hypothesis could account for the much higher tissue concentration when intracerebral is compared with systemic administration. In the rat it was found that 50 μg of morphine sulphate injected into the thermoregulatory centers gave a mean hypothermic response equivalent to 25 mg/kg intravenously[2]. The concentration of morphine in the brain of the rat following intravenous injection of 25 mg/kg was 3.4 μg/g wet weight[3]. The blood concentration following systemic administration is 3–4 times greater than the brain levels and would tend to maintain the concentration at the receptors. The initial rate of fall in temperature is the same with intracerebral doses ranging from 10–100 μg but the degree of hypothermia developing is dose dependent, indicating that the duration of action is the limiting factor[4].

It is possible that the lack of free diffusion is due to the physico-chemical properties of the brain tissue with its closely packed cellular elements and paucity of interstitial fluid space. No extracellular fluid space could be demonstrated in the rat cortex using electron microscopy[5] and it was suggested that transport in brain tissue is across astrocytic cells. Post-mortem swelling of the brain cells, however, may account in part for these appearances[6].

The technique of intracerebral injection of drugs would seem to provide a degree of localization comparable to the use of electrolytic lesions or stimulation of the brain[7].

Résumé. La distribution de la morphine radioactive après une injection intracérébrale de cette drogue a été étudiée. Les résultats indiquent que la drogue ne se diffuse pas du site de l'injection à travers le tissu cérébral. La morphine est enlevée par le sang et a une période de demi-vie dans le cerveau d'environ 20 min.

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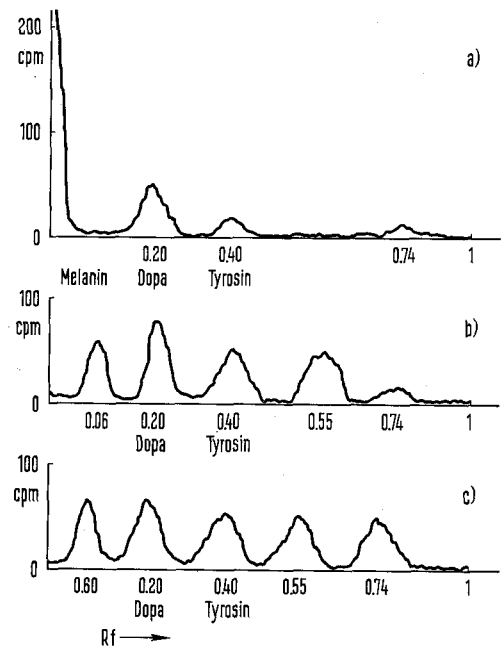
[2] V. J. LOTTI, P. LOMAX, and R. GEORGE, Int. J. Neuropharmacol. (1965), in press.
[3] J. C. SZERB and D. H. MCCURDY, J. Pharmacol. *118*, 446 (1956).
[4] V. J. LOTTI, P. LOMAX, and R. GEORGE, Int. J. Neuropharmacol. (1965), in press.
[5] R. L. SCHULTZ, E. A. MAYNARD, and D. C. PEASE, Am. J. Anat. *100*, 369 (1957).
[6] A. VAN HARREVELD, J. cell. comp. Physiol. *57*, 101 (1961).
[7] This research was supported by NSF Grant GB-1484.

Inhibition of Phenoloxidase Catalysed Transformation of Tyrosine to Melanin by Hydroxyphenylcarboxylic Acids

The significance of the presence of the enzyme phenoloxidase as well as melanin in certain regions of the mammalian central nervous system (CNS) is still unclear[1,2]. What has been definitely established is that disorders of melanogenesis are found in certain neurological disorders such as Parkinsons disease and oligophrenia phenylpyruvica[3,4]. In oligophrenia phenylpyruvica, an accumulation of phenylcarboxylic acids has been noted, but this is still unsuccessfully correlated with the pathogenesis of the disease. In the present paper, an inhibitory action of *p*-hydroxyphenylpyruvic and 3,4-dihydroxyphenylpyruvic acid on tyrosine oxidation to melanin by phenoloxidase, leading to accumulation of intermediate metabolites with potential toxic action on the nerve cells, will be described.

Materials and methods. Due to difficulties in obtaining phenoloxidase from brain in sufficient quantities, we used a preparation derived from the insect *Calliphora erythrocephala*[5].

10 Calliphora larvae were homogenized in 10 ml of 0.25 *M* sucrose containing 1% dehydroascorbic acid adjusted to pH 7. The homogenate was centrifuged 10 min at 1200 *g* and the supernatant thus obtained 10 min at 7000 *g*. The sediment was suspended in 0.25 *M* sucrose and centrifuged once more for 10 min at 7000 *g*. The sediment



Oxidation of tyrosine by phenoloxidase. (a) without inhibitor; (b) in the presence of $5.5 \cdot 10^{-3}$ *p*-hydroxyphenylpyruvic acid; (c) in the presence of $5.5 \cdot 10^{-3}$ 3,4-dihydroxyphenylpyruvic acid. Incubation time: 10 min at 37 °C. Paper chromatograms of the incubation mixtures in butanol/*n*-HCl.

[1] C. V. WENDE and M. T. SPOERLEIN, Life Sci. *6*, 386 (1963).
[2] G. C. COTZIAS, P. S. PAPAVASILIOU, M. H. VAN WOERT, and A. SAKAMOTO, Fedn. Proc. Socs exp. Biol. *23*, 713 (1964).
[3] J. W. HEATH, Archs Neurol. Psychiat. *58*, 484 (1947).
[4] J. A. N. CORSELLIS, J. Neurol. Neurosurg. Psychiat. *16*, 139 (1953). – J. H. FELLMANN, J. Neurol. Neurosurg. Psychiat. *21*, 58 (1958).
[5] C. E. SEKERIS and D. MERGENHAGEN, Science *145*, 68 (1964).

of this centrifugation was suspended in sucrose solution and used as enzyme preparation.

The standard incubation mixture consisted of 0.1 ml enzyme suspension, 0.1 ml of a 0.1% solution of C^{14}(G)-L-tyrosine (50,000 cpm) and 0.2 ml phosphate buffer pH 7. The incubation was carried out at 37 °C aerobically under constant shaking. When appropriate, *p*-hydroxy- and dihydroxyphenylpyruvic acids were added to a final concentration of $5.5 \cdot 10^{-3} M$. The reaction was stopped with methanol, and the radioactive metabolites separated by paper chromatography in butanol saturated with *n*-HCl and scanned with a gas flow detector.

Results and discussion. Incubation of tyrosine with the phenoloxidase in the absence of inhibitors leads to a very rapid transformation of tyrosine to melanin, without the accumulation of intermediary metabolites other than small amounts of DOPA and a substance with an Rf value of 0.75 (see Figure a). Addition of the phenylcarboxylic acids leads to an overall inhibition of melanin synthesis, as well as to accumulation of DOPA and three other intermediates with Rf values of 0.06, 0.5 and 0.75 (see Figure b and c). Metabolite 0.5 is still capable of indole ring closure (positive reaction with potassium ferricyanide) and has very probably a quinone structure (positive reaction with phenylhydrazine). Compounds 0.06 and 0.75 are strong reducing agents. The exact nature of these compounds remains to be elucidated.

Accumulation of intermediates of tyrosine oxidation in the CNS could be of pathogenetic importance in oligophrenia phenylpyruvica, in which phenylcarboxylic acids are accumulated.

Accumulation of an oxidation product of adrenalin, adrenochrome, has been found in humans and correlated to the pathogenesis of schizophrenia. If one considers that adrenalin as well as the other catecholamines must be the natural substrates of the brain phenoloxidase, an interesting correlation between disturbances of melanogenesis and schizophrenia arises. The nature of these intermediates and their effect on the nervous system is currently under study.

Zusammenfassung. *p*-Hydroxy und 3,4-Dihydroxyphenylbrenztraubensäure hemmt die von Phenoloxydase katalysierte Umwandlung von Tyrosin in Melanin unter Anhäufung von Zwischenprodukten. Das Ergebnis wird im Zusammenhang mit der Pathogenese nervöser Störungen diskutiert.

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Pteridine Derivatives in the Skin of *Lacerta muralis* Laur.

Pteridine derivatives have been demonstrated in the dorsal skin of amphibians[1-4], reptiles[5-7], and fishes[8]. In these vertebrates, as also in some invertebrates, the fluorescent substances may play a role in oxidation-reduction processes. Quantitative differences have been observed in animals (amphibians[3]) living for about one month under conditions of total darkness in comparison with animals subjected to normal photoperiodism. Recently, a relationship between pteridines and pigmentation has been established[9-12]. In the present investigation, the occurrence of fluorescent pteridine derivatives was demonstrated in the skin of *Lacerta muralis*. Pteridine determinations were made by paper chromatography after extraction with ethanol or methanol. After centrifugation of extracts, the supernatant was chromatographed ascendingly in *n*-butanol-acetic acid-water (4:1:5) or *n*-propanol 1% NH_3 (2:1) in a dark room at 23 °C. These experiments demonstrate the occurrence of four fluorescent pteridines: 2-amino-4-hydroxy-pteridine-6-carboxylic acid, isoxanthopterin, biopterin and riboflavin in the dorsal skin of adults of *Lacerta muralis*. Some characteristics of these substances are given in the Table.

Pteridine derivatives	Rf values at pH 7[a]	Fluorescence
2-Amino-4-hydroxy-pteridine-6-carboxylic acid	0.16	blue
Isoxanthopterin	0.22	violet
Riboflavin	0.43	yellow
Biopterin	0.48	blue

[a] Solvent: *n*-butanol-acetic acid-water (4:1:5).

Through oxidation or UV-irradiation of biopterin, 2-amino-4-hydroxy-pteridine-6-carboxylic acid with a bright blue fluorescence has been obtained. No 2-amino-4-hydroxy-pteridine has been found in the dorsal skin of the adult animals examined.

Zusammenfassung. Aus der Haut von *Lacerta muralis* wurden die folgenden Pterine isoliert und identifiziert: 2-Amino-4-hydroxy-pteridin-6-carbonsäure, Isoxanthopterin, Riboflavin und Biopterin.

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[1] T. Hama and M. Obika, Experientia *14*, 182 (1958).
[2] T. Goto, Jap. J. Zool. *19*, 68, 83 (1963).
[3] L. Liaci, Rass. Med. sper. *6*, 357 (1964).
[4] I. Ziegler-Günder, Experientia *15*, 429 (1959).
[5] J. Blair, Nature *180*, 1371 (1957).
[6] S. Odate, Y. Tatebe, M. Obika, and T. Hama, Proc. Japan Acad. *35*, 567 (1959).
[7] E. Ortiz and H. G. Williams-Aschman, Comp. Biochem. Physiol. *10*, 181 (1963).
[8] A. Dupont, Naturwissenschaften *11*, 267 (1958).
[9] T. Hama, Ann. N.Y. Acad. Sci. *100*, 977 (1963).
[10] T. Hama and S. Fukuda, *Pteridine Chemistry* (Pergamon Press, London 1964).
[11] M. Obika and T. Hama, Proc. Japan Acad. *36*, 151 (1960).
[12] M. Obika, Devl Biol. *6*, 99 (1963).