13799 P

Effect of Certain Split Products of Carcinogenic Azo Dyes on Melanin Formation.*

ANNE K. BAKER, B. E. KLINE AND H. P. RUSCH.

From the McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison.

During the course of some experiments concerned with the production of tumors by the subcutaneous injection of o-aminoazotoluene and 2,2'-azonaphthalene, it was noted that many of the black haired ABC mice developed grey hair following such treatment. The greying was at first local around the site of injection, and later became more general following the pattern described in the literature.[1] Since melanoblast pigmentation is concerned with melanin formation, this observation led to the present investigation which is concerned with the effects of certain oxidation products of these dyes on the tyrosine-tyrosinase reaction. Because there is considerably more information regarding the split products of butter yellow, an azo dye differing from o-aminoazotoluene only in the position of the CH_3 groups, the following compounds were studied: *p*-phenylenediamine, *p*-aminodimethyl aniline, and *p*-aminophenol.[2] These substances were used because aminoazotoluene and azonaphthalene are insoluble in water. Likewise as a possible oxidation product of azonaphthalene, B-naphthylamine was tested. As a check on our technic, modified after that of Martin, Wisansky and Ansbacher,[3] we also tested and confirmed their observations on sulfanilamide, *p*-aminobenzoic acid and hydroquinone. Because of their structural similarity, 3 other compounds were tested, *i. e.*, methyl *p*-phenylenediamine, quinone and *p*-aminoacetanilide.

The technic was briefly as follows: Amounts of tyrosine and of the compound to be studied sufficient to make a 0.001 molar solution of each substance were weighed out and were dissolved in M/15 Sorenson's phosphate buffer at pH 7.0. To 20 cc of this solution was added 0.5 cc of crude tyrosinase obtained from potato juice.

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1 Richter, C. P., and Clisby, K. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 684.

2 Stevenson, E. S., Dobrimer, K., and Rhoads, C. P., *Cancer Research*, 1942, **2**, 160.

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

The latter was prepared by grinding the potato, centrifuging and using the supernatant fluid. The reaction mixture was placed in 125 cc Erlenmeyer flasks and allowed to stand for 18 hours in air, and color observations were made after ½, 6 and 18 hours. Several controls were run with every experiment, *i. e.*, tyrosine plus tyrosinase, tyrosine and compound, compound and tyrosinase, and the compound alone. The end results are briefly summarized in Table I. Only the color and precipitation reactions at the end of the 18 hours are reported because at this time a good precipitate of melanin was invariably produced in the control tube.

Some of the compounds tested are autoxidizable (*p*-aminodimethyl aniline, *p*-phenylenediamine, methyl *p*-phenylenediamine and *p*-aminophenol) and gave similar reactions when added to the tyrosine-tyrosinase solution or when present in the various control runs (Table). Any interpretation of the inhibitory effect of these compounds on this reaction is therefore difficult to make. Sulfanilamide, *p*-aminobenzoic acid, *p*-aminoacetanilide, and B-naphthylamine are not autoxidizable and were not oxidized by the enzyme, yet they interfered with the production of melanin from tyrosine. These compounds might conceivably compete with the tyrosine for the enzyme and thus inhibit melanin formation, but this suggestion is by no means certain. Since the non-autoxidizable compounds, which in-

TABLE I.
Effect of Various Compounds on Melanin Formation.
Appearance of solutions after 18 hours.

Compound tested	Compound alone	Compound + Enzyme*	Compound + Tyrosine	Compound + Tyrosine + Enzyme*
Sulfanilamide	Colorless	Colorless	Colorless	Brown-black sol. Sl. ppt.
p-Aminobenzoic acid	"	"	"	Yellow-brown sol. No ppt.
p-Aminoacetanilide	"	Yellow pink cloudy	"	Red-brown sol. No ppt.
B-Naphthylamine	Pink sol.	Pink sol.	Pink sol.	Pink sol. No ppt.
	" ppt.		" ppt.	
p-Aminodimethyl aniline	Deep royal purple	Purple-black Black ppt.	Purple-black Sl. ppt.	Purple-black. Black ppt.
p-Phenylenediamine	Red-black	Red-black Sl. black ppt.	Red-black No ppt.	Red-black. Black ppt.
Methyl *p*-phenylenediamine	Royal purple Black ppt.‡	Red-purple Black ppt.‡	Royal purple black ppt.‡	Red-purple. Black ppt.‡
p-Aminophenol	Brown sol. ppt.	Brown sol. ppt.	Brown sol. ppt.	Brown sol. ppt.
Quinone	Orange red†	Deep reddish brown	Orange red	Brown-black. No ppt.
Hydroquinone	Yellow-pink	Deep reddish brown	Yellow-pink	Deep reddish brown. No ppt.
Tyrosine (control)	Colorless	Black ppt.		

* Enzyme = tyrosinase obtained from potato juice.

† Longer time — deep reddish brown.

‡ Observed after 48 hours.

hibit the reaction, show a close chemical relation to the substances which are autoxidizable, (*e. g.,* compare *p*-aminoacetanilide with methyl *p*-phenylenediamine) it is not improbable that the latter compounds may also have an inhibitory effect which is masked by their own color reactions.

The tendency of these autoxidizable substances to form colored compounds and finally a black precipitate may be due to a reaction similar to the formation of quinhydrone from quinone and hydroquinone and of phenoquinone from phenol and quinone as described by Karrer.[4] The question arises whether or not the formation of melanin could arise in a similar manner. Raper[5] reports that 3,4-dioxyphenyl alanine is formed upon the oxidation of tyrosine by tyrosinase and that after the oxidation the enzyme can be removed and melanin is still formed. Since the intermediate compound has a quinoid grouping, it seems possible that it might react to form melanin as indicated above.

Summary. The effect of certain split products of some carcinogenic azo dyes and related compounds on melanin formation was studied. It was observed that certain non-autoxidizable compounds such as *p*-aminoacetanilide, *B*-naphthylamine, *p*-aminobenzoic acid and sulfanilamide interfered with this reaction. When chemically related autoxidizable compounds (*p*-aminodimethylaniline, *p*-phenylenediamine, methyl *p*-phenylenediamine and *p*-aminophenol) were used, the color changes in both tyrosine-tyrosinase and control solutions were similar. It is, therefore, difficult to interpret the action of these latter chemicals on melanin formation.

13800 P

Effect of Low Calcium and Vitamin D-Deficient Diet on Bones and Teeth of Mature Rats.*

AXEL P. LUND AND W. D. ARMSTRONG.

From the Laboratory of Dental Research and Division of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.

Twenty male albino rats which had been raised to an age of 11 months on an adequate stock diet were fed for 5 weeks on a control

[4] Karrer, Paul, *Organic Chemistry,* New York, 1938.

[5] Raper, H. S., *Biochemical J.,* 1927, **21**, 89.

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