to α-amino nitriles needs to be reconsidered. It is widely believed, for example, that α-amino acids obtained from several types of simulation experiments were formed by the direct hydrolysis of corresponding α-amino nitriles[10–13]. In these alkaline reactions, any formation of α-amino nitriles would soon be followed by elimination of hydrogen cyanide. It therefore seems more likely to us that in such experiments most of the α-amino acids were products of alkaline hydrolysis of the peptides formed by the polymerization of hydrogen cyanide present either as a starting material or a reaction intermediate[3,4]. It has also been proposed[14–17] that the formation of triglycine from aminoacetonitrile (by heating with kaolinite and extracting with water)[14] occurred through a prior condensation between amine and nitrile groups yielding polyamidines. Since black solids were also a major product, it again seems more probable to us that the peptides were formed by base-catalyzed polymerization of hydrogen cyanide following decomposition of the starting material. We conclude, therefore, that in the reducing and basic environment of primitive Earth, α-amino nitriles played little or no direct part in the sequence of events leading to the prebiological synthesis of polypeptides and proteins. The main role, instead, was taken by hydrogen cyanide[3,4,18].

Zusammenfassung. Neutrale oder alkalische Hydrolyse von Aminoacetonitril gibt mindestens 6 α-Aminosäuren neben Glycin, welches das einzige Produkt bei saurer Hydrolyse ist. Die anderen α-Aminosäuren entstehen durch die Hydrolyse der peptidähnlichen Polymere, welche durch die Polymerisierung des aus dem Aminoacetonitril gebildeten Cyanwasserstoffes mit basischen Katalysatoren gebildet werden. Im Zusammenhang mit der chemischen Evolutionstheorie weisen diese Resultate darauf hin, dass α-Aminonitrile nur eine kleine oder gar keine direkte Rolle in der Aufeinanderfolge der Reaktionen gespielt haben, welche zur vorbiologischen Synthese von Polypeptiden und Proteinen führten.

R. E. Moser and C. N. Matthews

Central Research Department, Monsanto Company, Saint Louis (Missouri 63166, USA),
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Solid Phase Synthesis and Some Pharmacoogical Properties of 4-Ser-8-Gln-Oxytocin (Glumitocin)[1]

Glumitocin (4-Ser-8-Gln-Oxytocin) was first isolated from *Raia clavata*[2] and later identified in 3 additional elasmobranchs, *R. batis*, *R. fullonica* and *R. naevus*[3]. Until very recently the proposed structure had not been confirmed by laboratory synthesis[4]. As part of a continuing investigation on the phylogeny of the neurohypophyseal hormones the synthesis of 4-Ser-8-Gln-Oxytocin was undertaken using the adaptation of the Merrifield solid phase method[5] which has recently been applied toward the synthesis of oxytocin[6]. The synthetic product has been pharmacologically evaluated by methods previously described[7] and the results obtained are presented in the Table.

The required protected nonapeptide amide intermediate was synthesized in a stepwise manner beginning with 5.0 g of *t*-butyloxycarbonylglycyl resin containing 0.985 mmole of glycine according to the general procedure of Merrifield[8], using the modifications previously described[6] with one additional precaution, i.e. the trifluoroacetic acid, dimethylformamide and triethylamine used in the synthesis were all fractionally redistilled before use. 8 cycles of deprotection, neutralization and coupling were carried out with appropriate Boc-amino acids[9] producing the protected nonapeptide esterified to the resin. Boc-amino acids with protected side chains were S-Bzl-Cys, O-Bzl-Ser and O-Bzl-Tyr. The final cysteine residue was added as the *N*-Carbobenzoxy-S-Benzyl (*N*-Z-S-Bzl) derivative. All coupling reactions to form peptide bonds were mediated by dicyclohexylcarbodiimide[10] in methylene chloride except those involving the carboxyl groups of Asn and Gln, which were allowed to react in dimethylformamide (DMF) as their nitrophenyl esters[11].

Following the coupling of the final residue, the dried resin weighed 6.057 g. The weight increase of 1.057 g represents the incorporation of 0.81 mmole of protected nonapeptide in the resin. This is 82.2% of the amount expected, based on the original glycine content of 0.985 mmole of the esterified resin. Ammonolytic cleavage of the protected nonapeptide resin (2.9 g) was carried out as previously described[6] to give the protected nonapeptide amide Z-Cys(Bzl)-Tyr(Bzl)-Ile-Ser(Bzl)-Asn-Cys(Bzl)-Pro-Gln-Gly(NH_2) as a white amorphous powder, weight 464 mg; m.p. 243.5–245°, $[\alpha]_D^{22.5} - 29.0°$ (c, 1, dimethylformamide). Anal. calcd. for $C_{76}H_{92}N_{12}O_{15}S_2$: C, 61.77; H, 6.27; N, 11.37. Found: C, 61.97; H, 6.20; N, 11.28.

The yield of the protected nonapeptide amide from the cleavage was 82% of the amount expected based on the increase in weight of the resin. The overall yield based on the amount of glycine originally esterified to the resin

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was 67.5%. Amino acid analysis[12] gave: Asp, 1.00; Ser, 0.80; Glu, 1.00; Pro, 1.00; Gly, 1.00; Ile, 0.94; Tyr, 0.81; Bzl-Cys, 2.1; Cys, 0.05; NH_3, 3.2. Debenzylation of the protected nonapeptide was performed with sodium and liquid ammonia by the method of SIFFERD and DU VIGNEAUD[13] as used in the original synthesis of oxytocin[14]. The resulting dithiol was oxidized at pH 6 with an aqueous solution of 0.011 *M* potassium ferricyanide[15] (15 ml) and the solution was lyophilized. The glumitocin was purified by gel filtration[16,17] as follows: the lyophilizate was dissolved in 3 ml of 50% acetic acid and applied to a Sephadex G-15 (40–120 μ) column (1.2 × 110 cm) which was pre-equilibrated with 50% acetic acid[18]. The column was eluted with the same solvent at a flow rate of 10 ml/h and 85 fractions of 2 ml each were collected by an automatic fraction collector. The peptide material, as measured by both UV-absorption at 280 nm and by the FOLIN-LOWRY method[19], emerged from the column as 2 peaks clearly separated from inorganic salts. The second peak, which contained the majority of the peptide material, was located between fractions 33–41 with the maximum color value at fraction 37, whereas the chloride ion was detected between fractions 60–75. The fractions corresponding to the major peptide peak were pooled, diluted with twice the volume of water and the resulting solution was lyophilized. The lyophilized, desalted material was dissolved in 2 ml of 0.2 *N* acetic acid and subjected to gel filtration on a Sephadex G-15 (40–120 μ) column (1.2 by 110 cm) that had been pre-equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid at a flow rate of 10 ml/h and 90 fractions of 2 ml each were collected. A plot of FOLIN-LOWRY color values of the fractions showed a single symmetrical peak with a maximum at fraction 57. The fractions corresponding to this peak were pooled and lyophilized to give a white powder; weight 50 mg (50.5%); $[\alpha]_D^{22.0} - 4.0°$ (C, 0.5, 1 *N* acetic acid). Anal. calcd. for $C_{40}H_{60}N_{12}O_{13}S_2$ (981): C, 48.97; H, 6.16; N, 17.13. Found: C, 48.81; H, 6.25; N, 16.84. Amino acid analysis gave: Asp, 1.00; Ser, 0.83; Glu, 0.97; Pro, 0.95; Gly, 0.88; Cys, 1.96; Ile, 0.89; Tyr, 0.83; NH_3, 3.1.

Examination of aliquots (100 μg) by thin layer chromatography on silica gel H and by paper chromatography (ascending) on Whatman No. 1 paper in the solvent system butanol-acetic acid-water (4:1:5)[20] using ninhydrin and platinum reagent[21] for detection revealed only one component with an Rf of 0.16 in both instances. Likewise only one component in the direction of the cathode was observed when paper electrophoresis of a further aliquot (100 μg) in 2 pyridine acetate buffers of pH 3.5 and 6.5 was carried out using the same detecting reagents. The overall yield of pure product was 34% based on the initial glycine incorporation on the resin.

Synthetic 4-Ser-8-Gln-Oxytocin has specific activity on the isolated rat uterus (in the absence of Mg^{++}) comparable to that previously reported for natural[2] and synthetic[4] glumitocin. Potentiation of rat uterus activity by adding 0.5 m*M* Mg^{++} to the bath, however, was only about three-fold. This is in marked contrast to the ten-fold Mg^{++} potentiation reported by ACHER et al.[2] and CHAUVET et al.[22] for glumitocin isolated from *R. clavata* and *R. batis*. The reason for this discrepancy is not clear. The active principle (EOP II) isolated from *R. ocellata* pituitaries[23] had a Mg^{++} potentiation ratio of 2.6. Although it contained the same amino acid residues as glumitocin[23] the different Mg^{++} potentiation ratios suggested a difference in amino acid sequence. Examination of the activity ratios for synthetic 4-Ser-8-Gln-Oxytocin and the *R. ocellata* principle (Table) obtained previously by the same methods suggests that they may, after all, be identical. Direct comparisons by simultaneous assays are needed to settle this question.

Pharmacological activities[a] and activity ratios[b] with standard errors of 4-Ser-8-Gln-Oxytocin and EOP II[c]

	4-Ser-8-Gln-Oxytocin	EOP II
Rat uterus (RUsMg) (no Mg in bath)	9.8	18
Rat uterus (RUcMg) (0.5 m*M* Mg in bath)	29	
RUcMg/RUsMg = R_{Mg}	3.0 ± 0.1	2.6 ± 0.2
Rat vasopressor (RVP)	0.35	
RVP/RUsMg = R_{VP}	0.036 ± 0.003	< 0.02
Rat antidiuretic (RAD)	0.41	
RAD/RUsMg = R_{AD}	0.04 ± 0.006	0.04 ± 0.004
Isolated bull frog bladder water permeability (FB)	7.7	
FB/RUsMg = R_{FB}	0.8 ± 0.1	< 0.45
Rabbit milk-ejecting (ME)	53	
ME/RUsMg = R_{ME}	5.4 ± 0.3	6.9 ± 0.8

[a] Expressed in units/mg. [b] Biological assays were carried out and activity ratios were calculated as described in [7]. [c] See [7].

Zusammenfassung. Eine neue Synthese des Peptidhormons Glumitocin wird beschrieben, in der die Trägermethode von MERRIFIELD benutzt wird. Einige pharmakologische Daten dés Hormons werden mitgeteilt.

M. MANNING, T. C. WUU,
J. W. M. BAXTER and W. H. SAWYER

Department of Biochemistry, McGill University, Montreal (Quebec, Canada) and Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York (N.Y., USA), 5 April 1968.

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