time.

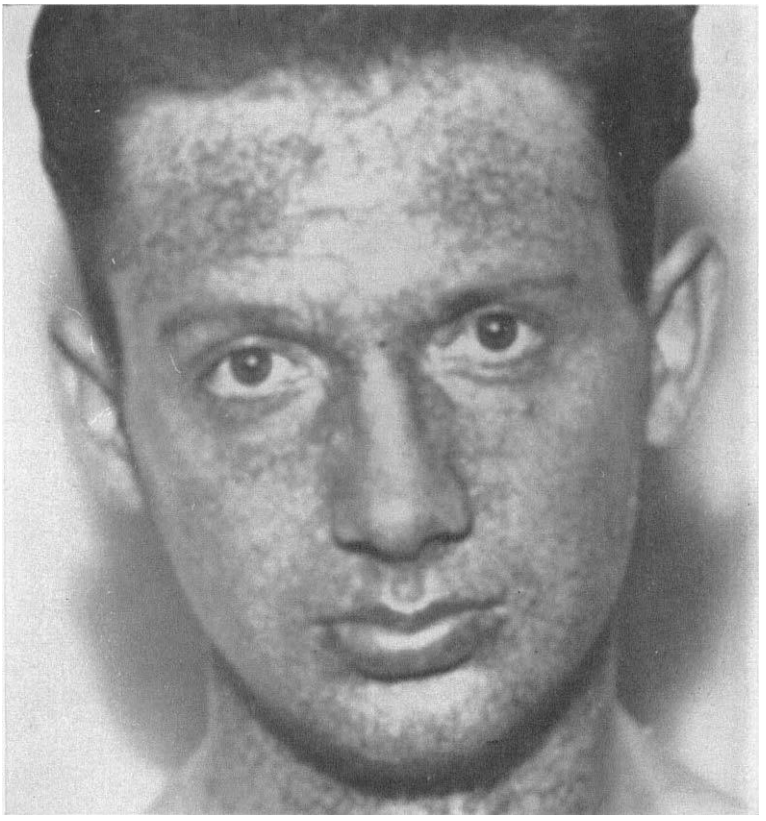

FIG. 11. Case 1, prior to the local application of 33 per cent monobenzylether of hydroquinone to the face.

General physical examination was essentially normal. Over the chin, cheeks, and forehead there was a circular band of well marginated hyperpigmentation with adjacent areas of hypopigmentation.

Basal metabolic rate was −19 per cent. Other laboratory reports were within normal limits.

The patient has consistently applied 30 per cent monobenzylether of hydroquinone in a vanishing cream to the lesions twice daily for thirty days. She noted a burning sensation lasting for thirty minutes after the preparation was applied. However, this feeling has been diminishing lately. The patient has shown definite lightening of her lesions after one month of therapy. There has been no dermatitis.

Case 4. N. S., a white female age 35, with Berlock dermatitis of the face. Thirty-three per cent monobenzylether of hydroquinone in yellow petrolatum was applied daily to the face for a period of two months with no evidence of depigmentation or dermatitis. Therapy is being continued.

Case 5. J. H., a white male age 25, with a macular pigmented nevus of the face. Thirty-three per cent monobenzylether of hydroquinone in yellow petrolatum was applied to the lesion daily for a period of two months with resulting depigmentation. Occasionally there was evidence of slight dermatitis during therapy.

FIG. 12. Case 1, following the local application of 33 per cent monobenzylether of hydroquinone to the face twice daily for eight months.

Case 6. F. Z., a white male age 31, with Riehl's melanosis of the face. Thirty per cent monobenzylether of hydroquinone in a vanishing cream base was applied twice daily for six weeks. To date there has been no change in pigmentation and no dermatitis. Therapy is being continued.

Case 7. A. L., a white male age 30, with ephelides of the arms. Thirty per cent monobenzylether of hydroquinone in a vanishing cream base was applied twice daily for three weeks. There has been some depigmentation, but pruritus and secondary lichenification from scratching in the treatment area occurred. Therapy has been stopped.

In addition to the above cases, nine more patients with various disorders of hyperpigmentation are being treated at the present time with 20 to 30 per cent monobenzylether of hydroquinone in a vanishing cream base applied twice

daily. While there has been no clinical follow-up as yet, we have not been notified of the occurrence of dermatitis. The accomplishment of depigmentation with the lack of dermatitis by the use of this compound is in accord with the findings of Oliver, Schwartz, and Warren (8) and Pollock (21).

Various bases and solvents for monobenzylether of hydroquinone were tried. It was decided that the most satisfactory preparations were a lotion (acetone seven parts and alcohol ten parts), a vanishing cream base, and yellow petrolatum, each containing 10 to 33 per cent monobenzylether of hydroquinone. When the lotion is used, the solvent quickly evaporates; and a very fine flesh colored powder of the compound remains on the treated area. This powder is not objectionable and often serves as an acceptable cosmetic covering for the lesions under treatment. The ointments are not particularly greasy and they remain very smooth. The lotion and the ointment preparations can be used together, the lotion during the day and the ointment at night.

Stolar (22) has had wide experience in treating cases of abnormal pigmentation with monobenzylether of hydroquinone. He found that when wetting agents are incorporated into the ointment, depigmentation extends beyond the area of application. In the preparations we used depigmentation occurred only at the site where the ointment was applied.

We have had no clinical experience with the oral use of p-hydroxypropiophenone in cases of hyperpigmentation. However, we hope to undertake a detailed clinical study of this compound in the near future.

## DISCUSSION

It is of interest to compare the mechanism of melanin inhibition effected by the three types of compounds which have been used clinically to depigment skin, namely, mercury compounds, ascorbic acid and p-hydroxyphenyl derivatives. Nealon (23) found that mercury preparations could decrease the color of skin by as much as 15 per cent. Darkly pigmented people showed the greatest change. However, in general it is not believed that these ointments are very effective depigmenting agents. Some dermatologists believe that mercury preparations act merely by producing an exfoliation which in turn causes depigmentation. This explanation is probably incorrect because many agents other than mercury compounds produce exfoliation without lightening of the skin color. A more plausible hypothesis is that mercury may replace the copper required for tyrosinase activity and thereby inactivate the enzyme. This concept is supported by recent *in vitro* experiments in which it was shown that mercury ions could compete with cupric ions for the active centers on tyrosinase (20). When mercury ions became bound to the enzyme an inactive preparation was obtained.

Ascorbic acid acts in a different manner to decrease the color of skin. Melanin pigment can exist in an oxidized or reduced state. In the oxidized state melanin is very dark, while in the reduced state it is light brown in color. Ascorbic acid can keep melanin in the reduced light colored form (3). In addition, ascorbic acid can inhibit melanin formation by preventing the oxidation of dopa quinone, an intermediate in the conversion of tyrosine and dopa to melanin. In all these

actions the effect of ascorbic acid is only temporary. Pigmentation returns upon discontinuation of the drug.

Para-hydroxyphenyl derivatives inhibit melanin formation in still another way. Hydroquinone and p-hydroxypropiophenone inhibit the enzymatic oxidation of tyrosine to dopa; that is, these compounds inhibit the first reaction of melanogenesis. The details of this inhibition are not known at the present time. These compounds may also serve as reducing agents to decrease skin color in a manner similar to the action of ascorbic acid. Hydroquinone is not a useful inhibitor of pigment formation clinically for two reasons. First, when applied locally to skin its action is weak and inconsistent because it is water soluble and probably cannot penetrate the skin surface to exert its effect. Second, hydroquinone is unstable and undergoes auto-oxidation to form deeply colored products. When hydroquinone is applied to the skin these darkly colored products form and adhere to the skin surface. Para-hydroxypropiophenone has little local action but is effective when used orally or parenterally. It is possible that p-hydroxypropiophenone may act indirectly through the pituitary gland to decrease melanin formation in addition to inhibiting the enzymatic formation of melanin. The mechanism of action of monobenzylether of hydroquinone remains obscure. This compound has no effect on the *in vitro* enzymatic oxidation of tyrosine or dopa to melanin. It is possible that monobenzylether of hydroquinone is converted to hydroquinone in the skin and that the hydroquinone formed prevents melanin formation. It is also possible that monobenzylether of hydroquinone can act as a reducing agent merely to maintain melanin in its lighter color. However, monobenzylether of hydroquinone does not act only as a reducing agent because some cases of occupational leukoderma resulting from contact with this chemical do not repigment completely upon cessation of exposure. Not all patients treated with monobenzylether of hydroquinone show depigmentation. Definite variations exist from person to person. It is not known whether this variability is due to differences in pH, hormonal activity, or differing enzyme concentrations in individual skins.

From the foregoing data as well as the reports of other investigators, it is evident that the hydroquinone derivatives, monobenzylether of hydroquinone and p-hydroxypropiophenone, are effective clinical agents for treating hyperpigmentation due to melanin. These agents are more effective and more useful than other agents used to date. When properly employed in 10 to 33 per cent concentration in lotions or ointments, monobenzylether of hydroquinone is a valuable local therapeutic agent. Undesirable side reactions such as dermatitis or sensitization do not preclude its controlled medical use. No serious side reactions have been encountered.

## SUMMARY

1. It has been shown through clinical trial, animal experiments, and *in vitro* studies that hydroquinone and p-hydroxypropiophenone are effective inhibitors of melanin formation. Monobenzylether of hydroquinone was the most effective inhibitor of melanin formation clinically, although it had no effect on melanin formation *in vitro*.

2. These compounds when administered orally to colored guinea pigs produce depigmentation.

3. The parenteral administration of hydroquinone and p-hydroxypropiophenone to black mice also produces depigmentation.

4. Monobenzylether of hydroquinone in 10 to 33 per cent concentration in lotions and ointments is an effective agent clinically for treatment of melanin hyperpigmentation.

5. The mechanism of action of these compounds has been discussed.

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## DISCUSSION

DR. PETER FLESCH: I would like to call Dr. Denton's attention to a recent paper by Foster (Proc. U. S. Nat. Acad. Sci., **36,** 606, 1950) who claims that there is a non-enzymatic conversion of tyrosine and of dopa in normal mouse skin. Unfortunately, it is impossible to evaluate this paper, because of lack of detailed experimental data. If Foster's findings should be confirmed, the mechanism of hydroquinone inhibition, as proposed by Dr. Denton, will have to be reexamined.

DR. ROTHMAN: I wonder whether p-hydroxypropiophenone can be classified as a hydroquinone derivative as it does not have two hydroxyl groups in para-position. The results presented here on the action mechanism of hydroquinone mono-benzyl ether are identical with those presented here a year ago by Dr. A. L. Lorincz (see J. Invest. Dermat. **15,** 425, December 1950).

DR. IRVIN BLANK: I may not be justified in rising to discuss Dr. Denton's paper because I shall not discuss the main body of the paper on pigmentation. However, in the last minute of Dr. Denton's paper, and also in Dr. Stolar's comment, there was a good deal of discussion of the clinical use of benzyl hydroquinone for producing de-pigmentation of the skin.

In a study of rubber dermatitis which we have been conducting in Boston, we have learned to have a rather healthy respect for this compound because of its sensitizing properties. It is probable that there have not yet been enough patients tested to draw any statistical conclusions, but one patient out of twelve, as in Dr. Denton's series, would indicate a rather high sensitization index.

I suspect this material can cause some rather bad sensitizations, and I think it may be dangerous to use it indiscriminately for the purpose of producing de-pigmentation.

DR. DENTON: In regard to the existence of tyrosinase in pigmented mouse skin we believe that all the studies reported show that the enzyme is present in that tissue.

For the treatment of our cases of hyperpigmentation we use a 20–30% concentration of monobenzylether of hydroquinone. A vanishing cream base works best for the ointment preparation. We found that a lotion consisting of ten parts of alcohol and seven parts of acetone with 20% monobenzylether of hydroquinone is also satisfactory.

Sensitivity is a consideration with the use of any local therapy particularly where a favorable cosmetic result is desired. Information gained from patch testing and clinical experience indicates that the preparations give a low incidence of sensitization.