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.

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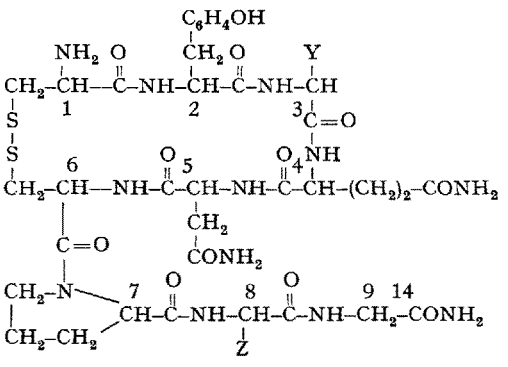
Arginine-Vasopressin, Lysine-Vasopressin, and Oxytocin, C^{14}-Labeled in the Glycine Residue[1,2]

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

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[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).

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Lab) was converted[7] to 3.2 mmoles (77%) of Boc-^{14}C-Gly [homogenous upon thinlayer chromatography on Silica gel G (Type Q1, Quantum Ind.) with *n*-BuOH:AcOH:H_2O (2:1:1, v/v/v) (S_1), Rf 0.9], which was esterified with chloromethylated copolystyrene-2% divinylbenzene resin (2.2 g resin containing 1.5 mmoles Cl/g, Mann Chem. Co.). The Boc-^{14}C-Gly-resin contained 0.55 mmoles ^{14}C-Gly/g as determined by Volhard titration[3]. An individual cycle for the incorporation of each additional amino acid involved acidolysis of the N-protecting group of the terminal amino acid residue followed by neutralization with triethylamine and subsequent acylation with the properly protected amino acid derivative. Between individual operations extensive washings were performed with CH_2Cl_2 or DMF, followed by absolute ethanol, and finally by glacial acetic acid. All amino acid derivatives were added in 5- to 7-fold excess and were allowed to react for 15 to 24 h. DCCI was used in the coupling of Boc-Arg(Tos)[7,8], Boc-Phe[7,9], and Tos-Cys(Bzl)[10], while the other amino acid derivatives were introduced in the presence of 1,2,4-triazole[11] via activated esters (i.e. Boc-Pro-OSu[12], Nps-Asn-OSu[13], Nps-Gln-OSu[13], Boc-Cys-(Bzl)-ONp[11]). Ammonolysis of the protected nonapeptide-resin (1.03 g) was carried out as described[6] to give the crude protected I (370 mg). This material redissolved in AcOH-abs. EtOH yielded 170 mg (22%)[14] of crystalline I [homogeneous upon thinlayer chromatography with $CHCl_3$:MeOH (8:2) (S_2); identical with authentic sample of unlabeled I, Rf 0.71]. Reduction of I with sodium in liquid ammonia as applied to the original[15] and subsequent[6] syntheses of AVP, followed by oxidative cyclization with ferricyanide and desalting with AG3X4[16], yielded ^{14}C-AVP, which was purified by partition chromatography on Sephadex G-25 with *n*-BuOH:EtOH:Pyr:0.1*N* AcOH (4:1:1:7). ^{14}C-AVP was detected by radioactivity measurements[17] as a symmetrical peak (Rf 0.33) identical to that of unlabeled AVP. Additional authentication of ^{14}C-AVP (yield 29 mg, 27%)[18] was achieved by chromatographic comparison with 'cold' AVP on Silica gel G [*n*-BuOH:AcOH:H_2O (4:1:5, upper phase) (S_3); single spot, Rf 0.4] and by bioassay (in the rat pressor assay[19]; the material exhibited an activity of 416 ± 16 U/mg, a value similar to that reported by other workers[6,20]).

Similarly, II was prepared in a stepwise manner except that DCCI was used as coupling agent for Boc-Lys(Z)[7], Boc-Pro[7], Boc-Cys(Bzl)[7], Boc-(Phe)[7] and Boc-Tyr[7] and that Z-Cys(Bzl) was introduced as its *p*-nitrophenyl ester[21]. When starting with 1.0 g of substituted polymer (0.30 mmoles of ^{14}C-Gly/g resin, 25 mCi/mmole, New England Nuclear) we obtained 146 mg (33%) of purified amorphous II, which was converted to hormone and upon purification gave 19 mg (20%) of ^{14}C-LVP [partition chromatography on Sephadex G-25 with *n*-BuOH:EtOH:Pyr:0.1% AcOH (5:1:1:8), Rf 0.17. Single spot, identical with cold LVP, upon thinlayer chromatography with S_1, Rf 0.4; 304 ± 22 U/mg rat pressor activity, which corresponds to the highest value reported to date for LVP[22]].

^{14}C-OT was obtained similarly. Nonapeptide-resin (0.5 g) [0.55 mmoles of ^{14}C-Gly/g resin; 30.7 mCi/mmole, ICN Tracer Lab. Boc-Leu[7] and Z-Cys(Bzl)[21] were introduced with DCCI and Boc-Ile as its *p*-nitrophenyl ester[11]] gave upon ammonolysis 120 mg (40%) amorphous III [thinlayer chromatography with S_2 (Rf 0.60); identical with unlabeled, protected nonapeptide of OT]. Conversion of III gave ^{14}C-OT, which was purified by partition chromatography as described for unlabeled oxytocin[23]. The resulting ^{14}C-OT [9 mg (10%)] gave upon thinlayer chromatography with S_3 a single spot, identical with oxytocin (Rf 0.47) and possessed an avian vasodepressor activity[24] of 482 ± 14 U/mg, a value reported for highly purified hormone[21,25].

Zusammenfassung. Die Synthese von Arginin-Vasopressin, Lysin-Vasopressin und Oxytocin, deren Glycinrest eine ^{14}C-Markierung trägt, wird mit Hilfe der Festkörpermethode nach MERRIFIELD beschrieben.

R. WALTER and R. T. HAVRAN[26]

Department of Physiology, Mount Sinai Graduate and Medical Schools of the City University of New York, 5th Avenue and 100th Street, New York (N.Y. 10029, USA), and Medical Research Center, Brookhaven National Laboratory, Upton (N.Y. 11973, USA), 25 January 1971.

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[18] After pooling the hormone-containing fractions and removal of the organic phase under reduced pressure the highly purified hormone was kept in an aqueous phase at 4 °C. The concentration of hormone (µ*M*/ml) was obtained from: decompositions/min/ml/2.2×10^6/specific activity.
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