that mere adsorption of insoluble compounds could take place, but probably not their active uptake into cytoplasmic granules. Phagocytosis in these experiments seems ruled out by the previous treatment by freezing, followed in some experiments by formalin-fixation or drying.

The fourth and fifth steps, melanin polymerization and binding to proteins, are so little understood that speculation about these reactions in the cells may be permitted. The fact that the pigment-uptake did not take place after subjecting to heat-inactivation which occurs with most proteins (5 min, 85°C), suggests that some cytoplasmic protein is involved in the phenomenon observed. Whether this protein would act enzymatically in the polymerization of melanin or by some other type of catalysis is not known. The same must be said about the fixation of polymerized melanin to proteins of the cytoplasmic granules.

Which stage of oxidation of dopa was taken up by the cells, dopachrome or a later step on the way to the subsequent formation of melanin, is obscure. Under the experimental conditions in an atmosphere of nitrogen, no direct aerobic oxidation could have taken place; nevertheless, active pigment-uptake from previously partially autoxidized substrate solutions was obtained. This was seen with autoxidized solutions of dopa, dopamine, and epinephrine. With dopa, dark brown or black melanin-like pigments were observed in the cells, whereas after incubation with epinephrine, yellow or yellow-brown pigmentation was obtained. This was typical also for the iodine-oxidized solutions, where rapid and complete oxidation of the substrates to dopachrome or adrenochrome had been produced. Two explanations for this difference in color are possible. The final melanins produced from dopa and epinephrine may be significantly different in this respect. Alternatively, soluble compounds, like adrenochrome, may be taken up by some cytoplasmic proteins and then not altered, stay yellow, whereas in the case of dopa further oxidation occurs to black dopa melanin.

Present findings indicate that the pigment-production in the intestinal cells, or in skin or in leucocytes, is not highly specific, as has been claimed to be the case for the histochemical dopaoxidase reaction in the skin.

The pigmentation developed in previously autoxidized solutions over a pH range of 5 to 9 or higher. It has also been shown recently in a critical study of the dopa-factor by VAN DUIJN[15] that mushroom-phenoloxidase-oxidized dopa solutions give a similar positive reaction at a low pH (4·65).

The epidermis reaction was claimed to be specific for L-dopa and negative for D-dopa (BLOCH and SCHAAF[16]); however dopamine, which has no asymmetric carbon atom was reported to give the same reaction (MULZER and SCHMALFUSS[17]). In the present study it was found that intestinal cells give the same strong reaction with pre-oxidized epinephrine or its dextro-isomer, suggesting that no such specific phenolase was involved in this step.

The possibility was considered that this reaction could be related to some peroxidase-activity. If in some of the oxidation-steps before or after the formation of dopachrome a peroxide should be formed (MASON[9]), and if these cells should contain peroxidase activity, it is conceivable that an amount of melanin could be formed out of the partly oxidized substrate even in an atmosphere of nitrogen to a sufficient extent to lead to cytoplasmic pigmentation. In a few experiments a strong benzidine peroxidase reaction was found with pigmented cells, and also in similar cells of sections not previously treated. This observation needs further confirmation. Peroxidases are known to be HCN-sensitive, whereas the pigment-uptake is very little affected by HCN according to VAN DUIJN[18].

A number of the present observations obviously require further study for their elucidation. It is so far not clear why with incubation media containing tryptamine, which remained clear and visibly colorless for a number of days, similar pigment-uptake was found as with tryptophane, hydroxytryptamine, etc. Reaction media which have been allowed to stand at room temperature for over 2–3 h or overnight have not been checked for bacterial growth, which could possibly account for some of the 'spontaneous' oxidation taking place in them.

Nothing is known regarding the further fate of these pigmented cells, and whether they are capable of eliminating the pigment. It was reported that in cases of melanosis coli of man, ascribed to the prolonged use of cascara sagrada medication (anthraquinone or emodin), the pigmentation was reversible (BOCKUS, WILLARD, and BANK[19]).

A re-evaluation of the histochemical dopaoxidase reaction in skin is desirable, according to these results and to those which have been obtained by VAN DUIJN[15]. In spite of these differences between the classical melanocytes in the epidermal part of the skin and the cells in the intestine or in the dermis described here, the possibility that some stages of the pigment development and uptake by these cells may be related or identical cannot be ruled out. It would not seem unlikely, that the reaction found in epidermal melanocytes is but a cytologically specialized or adapted form of a more general phenomenon of the possibly non-enzymatic oxidation of dopa, epinephrine, or tryptamines. If this reaction, which is quite rapid, occurs *in vivo*, and if quantitation could be achieved, it might provide information about the oxidative metabolic pathways of the physiologically and pharmacologically important compounds mentioned above.

Zusammenfassung

Gefrierschnitte von frischem oder formalin-fixiertem Dünndarm verschiedener Tierarten werden in teilweise autoxydierten, 2×10^{-3}-molaren Lösungen von Dopamin, Dopa, Adrenalin, Hydroxytryptamin und verwandten Verbindungen bei pH = 8 inkubiert. Gewisse Zellen der tunica propria der Schleimhaut zeigen dann nach relativ kurzer Zeit eine zum Teil sehr starke granuläre Pigmentierung ihres Zytoplasmas.

[15] P. VAN DUIJN, J. Histochem. Cytochem. *1*, 143 (1953); Acta physiol. pharmacol. Neerland *5*, 413, 428 (1957).

[16] B. BLOCH and F. SCHAAF, Klin. Wschr. *11*, 11 (1932).

[17] P. MULZER and H. SCHMALFUSS, Med. Klinik *27*, 1099 (1931).

[18] P. VAN DUIJN, Acta physiol. pharmacol. Neerland *5*, 413, 428 (1957).

[19] H. L. BOCKUS, J. H. WILLARD, and J. BANK, J. Amer. med. Ass. *101*, 1 (1933).

Corrigendum

G. ZBINDEN und A. STUDER: *Histochemische Untersuchungen über den Einfluss von Ipronazid (Marsilid) auf die durch Reserpin erzeugte Freisetzung von Adrenalin und Noradrenalin aus dem Nebennierenmark.* Exper. *14*, fasc. 6, 201 (1958).

Die beiden letzten Sätze der englischen Zusammenfassung müssen richtigerweise wie folgt lauten:

In animals pretreated with equimolar doses of isoniazid, however, histochemical catecholamine reactions show a marked decrease in all cells of the adrenal medulla. These results suggest that monoamine oxidase plays a part in the reserpine-induced release of catecholamine.