and MAALOE[7] and is supported by the findings of GROSS et al.[8] in a cell-free system.

The growth rate, protein and RNA syntheses of *B. subtilis*, exposed to boseimycin (0.2 µg/ml), were affected keeping DNA synthesis unimpaired even during 3 h of treatment. It is suggested that the drug affects the translational level of the protein synthesizing system. The normal growth of the bacteria restored immediately after the removal of the drug suggests only a transient inhibition of macromolecular synthesis rather than any irreparable damage caused to cellular components.

Zusammenfassung. Unmittelbar nach Zugabe von Boseimycin zu *B. subtilis* werden Wachstum und Proteinsynthese verhindert. DNA und RNA werden erst später beeinflusst. Die hemmende Wirkung von Boseimycin auf die behandelten Zellen ist nach Waschung mit Phosphatpuffer reversibel.

T. K. MISRA and R. K. SINHA[9]

Department of Microbiology, Bose Institute, Calcutta 9 (India), 7 August 1970.

[9] Present address: Department of Molecular Biology and Microbiology, School of Medicine, Tufts University, Boston (Massachusetts 02111, USA).

Widespread Occurrence of Inhibitors of Melanoma Tyrosinase in Plant and Mammalian Tissues

Inhibitors of tyrosinase have been detected in melanotic melanomas[1–5]. Partial purification of some of these inhibitors has been reported[4]. These studies have implied that these inhibitory factors have a limited distribution being rather specific for melanoma. They have also been considered to have a role in regulating melanogenesis. It was thought of interest to study the natural distribution of inhibitors of tyrosinase to see whether they are specific to melanoma tissue.

Materials and methods. B16 melanoma was transplanted in C57 BL/6J mice as previously described[6]. Cloudman S91 melanoma obtained from Jackson Laboratories, Bar Harbor, was found to be mostly amelanotic. This tumour was maintained by transplantation into DBA/1J mice.

Tyrosinase activity was determined as previously described[7]. The major source of tyrosinase for these experiments were homogenates (15% prepared in water) of previously frozen B16 melanoma. Serum from mice bearing B16 melanoma and an extract of mushroom was also used. The tissue extracts were homogenates (90% prepared in water).

Results. Table I shows the effect of extracts from a variety of tissues on tyrosinase activity. It can be seen that homogenates of liver, kidney, spleen and brain from rats and mice inhibited the tyrosinase activity. This inhibitory effect varied from 20 to 70% depending on the tissues. Heated extract of mushroom also decreased the tyrosinase activity. Extract from an amelanotic Cloudman S91 melanoma also inhibited the tyrosinase activity. High concentrations of the tissue extracts produced increasingly greater inhibition. Human sera and similarly prepared extracts from human spleen, liver, skin, kidney and breast gave similar results.

Table II shows some of the properties of the inhibitory factor(s) in rat liver. Either heating at 100 °C for 15 min or dialysis partially abolished the inhibitory effect, whereas when the extract was dialyzed and then heated, the inhibitory effect was absent. Similar results were also obtained with the following tissues – liver, kidney, spleen and brain from mice and rats and Cloudman S91 melanoma. These tissues were also found to inhibit tyrosinase activity of purified mushroom tyrosinase (Sigma Chemical Laboratory) and of serum from mice bearing B16 melanoma.

Discussion. The above results demonstrate the widespread distribution of inhibitor(s) of tyrosinase in a variety of sources. That there is more than one inhibitory factor is shown by the observation that either heating or dialysis alone only partially decreases the inhibitory effect, whereas the combination of dialysis and heating almost completely abolishes any inhibitory effect.

Table I. Effects of extracts from various tissues on tyrosinase activity of B16 melanoma homogenate

Experiment No.	Animal	Tissue	Tyrosinase activity[a]	Decrease (%)
1	–	None	18.2	–
	Rat	Liver	7.2	60
	Rat	Kidney	7.2	60
	Rat	Spleen	11.2	39
	Rat	Brain	6.8	62
2	–	None	15.2	–
	Mouse	S91 melanoma	9.8	35
4	–	None	3.9	–
	Mouse	Liver	2.6	33
	Mouse	Kidney	1.2	69
	Mouse	Spleen	2.9	25
	Mouse	Brain	1.3	33
5	–	None	11.2	–
	Mouse	Serum	6.8	39
6	–	None	19.6	–
	Mushroom	Shoot	14.6	25
7	–	None	15.2	36
	Mouse	Cloudman S91 melanoma	9.8	

[a] nmoles of tyrosine oxidized.

1 M. COOPER and Y. MISHIMA, Nature, Lond. *216*, 189 (1967).
2 E. DERNALOWICZ, J. TROJANOWSKI, A. BOMIRSKI and T. DOMINICZAK, Nature, Lond. *215*, 188 (1967).
3 G. J. Z. SATOH and Y. MISHIMA, J. Invest. Dermat. *48*, 301 (1967).
4 G. F. WILGRAM, P. LYNCH, J. A. MABEL and D. A. EVANS, Fedn Proc. *29*, abst. 413 (1970).
5 S. SILAGI, Abstracts of the VIIth International Pigment Cell Conference, Seattle 1969, p. 23.
6 I. A. MENON and H. F. HABERMAN, Cancer Res. *28*, 1237 (1968).
7 I. A. MENON and H. F. HABERMAN, Arch. Biochem. Biophys. *137*, 231 (1970).

The widespread distribution of inhibitor(s) of tyrosinase casts doubts upon the specificity of inhibitor(s) present in amelanotic melanomas and makes it difficult to interpret the role of inhibitors as controlling factors in melanogenesis[8].

Table II. Effects of heating and dialysis on the inhibitory effect of liver homogenate on tyrosinase activity of B16 melanoma homogenate

Experiment No.	Tissue	Treatment	Tyrosinase activity[a]	Decrease (%)
1	–	–	17.0	–
	Liver	None	4.2	75
	Liver	Dialysed	9.2	46
	Liver	Heated	7.2	58
2	–	–	40.6	–
	Liver	None	25.6	37
	Liver	Dialyzed and heated	37.4	8

[a] nmoles of tyrosine oxidized.

Résumé. Les inhibiteurs de tyrosinase ont été trouvés dans des extraits de foie, de rein, de rate et de cerveau de rats et de souris, ainsi que dans des tumeurs amélanotiques S91, des sérums humains, des extraits de foie, de rein, de rate, de sein, et de peau humaine. Il y a au moins deux inhibiteurs: le premier, stable à la chaleur et dialysable, tandis que le second est labile à la chaleur et non-dialysable.

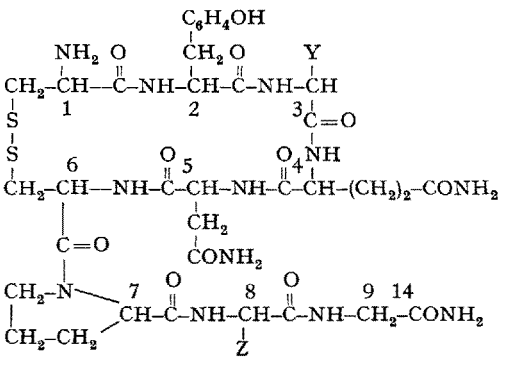
I. A. Menon and H. F. Haberman

Departments of Medicine and Biochemistry, Medical Sciences Building, University of Toronto, and Toronto Western Hospital, Toronto 181 (Ontario, Canada), 8 December 1970.

[8] This investigation was supported by the Medical Research Council of Canada, and the Ontario Cancer Treatment and Research Foundation. One of us (H.F.H.) is a Fellow of the Ontario Cancer Treatment and Research Foundation. The authors thank Mrs. H. Li and Miss E. Santos for their skilful technical assistance.

Arginine-Vasopressin, Lysine-Vasopressin, and Oxytocin, C^{14}-Labeled in the Glycine Residue[1,2]

Solid-phase synthesis[3] is a useful procedure for the preparation of peptides[4] and polynucleotides[5] possessing structures of great diversity. Insolubility of intermediary products attached to a solid support in conjunction with automation of the repetitive synthetic cycle render this method ideal for the preparation of radioactively labeled, naturally occurring biopolymers. In this communication we report on the application of the stepwise solid-phase procedure to the synthesis of [9-glycinamide-1-^{14}C]-arginine-vasopressin (^{14}C-AVP), [9-glycinamide-1-^{14}C]-lysine-vasopressin (^{14}C-LVP), and [9-glycinamide-1-^{14}C]-oxytocin (^{14}C-OT) with specific radioactivities of about 30, 25 and 30 mCi/mmole, respectively (Figure).



8-Arginine-vasopressin:

$$Y = -CH_2-C_6H_5;\quad Z = -(CH_2)_3-NH-\overset{\overset{NH}{\|}}{C}-NH_2$$

8-Lysine-vasopressin:

$$Y = -CH_2-C_6H_5;\quad Z = -(CH_2)_4-NH_2$$

Oxytocin:

$$Y = -\underset{\underset{CH_3}{|}}{CH}-CH_2-CH_3;\quad Z = -CH_2-\underset{\underset{CH_3}{|}}{CH}-CH_3$$

The preparation of the protected nonapeptides, i.e. for ^{14}C-AVP, S-Bzl-N-Tos-Cys-Tyr-Phe-Gln-Asn-S-Bzl-Cys-Pro-N^G-Tos-Arg-^{14}C-Gly-NH_2 (I); for ^{14}C-LVP, S-Bzl-N-Z-Cys-Tyr-Phe-Gln-Asn-S-Bzl-Cys-Pro-N$^\varepsilon$-Z-Lys-^{14}C-Gly-NH_2 (II); and for ^{14}C-OT, S-Bzl-N-Z-Cys-Tyr-Ile-Gln-Asn-S-Bzl-Cys-Pro-Leu-^{14}C-Gly-NH_2 (III) was performed according to the procedure used for the solid-phase synthesis of arginine-vasopressin[6]. For the synthesis of I, 4.2 mmoles of glycine-1-^{14}C (^{14}C-Gly) (specific radioactivity 30.7 mCi/mmole, Lot 9037-53, ICN Tracer

[1] Supported by National Institutes of Health grants No. AM-13567 and No. AM-10080 and the Atomic Energy Commission.

[2] Abbreviations follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature in Biochemistry *5*, 2485 (1966). All optically-active amino acids are of L-configuration. The following additional abbreviations were used: N-hydroxysuccinimide ester (OSu), ethanol (EtOH), methanol (MeOH), acetic acid (AcOH), *n*-butanol (*n*-BuOH), pyridine (Pyr) and N,N'-dicyclohexylcarbodiimide (DCCI). Protected peptides and hormones were visualized on thinlayer plates according to the procedure by H. Zahn and E. Rexroth, Z. analyt. Chem. *148*, 181 (1955). The biological activities of the hormones were measured against the U.S.P. Posterior Pituitary Reference Standard; the four-point design was used for these bioassays and standard errors were calculated according to the method of C. I. Bliss, *The Statistics of Bioassay* (Academic Press, New York, N.Y. 1952).

[3] R. B. Merrifield, J. Am. chem. Soc. *85*, 2149 (1963).

[4] R. B. Merrifield, in *Recent Progress in Hormone Research* (Ed. G. Pincus; Academic Press, New York 1967), vol. 23, p. 451.

[5] H. Hayatsu, Protein Nucleic Acid Enzyme *13*, 869 (1968). – F. Cramer and H. Köster, Angew. Chem. *80*, 488 (1968). – H. Hayatsu and H. G. Khorana, J. Am. chem. Soc. *89*, 3880 (1967). – L. R. Melby and D. R. Strobach, J. Am. chem. Soc. *89*, 450 (1967).

[6] J. Meienhofer, A. Trzeciak, T. Douša, O. Hechter, R. T. Havran, I. L. Schwartz and R. Walter, in *Peptides 1969* (Ed. E. Scoffone; North-Holland Pub. Co., Amsterdam, in press). – J. Meienhofer, A. Trzeciak, R. T. Havran and R. Walter, J. Am. chem. Soc., *92*, 7199 (1970).