cerebral organs where transformation into ^{14}C-dopa may occur. Part of this ^{14}C-dopa might be taken up by the brain and serve as precursor for ^{14}C-catecholamines and their metabolites. However, according to previous results[5], the small amounts of radioactivity injected intraventricularly in the present experiments would hardly lead to a measurable rise of ^{14}C-catechols if administered extracerebrally (e.g. subcutaneously).

The missing effect of chlorpromazine on the cerebral content of ^{14}C-dopa might be due to the fact that dopa decarboxylase is a highly active enzyme which does not allow dopa to accumulate. Also a slight activation of dopa decarboxylase by chlorpromazine has recently been reported[17]. Acceleration of dopa decarboxylase, however, is hardly a major cause for the increased formation of ^{14}C-catecholamines seen in the present experiments. Thus, according to previous findings[9], chlorpromazine does not enhance the in vivo transformation of ^{14}C-dopa into cerebral ^{14}C-dopamine. Therefore, the increase of cerebral ^{14}C-catecholamines and metabolites after chlorpromazine is probably the consequence of an enhanced hydroxylation of ^{14}C-tyrosine which is thought to be the limiting step in the biosynthesis of catecholamines.

In rats in which the chlorpromazine-induced hypothermia is not prevented the results are less clear. These animals also show a marked rise of ^{14}C-catecholamines and their metabolites in the brain compared with normothermic controls, but at the same time the overall radioactivity of the brain increases.

In conclusion, the present results with the intraventricular injection of ^{14}C-tyrosine in normothermic rats strongly support the hypothesis that chlorpromazine enhances the hydroxylation of tyrosine within the brain. As previously suggested[3,5–8], this effect may be due to a feedback mechanism in consequence of a primary blockade of catecholaminergic receptors by chlorpromazine. A direct activation of tyrosine hydroxylase is unlikely[18,19].

Zusammenfassung. Im Hirnstamm (inklusive basale Ganglien) von normothermen Ratten verstärkt Chlorpromazin die Bildung von Noradrenalin, Dopamin und Catecholaminmetaboliten aus Tyrosin, welches in die Hirnventrikel eingegeben wurde. Die Gesamtradioaktivität sowie die ^{14}C-Dopa-Konzentration im Gehirn werden durch das Medikament nicht verändert. Diese Befunde sprechen für eine zerebrale Aktivierung der Tyrosinhydroxylierung durch Chlorpromazin.

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Forschungsabteilung der F. Hoffmann-La Roche and Co. AG, CH-4002 Basel (Switzerland), 5 June 1969.

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A Cytochemical Study of Phaeomelanin Formation in Feather Papillae of New Hampshire Chick Embryos

Phaeomelanins are alkali-soluble pigments which as yet have been found only in hair and feathers[1,2]. Investigation of these compounds has proceeded extremely slowly, and it is only recently that the isolation of a number of phaeomelanins from the feathers of New Hampshire chickens has been reported[3,4]. Chemical studies of the isolated pigments, which contain C, H, N, O and S, have suggested that they derive biogenetically from tyrosine and cysteine and that 5-S-cysteinyldopa[5] is an important intermediate in their biosynthesis[6,7].

In order to substantiate this finding we have studied by autoradiography the incorporation of cysteine-3-C^{14} as well as tyrosine-2-C^{14} in the feather papillae of embryonic New Hampshire chick; up till now only this phaeomelanin has been characterized.

Material and methods. Patches of skin were removed from the backs of 19-day-old embryos and sliced into strips about 1 mm wide. Tissue slices were placed in 2.5 ml of 0.1 M phosphate buffer at pH 6.8 containing 2500 units of Penicillin G and incubated for 24 h at 37 °C in a Dubnoff shaker either with 0.3 µc of DL-cysteine-3-C^{14} (34.7 mc/mM) or with 0.25 µc of DL-tyrosine-2-C^{14} (50.0 mc/mM). As a control, slices from the same specimen were treated identically, but in addition to the substrates, sodium diethyldithiocarbamate (0.01 M), a tyrosinase inhibitor, was present. For a second control, 2 other specimens were incubated respectively with 0.3 µc of glycine-2-C^{14} (21.5 mc/mM) and with 0.3 µc of DL-tryptophane (methylene-C^{14}, 52.0 mc/mM). Following incubation, the tissue slices were removed, washed with Hanck's solution and fixed in Carnoy. The specimens were then imbedded in paraffin, sectioned at 5 µ, and autoradiographs made with Kodak NTB-3 liquid emulsion. After 8 days exposure slices were stained with Mayer's hemalum. In other experiments 0.25 µc of labelled cysteine were injected directly into the yolk sac during the 8th day of development. 8 days after the injection the embryos were killed and autoradiographs made as described above.

Results and discussion. Figure 1a and 1b are autoradiographs of epithelial melanocytes incubated with C^{14}-cysteine and C^{14}-tyrosine respectively. It was found that both labelled compounds are incorporated more into melanocytes than into epithelial cells. Also a qualitative comparison revealed no remarkable difference in the uptake of these labelled compounds by the melanocytes.

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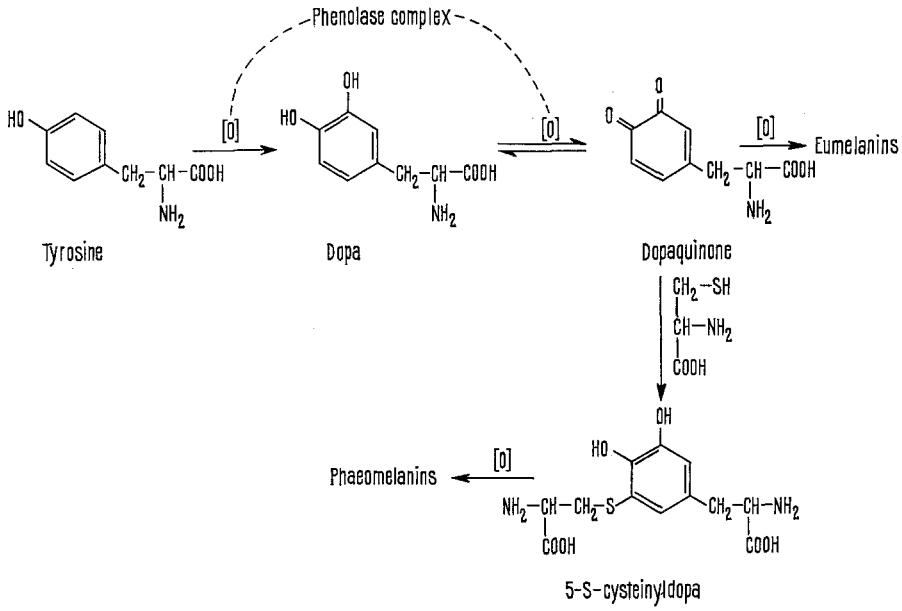

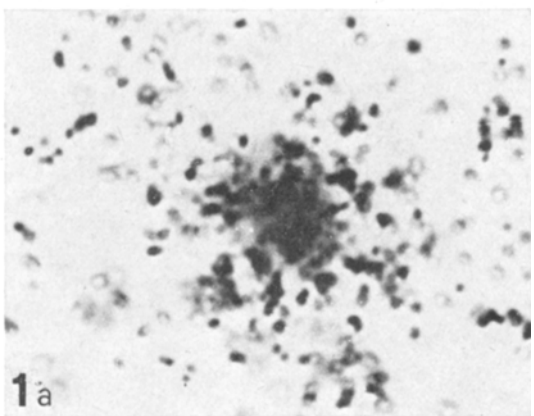

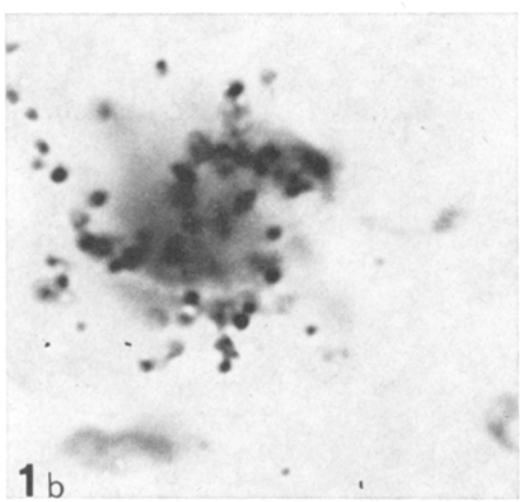

Fig. 1. Autoradiographs of epidermal melanocytes of chick embryo, incubated in C^{14}-cysteine (a) and C^{14}-tyrosine (b). The focus is on silver grains. Pigment granules and stained nuclei are out of focus. (a) × 1500; (b) × 2000.

In the same experimental conditions labelled glycine and tryptophane, which are not specifically related to the formation of phaeomelanins[8], were found in melanocytes in the same concentration as in epithelial cells. Moreover, in the presence of an inhibitor of tyrosinase, the uptake of both cysteine (Figure 2a) and tyrosine (Figure 2b) by pigment cells was low and the amount incorporated negligible.

In order to find out to what extent cysteine is incorporated into the pigment itself, a long term experiment has been done. The labelled compound was injected into the yolk sac during the 8th day of development when melanocytes of feather papillae are not yet pigmented. 8 days after the injection, autoradiographs showed that labelled cysteine was taken up by melanocytes preferentially (Figure 3). Under these conditions the labelling is probably due to the incorporation of the injected substance into phaeomelanic granules since they are not subjected to a rapid turnover as other cytoplasmic compounds, e.g. proteins.

The results reported in this study support our view that phaeomelanins are formed in vivo by a deviation of eumelanin pathway involving a reaction between cysteine and dopaquinone produced by enzymatic oxidation of tyrosine[3,10]. Accordingly 2 separated, but interrelated, metabolic pathways for eumelanin and phaeomelanin are suggested (Figure 4). This is consistent with the fact that both tyrosinase[11] and dopa oxidase[12,13] activity have been detected in melanic and phaeomelanic hair follicles and feather papillae[2]; moreover, a change in the cysteine content of the melanocytes can explain the

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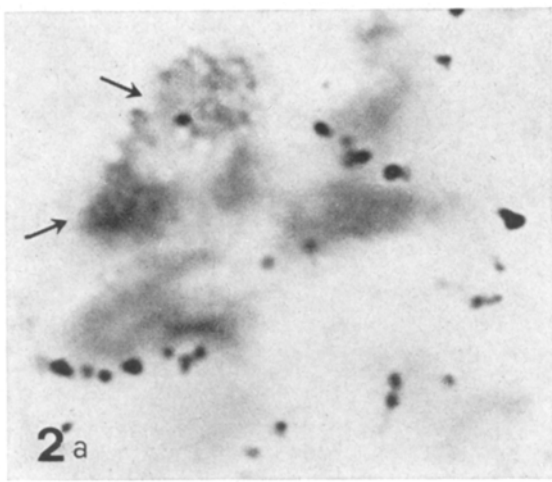


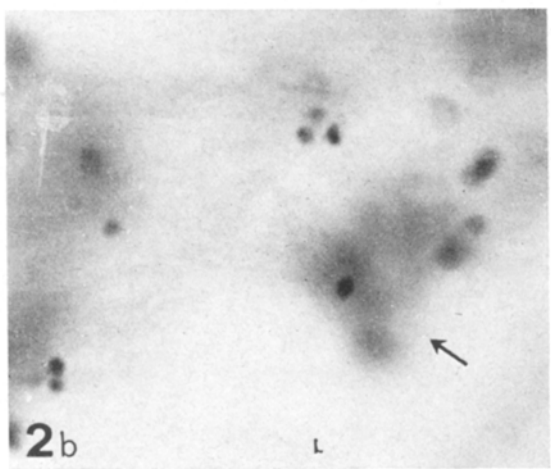
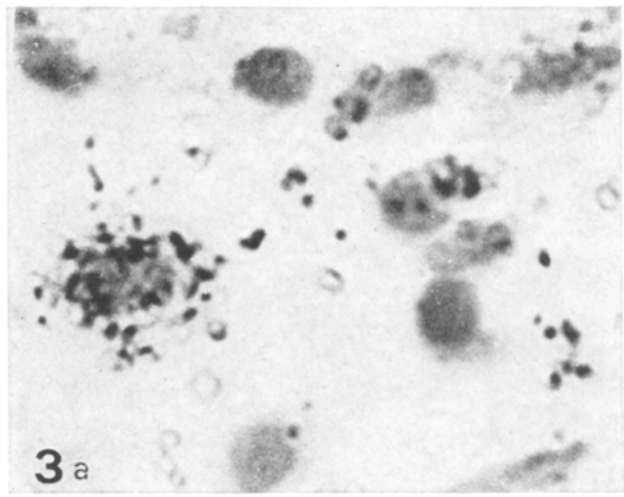


Fig. 2. Autoradiographs of epidermal melanocytes (arrows) of chick embryo incubated in C^{14}-cysteine (a) and C^{14}-tyrosine (b) in the presence of sodium diethyldithiocarbamate. Note the almost complete lack of labelling in the melanocytes. × 1500.



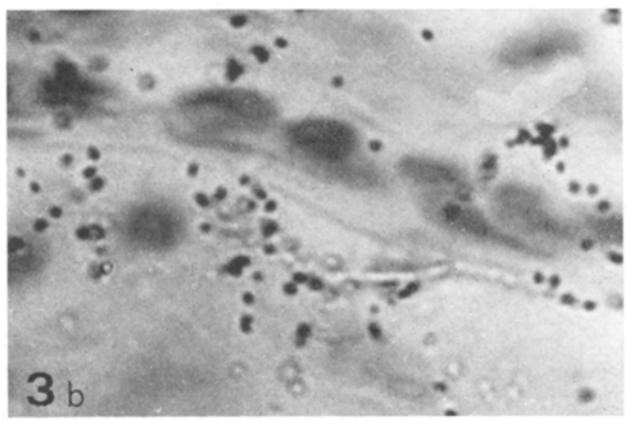


Fig. 3. In vivo, incorporation of C^{14}-cysteine: (a) labelled melanocyte, × 1500; (b) labelled dendrite, × 2000.

pigmentary switch mechanism leading either to eumelanins or phaeomelanins. In the agouti pigment cells this has already been demonstrated by CLEFFMANN[14], who showed that the increase in sulfhydryl content of melanocyte is one of the initial events leading to the change from the production of eumelanin to that of phaeomelanin.

Since the works of ROTHMAN et al.[15] and FLESCH[16], sulfhydryl compounds have been known to be involved in melanin formation and it has been suggested their role is the control of tyrosinase activity to oxidize and polimerize eumelanin precursors[14,17,18]. In contrast with this view, our results suggest that cysteine does not act as an inhibitor of tyrosinase but that it can react with dopaquinone yielding a new intermediate, 5-S-cysteinyldopa, whose further oxidation leads to the formation of phaeomelanins.

Zusammenfassung. Die Inkorporation von C^{14}-Cystein und C^{14}-Tyrosin in die Federpapillen von New-Hampshire-Embryonen wurde autoradiographisch untersucht. Chemie und Biogenese der rotbraunen Pigmente der Haare und der Federn, der Phäomelanine, werden diskutiert.

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