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Para-Aminobenzoic Acid and Dopa Reaction.

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Melanoblast pigmentation has been considered by Bloch[1] to be due to a specific dopa oxidase causing the formation of melanin by its action on dopa (3,4-dihydroxy-phenyl alanine). Since p-aminobenzoic acid has chromotrichial activity,[2, 3] it probably plays a rôle in the formation of melanin which can be demonstrated experimentally. Therefore, the influence of this acid was studied in the reactions yielding melanin and catalysed by the readily available potato tyrosinase, namely in tyrosine-tyrosinase, dopa-tyrosinase, and catechol-tyrosinase systems. Other substances were investigated under comparable conditions, such as, *e. g.*, sulfanilamide, the bacteriostatic effect of which is nullified[4, 5, 6] by p-aminobenzoic acid; aniline, the simplest aromatic amine; and calcium pantothenate, because its curative effect has been observed in gray-haired animals reared on pantothenic acid-deficient diets.[7, 8]

In the experiments recorded in Table I, tyrosine and tyrosinase passed through the typical red[9] stage before producing the black, flocculent melanin precipitate. When p-aminobenzoic acid was present, the intermediate red phase was not developed, the black melanin precipitate was not formed, but a brownish reaction mixture was produced. Similar phenomena were observed when sulfanilamide was employed in place of the acid. The experiments were repeated with dopa-tyrosinase as well as with catechol-tyrosinase and comparable data obtained. However, as is well known, the speed of reactions is more rapid in the dopa-tyrosinase and in the catechol-tyrosinase than in the tyrosine-tyrosinase system. Furthermore, the color changes observed in the catechol experiments were not identical with those in the other systems. Nevertheless, the influence

1 Bloch, B., *Z. physiol. Chem.*, 1917, **98**, 226.
2 Ansbacher, S., *Science*, 1941, **93**, 164.
3 Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.
4 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.
5 Landy, M., and Wyeno, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 59.
6 Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1941, **20**, 189.
7 Unna, K., and Sampson, W. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 309.
8 György, P., and Poling, C. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 773.
9 Raper, H. S., and Wormall, A., *Biochem. J.*, 1925, **19**, 84.

of p-aminobenzoic acid was definite, and it appeared to be the same in the case of the 3 substrates, tyrosine, dopa, and catechol. This latter observation was to be expected, the first step in the oxidation of tyrosine by tyrosinase being the formation of dopa, as pointed out by Raper.[10]

TABLE I.
Tyrosine-Tyrosinase System.*

Addition compounds	Intermediate phase		Reaction product in 18 hr	
	Color	Time, min.	Color	Precipitate
None (control)	red	30	black	flocculent
Tryptophane	red-purple	35	"	"
Calcium pantothenate	" "	35	"	"
Benzoic acid	red	90	"	"
o-Cresol	red tint	90	"	"
Ascorbic acid			red	none
Cysteine			faint red	"
p-Hydroxy-benzoic acid	red	30	brownish with red tint	"
p-Hydroxy-phenyl acetic acid	"	30	" " " "	"
Hydroquinone	"	35	brownish	"
p-Aminobenzoic acid	faint brownish	35	"	"
Sulfanilamide	" "	40	" with green tint	"
Aniline	red-gray	90	slight-brown	"

*The pH of 20 mg of addition compound and 20 mg of tyrosine in 10 ml of saline solution was adjusted to 7.0; then 10 ml of phosphate buffer and potato slices (or juice) were added.

The data as a whole seem to show conclusively that, under identical experimental conditions, calcium pantothenate has no influence and that p-aminobenzoic acid and sulfanilamide are effective in modifying melanin formation. It appears, therefore, that an enzyme system has been found in which the 2 aromatic amines attack at the same point. This is particularly interesting, because p-aminobenzoic acid inhibits the antibacterial action of sulfanilamide,[4, 5, 6] a fact which suggests a common point of attack for the 2 substances in bacteria systems.

Raper and Wormall[9] have shown that melanin formation occurs in 3 stages, that the enzyme is important in the first part, the result of which is a red pigment, and that the 2 subsequent phases, involving decolorization of the red pigment and oxidation to melanin, do not require an enzyme. Since the oxidation of tyrosine by tyrosinase does not go through the characteristic red phase when p-aminobenzoic acid or sulfanilamide is present in the dopa enzyme system, it appears that aromatic amines can play a rôle in the non-enzymatic

[10] Raper, H. S., *Biochem. J.*, 1926, **20**, 735; 1927, **21**, 89.

portion of the process. It may be that the intermediate phase is not observed, because the amino group of p-aminobenzoic acid reacts with the red substance (halochrome) to give soluble anilino-like compounds which undergo auto-oxidation to a brownish melanin more soluble than that derived from tyrosine alone. Pugh and Raper[11] did not observe aniline to form an anilino compound with tyrosine oxidized by tyrosinase, but isolated anilino compounds when catechol or p-cresol was oxidized by tyrosinase and oxygen. Moreover, they found that aniline alters melanin formation, an observation confirmed by the data of Table I, since no black pigmentation occurred when tyrosine was acted upon by tyrosinase in the presence of the aromatic amine.

In view of the above, it is conceivable that p-aminobenzoic acid modifies in the animal organism the type of melanin produced from the oxidation of dopa by dopa oxidase, an enzyme which has been found in skin by Bloch and Schaaf.[12] The nature of this enzyme is not clearly defined. Whether it will eventually be found to be a specific dopa oxidase[1] or an inh'bited tyrosinase,[13, 14] is immaterial as far as the data presented are concerned, because p-aminobenzoic acid appears to enter the non-enzymatic portion of the reactions leading to the formation of melanin.

Summary. Para-aminobenzoic acid modifies the formation of melanin; sulfanilamide has an apparently similar effect; under identical conditions, calcium pantothenate has no influence.

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Inability of Desoxycorticosterone to Maintain Lactation.*

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The following facts have established the adrenal cortex as an important factor in lactation: (1) adrenalectomized rats do not

[11] Pugh, C. E. M., and Raper, H. S., *Biochem. J.*, 1927, **21**, 1370.

[12] Bloch, B., and Schaaf, F., *Biochem. Z.*, 1925, **162**, 181.

[13] Raper, H. S., *Physiol. Rev.*, 1928, **8**, 245.

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

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