ten, olivbräunlichen oder olivgrünen Farbtöne im ÜE an, während die zur Kontrolle synchron-inkubierte Skelettmuskulatur tiefdunkelblau mit Jod reagierte. Ohne Äthanolzusatz verlief die Polysaccharidsynthese bis zu dem mit Jod mahagonibraun sich anfärbenden Glykogen. Das Verteilungsmuster des enzymatisch aufgebauten Polysaccharides entspricht weitgehend dem natürlichen Glykogenvorkommen im ÜE im Sinne einer Zunahme von der Epithelbasis zur -oberfläche[11].

Die Ergebnisse werden an anderer Stelle ausführlich dargestellt.

Summary. Histochemical investigations on enzyme activities in the transitional epithelium showed positive reactions with succinic dehydrogenase, esterase, alkaline phosphatase, acid phosphatase and amylophosphorylase (incl. branching factor). Negative results were drawn from cholinesterase, 5′-nucleotidase, glucose-6-phosphatase, and carbonic anhydrase.

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The Biological Activities of Arginine-Vasotocin Obtained by a New Synthesis

Arginine-vasotocin (= Arg^8-oxytocin = Ile^3-arginine-vasopressin) was first synthesised in 1958[1]. One year later, a peptide of this constitution was discovered in the pituitary of some non-mammalian vertebrates[2,3]. As the data of the literature[1,4] on the biological activities of arginine-vasotocin do not entirely agree, we have undertaken a new improved synthesis[5] of this peptide and examined the biological properties of the pure synthetic product thereby obtained.

N-CBO-*L*-proline was condensed by the mixed anhydride procedure with *p*-nitro-phenyl G-tosyl-*L*-arginate and the dipeptide thus obtained was reacted with glycinamide, giving N-CBO-*L*-prolyl-G-tosyl-*L*-arginyl-glycinamide. Removal of the CBO protecting group and condensation with N-CBO-*L*-glutaminyl-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-azide[6] afforded N-CBO-*L*-glutaminyl-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-*L*-prolyl-G-tosyl-*L*-arginyl-glycinamide. After removal of the CBO protecting group this hexapeptide was condensed with *p*-nitro-phenyl N-tosyl-S-benzyl-*L*-cysteinyl-*L*-tyrosyl-*L*-isoleucinate[7] to N-tosyl-S-benzyl-*L*-cysteinyl-*L*-tyrosyl-*L*-isoleucyl-*L*-glutaminyl-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-*L*-prolyl-G-tosyl-*L*-arginyl-glycinamide (m.p. 236–238°. $[\alpha]_D^{22} = -56°$ in 95% acetic acid. Analysis calculated for $C_{71}H_{93}O_{18}N_{15}S_4$: C 55.3; H 6.1; O 16.6; N 13.6; S 8.3. Found: C 55.1; H 6.3; O 17.0; N 13.4; S 8.4). Removal of the protecting groups by treatment with sodium in liquid ammonia, followed by oxydation with air, purification by counter-current distribution in the system sec.-butanol/water/trifluoroacetic acid (120:160:1), conversion in the acetate form and lyophilisation, afforded homogeneous arginine-vasotocin. A sample was dried for 8 h at 100° and gave correct amino acid and elementary analysis (diacetate).

The pharmacological qualities of arginine-vasotocin obtained by the above described synthesis were investigated in different experimental sets generally adopted in studies on the biological activities of the neurohypophysial hormones. Three tests were used to demonstrate and measure effects peculiar to oxytocin. These included the rat uterus *in vitro*[8], the blood pressure of the chicken[9,10] and the mammary gland of lactating rabbits[11,12]. Two tests were used to detect and measure biological activities characteristic of vasopressin, namely the blood pressure of rats[13] and the diuresis of rats in alcohol anaesthesia[14–16]. The Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances[17] served as a reference standard and the activities were expressed in terms of International Units per mg.

The values obtained in the present study are summarised in the first line of the Table. The figures given for another synthetic preparation of this hormone[1] are shown

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Biological activities of arginine-vasotocin in international units per mg[a]

	Rat uterus (isolated)	Chicken blood pressure	Rabbit mammary gland	Rat blood pressure	Rat antidiuresis
Our synthetic product	**115 ± 15**	**285 ± 40**	**ca. 210**	**245 ± 15**	**250 ± 35**
	90 ± 12	224 ± 32	ca. 165	193 ± 12	197 ± 28
KATSOYANNIS and DU VIGNEAUD[1]	75	150		125	
HELLER and PICKERING[4]	40		119	71	71

[a] The activities printed in normal type refer to lyophilised peptide, those printed in heavy type to the peptide itself (free base, without solvent).

in the second line and those published for a purified preparation of arginine-vasotocin of natural source[4] in the third line of the Table. The activities printed in normal type refer to 1 mg powder obtained by lyophilisation of a solution containing the peptide. Such values depend on the solvents used and on the conditions under which the lyophilisation is carried out. The activities printed in heavy type refer to 1 mg of the peptide itself (free base, without solvent). These values are obtained on the basis of nitrogen analysis and are therefore independent of the conditions under which the lyophilisation is performed. The values obtained in the present study are in all tests higher than the activities previously published for arginine-vasotocin.

It is fully appreciated that the expression of the activities of a compound in terms of those of another compound is never fully satisfactory. The Third International Standard containing oxytocin and arginine-vasopressin is therefore not an ideal reference standard for arginine-vasotocin although it is the best we can use to-day. Even bearing this in mind it is felt that the quantitative difference between the biological activities of arginine-vasotocin found in the present study and those published previously can hardly be due to mere chance. It rather suggests a higher degree of purity of our sample of arginine-vasotocin.

Zusammenfassung. Eine neue Synthese von Arginin-Vasotocin (= Arg^8-Oxytocin = Ile^3-Arginin-Vasopressin) wird beschrieben. Das reine synthetische Peptid erwies sich in verschiedenen biologischen Testen wirksamer als nach früheren Literaturangaben zu erwarten war.

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The Synthesis and some Pharmacological Effects of Serine^4-Isoleucine^8-Oxytocin, a Probable Neurohypophysial Hormone

Pharmacological evidence has been advanced for the presence in the neurohypophysis of two species of teleost fish—namely *Pollachius virens*[1] and *Gadus luscus*[2]—of a hitherto unknown hormone similar to but not identical with oxytocin. The isolation of a hitherto unknown peptide from the neurohypophysis of three species of teleost fish (*Pollachius virens, Gadus luscus*, and *Merluccius merluccius*) has recently been published[3]. On the basis of amino acid analysis and preliminary enzymatic degradation studies it was postulated[3] that this peptide is an analogue of oxytocin, namely Ser^4-Ile^8-oxytocin. We have synthesised Ser^4-Ile^8-oxytocin and tested some of its pharmacological activities.

L-Prolyl-*L*-isoleucyl-glycinamide[4] was condensed with *p*-nitrophenyl N-CBO-S-benzyl-*L*-cysteinate[5] to N-CBO-S-benzyl-*L*-cysteinyl-*L*-prolyl-*L*-isoleucyl-glycinamide. After removal of the CBO protecting group, condensation with *p*-nitro-phenyl N-CBO-asparaginate[6] yielded the expected protected pentapeptide. After removal of the CBO protecting group this was reacted by the dicyclohexyl-carbodiimide procedure[7] with N-trityl-*L*-serine, giving the protected hexapeptide. Removal of the trityl protecting group and condensation with *p*-nitro-phenyl N-tosyl-S-benzyl-*L*-cysteinyl-*L*-tyrosyl-*L*-isoleucinate[8] yielded N-tosyl-S-benzyl-*L*-cysteinyl-*L*-tyrosyl-*L*-isoleucyl-*L*-seryl-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-*L*-prolyl-*L*-isoleucyl-glycinamide (m.p. 222°. $[\alpha]_D^{22} = -24°$ in dimethylformamide. Analysis calculated for $C_{62}H_{83}O_{14}N_{11}S_3$: C 57.2; H 6.4; O 17.2; N 11.8; S 7.4. Found: C 56.9; H 6.8; O 17.3; N 11.6; S 7.3). Removal of the protecting groups by treatment with sodium in liquid ammonia, followed by oxydation with air and purification by counter-current in the system sec-butanol/water/acetic acid 100:120:1 yielded homogeneous Ser^4-Ile^8-oxytocin (K = 0.57).

This synthetic peptide was compared with the Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances[9] in five tests generally used for the quantitative pharmacological characterisation of neurohypophysial hormones. The oxytocic effect was assayed on the isolated rat uterus[10], the avian depressor activity on the blood pressure of white Leghorn cockerels[11,12] and the milk ejecting potency on the mammary glands of lactating rabbits *in situ*[13,14]. The antidiuretic effect was tested in water-loaded rats anaesthetised with alcohol[15–17] and the pressor activity in rats pretreated with an adrenergic blocking agent[18,19].

The results are summarised in the Table; the biological potencies of Ser^4-Ile^8-oxytocin in the different tests are given in International Units per mg pure peptide (free base).

Pharmacological activities of Ser^4-Ile^8-oxytocin in International Units per mg

Rat uterus (isolated)	Chicken blood pressure	Rabbit mammary gland	Rat blood pressure	Rat antidiuresis
150 ± 12	320 ± 15	300 ± 15	0.06 ± 0.01	0.18 ± 0.03

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