PA II in the hepatic artery, so that the percentage ratio of PA II across the liver was higher than 55.1%. Indeed, we demonstrated a significant extraction of angiotensin II across the spleen ($P < 0.01$) and across the splanchnic region with exclusion of the spleen ($P < 0.05$; group 1), for which no values are reported. After the exclusion of the hepatic circulation (group 2) the percentage ratio of angiotensin II decreased from 75.5% to 43.4% ($P < 0.01$) across the spleen and from 74.4% to 45.1% ($P < 0.05$) across the splanchnic region, indicating a significant increase in angiotensin II extraction. The data of this study do not allow to conclude if changes in haemodynamics account for this increase in the inactivation of angiotensin II within the spleen and the splanchnic region or if the absence of the liver is a stimulus to both vascular beds to increase the inactivation of angiotensin II.

Résumé. Cette étude démontre que la rénine est inactivée par le foie, et que les poumons, la rate et la région splanchnique ne la metabolisent pas. Les poumons forment de l'angiotensine II, tandis que le foie, la rate et la région splanchnique sont capables de l'inactiver. Après exclusion de la circulation hépatique, l'inactivation de l'angiotensine II par la rate et par la région splanchnique est augmentée.

R. FAGARD, E. FOSSION, M. CAMPFORTS, R. DERUYCK and A. AMERY

Departments of Medicine and Surgery, Akademisch Ziekenhuis St. Rafaël, Kapucijnenvoer, B–3000 Leuven (Belgium),
13 November 1973.

Melanogenesis from Tryptophan. Biogenetic Experiments with Harding-Passey Mouse Melanoma

The possibility that tryptophan is involved in melanogenesis has already been proposed. CHEN and CHAVIN[1] observed that, not only tyrosine, but tryptophan and other aromatic amino acids can be utilized as substrates from goldfish skin tyrosinase. NICOLAUS[2] reported that radioactive melanin was obtained in cuttlefish after injection of D,L-tyrosine-2-^{14}C or L-phenylalanine-^{14}C (U) or D,L-tryptophan [benzene ring-^{14}C (U)]. With the latter compound, the radioactivity of the ink sac gland was about 1/40 of that obtained when L-tyrosine-^{14}C (U) was injected. VISCONTINI and MATTERN[3,4], studying hydroxylation of tryptophan-3-^{14}C by means of tetrahydropterin in the presence of ferrous ions, observed that radioactive melanin was formed via 5-hydroxytryptophan.

Our previous research[5–7] on the action of polyphenol oxidase from potatoes, and from the *Psalliota campestris* mushroom, and of tyrosinase from *Sepia officinalis*, on tryptophan and on several of its metabolic derivatives showed that only kynurenines and some indolic compounds (5-hydroxytryptamine, 5-hydroxytryptophan and tryptamine) give a formation of black-brown pigments. The highest yield was had with 3-hydroxykynurenine.

As it was impossible with degradation to demonstrate differential features among melanins derived from tyrosine and from tryptophan and its metabolites, we have decided to follow a biogenetic route for the purpose of investigating further the role of tryptophan in melanin formation, and therefore the Harding-Passey mouse melanoma was chosen as an experimental model.

We have therefore administered D,L-tryptophan[benzene ring-^{14}C(U)] (specific activity: 95 mCi/mmol), D,L-tryptophan (methylene-^{14}C) (specific activity: 51 mCi/mmol), D,L- 5-hydroxytryptophan (methylene-^{14}C) (specific activity: 51 mCi/mmol) and 5-hydroxytryptamine-3^{1}-^{14}C (serotonin) creatinine sulphate (specific activity: 57 mCi/mmol) in mice with Harding-Passey melanoma and the incorporated radioactivity into melanin was determined after isolation from melanoma. As a comparison we have also experimented with D,L-tyrosine-2-^{14}C (specific activity: 45.4 mCi/mmol).

For each labelled compound considered, 2 groups of mice of approximately the same weight (18–22 g) were used. The mice were of Swiss albino (Arsal–Rome) strain subsequently transplanted with Harding-Passey deeply pigmented melanoma. 14 days after tumour transplanta-

1 Y. M. CHEN and W. CHAVIN, Analyt. Biochem. *13*, 234 (1965).
2 R. A. NICOLAUS, *Melanins* (Ed. E. LEDERER; HERMANN, Paris 1968), p. 72.
3 M. VISCONTINI and G. MATTERN, Helv. chim. Acta *50*, 1664 (1967).
4 M. VISCONTINI and G. MATTERN, Helv. chim. Acta *53*, 832 (1970).
5 L. MUSAJO, C. A. BENASSI, G. ALLEGRI, E. LEVORATO and A. DE ANTONI, Chimica Ind., Milano *48*, 1221 (1966).
6 L. MUSAJO, A. DE ANTONI, G. ALLEGRI, E. LEVORATO and C. A. BENASSI, Convegno C.N.R. Chimica del Farmaco e dei Prodotti Biol. Attivi, 30–31 gennaio-1 febbraio 1967, Roma (1969), p. 233.
7 A. DE ANTONI, G. ALLEGRI and C. COSTA, Gazz. chim. ital. *100*, 1039 (1970).

Radioactivity in Harding-Passey mouse melanoma melanin after injection of ^{14}C-labelled compounds

^{14}C-labelled compound	No. of animals	Administered radioactivity (μCi)		Tumor weight	Melanin isolated	Incorporated radioactivity (dpm)	
	treated	Per mouse	Total	total (g)	total (mg)	Total	Per mg melanin
D,L-tryptophan [benzene ring-^{14}C(U)]	11	2.5	27.5	11.5	23.4	24,470	1,046
	11	10.0	110.0	13.3	36.4	186,520	5,124
D,L-tryptophan-methylene-^{14}C	4	2.5	10.0	7.5	5.0	7,560	1,512
	4	10.0	40.0	5.9	8.0	41,584	5,198
D,L-5-hydroxytryptophan-methylene-^{14}C	4	2.5	10.0	3.9	9.1	3,600	396
	4	10.0	40.0	3.2	8.3	2,230	269
5-Hydroxytryptamine-3′-^{14}C	4	2.5	10.0	4.5	10.6	2,790	263
	4	10.0	40.0	2.5	6.9	2,640	383
D,L-tyrosine-2-^{14}C	4	2.5	10.0	2.9	7.6	9,211	1,212
	4	10.0	40.0	7.9	24.4	128,686	5,274

tion, one group of mice were injected i.p. with 2.5 μCi (administered in 3 equal doses in the first 3 days) and the other with 10 μCi (administered in 5 equal doses in the first 5 days) of labelled compound in 0.1 ml of an isotonic saline solution for each animal. 13 days after the first injection, the 2 groups of mice were sacrificed, the melanomas removed and the melanin was isolated according to NICOLAUS et al.[8]. As a control the melanin from melanoma of untreated animals was also isolated.

The radioactivity was measured in a liquid scintillation spectrometer Beckman LS-150, suspending 2 mg samples of melanin in 6 ml of Insta-Gel emulsifier (Packard Instrument Company, Inc.) and 4 ml of water. Efficiency was determined by using *n*-hexadecane-1-^{14}C as an internal standard. The Table summarizes our results.

It appears that tryptophan is incorporated into melanin of Harding-Passey mouse melanoma, either by administration of D,L-tryptophan-benzene ring-^{14}C, or D,L-tryptophan-methylene-^{14}C. In both cases the incorporated radioactivity per mg melanin (see the last column of Table) is related to the radioactivity administered and it is almost equal among the group of animals treated with the same amount of the two substances. This shows that tryptophan is incorporated into melanin not only with its benzene ring, but also with alanine chain or at least with a part of it.

Taking all this into consideration, there seem to be two pathways through which tryptophan is involved in the melaninic synthesis: the 'via tryptophan → kynurenine → 3-hydroxykynurenine'[5-7] and the 'via 5-hydroxytryptophan'[3,4]. We found (see Table) that the administration of 5-hydroxytryptophan and 5-hydroxytryptamine gives an incorporation of radioactivity into melanin significantly lower than tryptophan, whether in total value or expressed as radioactivity per mg melanin. Besides we did not note any relationship between the amount of radioactive 5-hydroxytryptophan derivatives administered and the amount of radioactivity incorporated into melanin, as observed with tryptophan.

These results seem to show that the tryptophan does not follow the 'via 5-hydroxytryptophan' for the formation of melanin in Harding-Passey mouse melanoma, but that it follows its metabolic pathway through the kynurenine.

We were also able to show (see Table) that the administration of D,L-tyrosine-2-^{14}C to Harding-Passey melanoma mice gives an incorporation of radioactivity into melanin (expressed as dpm/mg pigment) almost equal to that obtained with tryptophan. Therefore from the biogenetic comparison with tyrosine, tryptophan too must be considered as an important precursor in the biogenesis of melanins.

Riassunto. Il triptofano ^{14}C- uniformemente marcato nell'anello benzenico e il triptofano marcato ^{14}C nel metilene sono incorporati in modo significativo nella melanina del melanoma di Harding-Passey del topo. 5-ossitriptofano e 5-ossitriptamina non mostrano invece una significativa incorporazione nella stessa melanina. L'incorporazione ottenuta con il triptofano marcato sia nel nucleo che nel metilene è all'incirca uguale a quella ottenuta per somministrazione di tirosina-2-C^{14}, per cui anche il triptofano deve essere considerato un precursore delle melanine.

A. DE ANTONI, G. ALLEGRI, C. COSTA and F. BORDIN

Istituto di Chimica Farmaceutica e Tossicologica dell'Università di Padova, Via Marzolo 5, Padova (Italy), 28 December 1973.

[8] R. A. NICOLAUS, M. PIATTELLI and E. FATTORUSSO, Tetrahedron *20*, 1163 (1964).

Effect of Methylene Blue on P_{50} and 2,3DPG of Human Blood in vitro

The redox dye, methylene blue, is used in the treatment of methemoglobinemia[1] and it has been proposed for the treatment of lactic acidosis[2]. More recently, KOCHOLATY and DAWSON[3,4] have suggested the use of inosine and methylene blue (which exert a cooperative effect in the maintenance of 2,3DPG) in the preservation of human red cells for transfusion purposes.

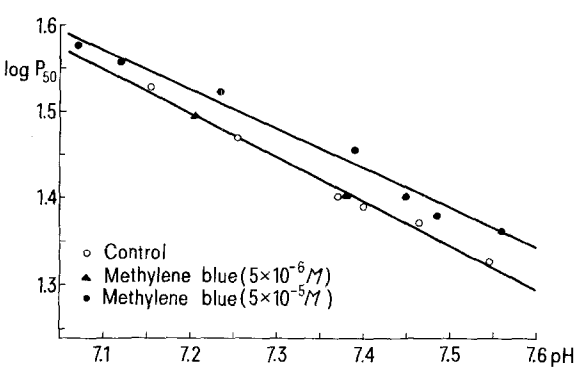

Fig. 1. Effect of different concentration of methylene blue on the oxygen affinity of human blood at various plasma pH.

The present work deals with the addition of varying concentrations of methylene blue at different pH to fresh human blood and its effect on the erythrocyte content of 2,3DPG, H^+, K^+ and Na^+.

Material and methods. Heparinized venous blood was collected in plastic syringes from 3 normal non-smoking subjects and placed in an ice bath. The P_{50} values were determined with a direct method: i.e. equilibrating the blood samples with a gas mixture containing a partial pressure of oxygen at which hemoglobin was 50% saturated. This was obtained by using a gas mixing Control Module I.L. 208/1 composed of a flowmeter system mixing the content of 2 gas cylindres respectively containing 5.7% of CO_2 in N_2, and 5.7% of CO_2 in air. The outlet composition of the gas mixture was continuously checked by an Oxygen Monitor I.L. 208/2. Then, the gas flowed into an 'open-type' equilibrating vessel

[1] E. JAFFE and P. MELLER, *Progress in Hematology*, (Eds. C.V. MOORE and E. B. BROWN; Grune and STRATTON, New York–London 1964), p. 48.
[2] R. E. TRANQUADA, S. BERNSTEIN and W. GRANT, Arch. intern. Med. *114*, 13 (1964).
[3] W. F. KOCHOLATY and R. B. DAWSON, Vox sang. *22*, 236 (1972).
[4] R. B. DAWSON and W. F. KOCHOLATY, Adv. exp. Med. Biol. *28*, 495 (1972).