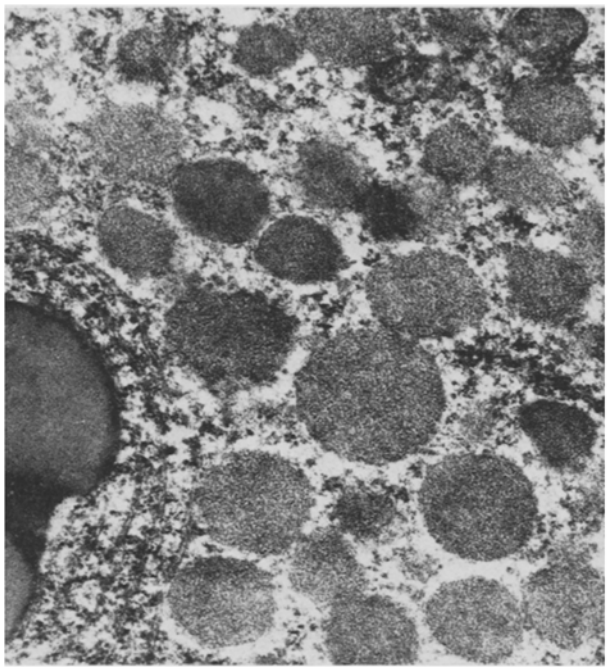

Fig. 1. D cell of the human pancreatic islets. ×31,000.

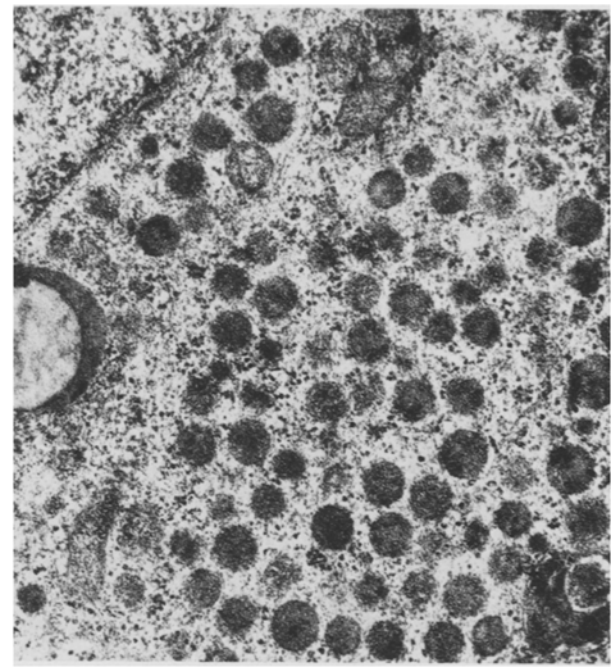

Fig. 2. A new cell type of the human islets. ×31,000.

light cytoplasm. This new cellular type (VI) has so far been probably included in the population of the D cells, from which it differs distinctly by the size of the secretory granules (the diameter of the secretory granules of the D cells ranges from 290–620 nm). This new type of endocrine cell seems to be less frequent than the D cells, but more frequent than the G cells and the 'enterochromaffin' cells.

Zusammenfassung. Es wird neben den bereits beschriebenen A, B, D, G und den «enterochromaffinen» Zellen, in den Langerhansschen Inseln des menschlichen Pankreas ein neuer Zelltyp mit charakteristischer, besonders kleiner Sekretionsgranula und von granulärer Struktur gefunden.

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13 December 1971.

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Natriferic and Hydroosmotic Potencies of Deamino-Oxytocin Analogues with no Disulphide Bridge

It has been suggested that the presence of the disulphide bridge in the vasopressin molecule was indispensable for the interaction with the receptor involved in the hormones' effect on toad bladder water permeability. The findings of Schwartz et al.[1] indicated that tritiated vasopressin could bind to the toad urinary bladder through s-s bridges. Rasmussen et al.[2] suggested a two-step binding process: a stereospecific interaction between the hormonal molecule and its receptor site, followed by a sulphhydryl-disulphide exchange reaction between the s-s bridge of the hormone and SH groups of the receptor, the latter reaction being directly responsible for the permeability changes. This theory was challenged by the finding that deamino carba-oxytocin was able to increase toad bladder water permeability[3] and stimulate sodium active transport by the frog skin[4] despite the fact that a sulphhydryl-disulphide exchange reaction was impossible in this case. However, replacement of sulphur atoms by CH_2 groups in the oxytocin molecule led to a sharp drop in the biological activities compared to those of the parent oxytocin. This ob-

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servation suggests that the disulphide bridge plays an important part in maintaining the biologically active conformation of the hormonal molecule.

More recently, a series of oxytocin analogues with modified ring structure and size were synthetized; they may help to elucidate the specific role of this part of the hormonal molecule in its biological activities. The purpose of the present study was to determine the hydroosmotic (increase in frog urinary bladder water permeability) and natriferic (stimulation of frog skin active sodium transport) potencies of this series of analogues.

Table I. Ring structure of oxytocin analogues tested.

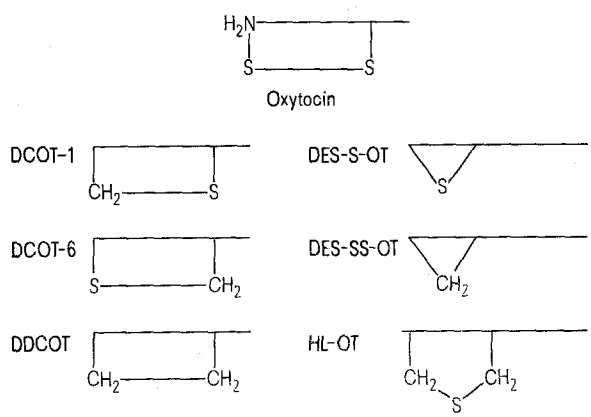

Table II. Natriferic and hydroosmotic activities of oxytocin analogues with modified ring structure

Compound	Natriferic activity (Units per mg)	Hydroosmotic activity (Units per mg)
Oxytocin	450	450
DCOT-1	7.23± 1.01 (10)	8.61± 2.67 (12)
DCOT-6	49.46± 7.67 (11)	40.21± 6.98 (14)
DDCOT	26.18± 3.25 (6)	11.61± 1.71 (14)
DES-S-OT	39.97±12.16 (7)	30.39± 4.56 (16)
DES-SS-OT	8.80± 1.22 (9)	10.94± 2.77 (13)
HL-OT	42.85±11.83 (7)	31.26±11.37 (12)

Activities were determined from the threshold doses ratio (see Methods). One natriferic or hydroosmotic unit is defined as the activity of one international unit of synthetic oxytocin. Values are means±SD. The number of experiments is indicated in parentheses.

Experimental. The following analogues[5] were used (see Table I): DCOT-1[6] = deamino-carba-1-oxytocin (α deamino cystathionine-oxytocin)-DCOT-6[7] = deamino-carba-6-oxytocin (β deamino-cystathionine-oxytocin)-DDCOT[7] = deamino dicarba 1,6-oxytocin (α aminosuberic acid 1,6-oxytocin)-DES-S-OT[8] = deamino lanthionine 1,6-oxytocin-DES-SS-OT[8] = amino pimelic acid 1,6-oxytocin and HL-OT[9] = deaminohomolanthionine 1,6-oxytocin. They were synthetized by Dr. K. JOŠT[5] and coworkers, of the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague. Synthetic oxytocin used as the standard for the biological assays was purchased from Sandoz and Spofa.

The hydroosmotic effect (increase in the net water flow along an osmotic gradient) was measured on the isolated frog bladder (*Rana esculenta*) using a previously described technique[10]. In the course of each experiment, dose-response relationships (cumulative doses technique) were successively determined 1. for oxytocin used as a standard, 2. for the analogue tested and 3. for oxytocin again, to test the maintenance of the bladder's reactivity. The natriferic effect (stimulation of active sodium transport) was measured by the increase in current flowing through the short-circuited[11] frog skin. The experimental procedure was similar to that used for the hydroosmotic experiments.

The biological potency of the individual compounds in relation to that of oxytocin was calculated as the ratio of the threshold doses of oxytocin and analogue determined on the same preparation.

Intrinsic activities are measured by the magnitude of the maximum response estimated from the double reciprocal plot. They were expressed as a percentage of the intrinsic activity of oxytocin. Affinity was measured according to EGGENA et al.[12] by the pD_2 value (negative logarithm of the hormone concentration in the medium eliciting half the maximum response).

Results. As indicated in Table II, the substitution of one or both sulphur atoms in the molecule of oxytocin as well as the modification of the ring size are compatible with

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Table III. Affinities and intrinsic activities of oxytocin analogues with modified ring structure

Compound	Natriferic test		Hydroosmotic test	
	pD2	IA	pD2	IA
Oxytocin	8.13	100 ± (15)	8.13	100 ± (17)
DCOT-1	6.66	103 ±7.20. (5)	6.45	48.8± 5.4 (7)
DCOT-6	7.20	83.6±5.0 (6)	7.02	7.02±17.4 (7)
DDCOT	6.55	74.6±8.3 (5)	6.62	69.4± 6.0 (7)
DES-S-OT	6.77	82.2±3.7 (6)	7.08	85.6± 5.6 (7)
DES-SS-OT	6.24	69.8±5.8 (8)	6.71	85.3± 5.6 (7)
HL-OT	6.64	78.5±3.3 (6)	6.94	87.1± 4.8 (8)

Intrinsic activities (IA) are expressed as % of oxytocin intrinsic activity used as a standard. Values are means ±SD. The number of experiments is indicated in parentheses.

the maintenance of both natriferic and hydroosmotic activities. However, the biological potencies of the analogues tested decrease by at least one order of magnitude compared to that of oxytocin. Furthermore, the loss in natriferic activity is very similar to the loss in hydroosmotic activity.

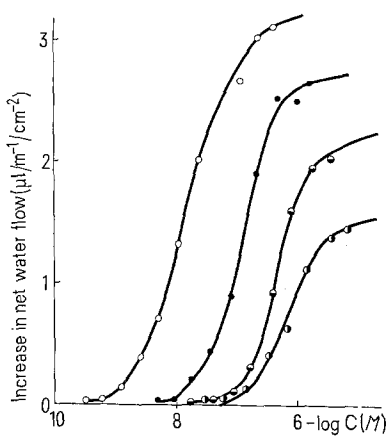
The data given in Table III indicate that the modifications introduced in the structure of the oxytocin ring affect the apparent affinity for the receptor and the intrinsic activity of the hormone receptor complex in different ways. Comparison of DCOT-1, DCOT-6 and DDCOT shows that the two sulphur atoms are not equivalent. In both the natriferic and hydroosmotic tests, the replacement of the S atom in position 1 by a CH_2 group leads to a more pronounced decrease in affinity than the same replacement in position 6. The decreases in affinity induced by replacement of one or other of the S atoms are not cumulative; pD_2 values for DDCOT are very close to those of DCOT-1. On the other hand, natriferic and hydroosmotic intrinsic activities are unequally modified. The intrinsic hydroosmotic activity of $DCOT_1$ decreases by a factor of 2 while its intrinsic natriferic activity (Figure) remains unchanged. In both tests, the intrinsic activity of DDCOT is significantly lower than that of oxytocin. Finally (Table III) modification of the ring size in DES-S-OT, DES-SS-OT and HL-OT is compatible with the maintenance of at least 80% of the intrinsic activity of the parent oxytocin.

Discussion. The observation that deamino carba-analogues are able to elicit hydroosmotic and natriferic responses by frog epithelial cells confirms the previous conclusion[3,4] that the presence of a disulphide bridge in the hormonal molecule is not a prerequisite for activity.

Comparison of the activities of the mono and dicarba analogues on the one hand, and of $DCOT_1$ and $DCOT_6$ on the other, shows that the position of the sulphur atom replacement plays an important part. Although the precise conformation of these analogues is not yet known, circular dichroism data for deamino carba analogues indicate that their basic structural parameters are similar[13]. Thus the differences observed between these analogues might reflect a specific role of sulphur atoms in the hormone receptor interaction rather than the consequences of important structural modifications of the whole molecule induced by $S–CH_2$ interchange.

Frog skin and bladder receptors are less sensitive to a modification of the size of the hormone ring than are the rat uterus and kidney receptors[13]. Thus DES-S-OT, an analogue with a reduced ring size, and HLOT, an analogue with an enlarged ring size, have similar natriferic and hydroosmotic activities, while[14] the uterotonic and antidiuretic activities of HL-OT are respectively 6, 7 and 3 times higher than those of DES-S-OT.

Despite the fact that hydroosmotic and natriferic responses were measured on different structures, the observation that $DCOT_1$ exhibits different intrinsic activities in the two tests in in line with the previous proposal (for review, see[15]) that different receptors are involved in the hydroosmotic and natriferic responses.



Hydroosmotic dose-response relationships for oxytocin deamino-carba analogues. Ordinates: increase in net water flow above the resting value. (Frog bladder incubated with Ringer inside and 20-fold diluted Ringer outside: osmotic gradient: 230 mOsm/l). ○, Oxytocin; ●, DCOT-6; ◐, DDCOT; ◒, DCOT-1.

Résumé. Des analogues déaminés de l'ocytocine, dans lesquels l'atome de S en position 1, 6 ou 1 et 6 a été remplacé par un radical CH_2, de même que des analogues déaminés dont la partie cyclique a été raccourcie ou allongée, restent capables d'augmenter la perméabilité à l'eau de la vessie ou le transport actif du sodium par la peau de la grenouille. L'ensemble des substitutions étudiées réduit de manière importante (85 à 300 fois) l'affinité du peptide pour son récepteur et affecte de manière variable son activité intrinsèque.

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Chromosomal Polymorphism in the Phyllostomatid Bat, *Mimon crenulatum* (Geoffroy)

The chromosomes of 49 species of bats of the family Phyllostomatidae have been described based on the examination of 441 specimens[1–13]. In only two species, *Mesophylla macconelli*[8,9] and *Uroderma magnirostrum*[10], have chromosomal polymorphisms been reported. We have found an addition polymorphic chromosomal system in specimens of *Mimon crenulatum* collected from localities in Trinidad, Colombia, and Peru, spanning a distance of more than 1500 miles.

Specimens were obtained by use of mist nets from natural populations and karyotypic preparations were made from in vivo cultures of bone marrow[9]. A minimum of 25 spreads from each individual was examined. Voucher specimens from Peru were deposited in the collections of the Louisiana State University Museum of Zoology (LSUMZ)[14], and the Museum of Vertebrate Zoology, University of California (MVZ) and the material from Colombia and Trinidad is in The Museum, Texas Tech University (TT).

A total of 20 specimens was examined (8 from Trinidad, 6 from Colombia, and 6 from Peru) and all had a diploid number of 32. Chromosomal data for the 20 specimens are