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Relationships Between the Chemical Structures and the Biological Properties of the Posterior Pituitary Hormones and their Synthetic Analogues

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During the past five years, the chemistry and the pharmacology of peptides related to the posterior pituitary hormones have aroused considerable interest. In no other field of biologically active peptides have so many analogues and homologues been synthesized and submitted to such an extensive pharmacological testing.

In an attempt to find some relationships between the chemical structures of these compounds and their biological properties, we have drawn up a series of Tables which permit a systematic study of the pharmacological changes brought about by limited modification of the structure of the natural hormones, oxytocin and lysine-vasopressin.

In addition to the name and the abbreviated chemical formula of the compounds presented for comparison in the Tables, we have, in most cases, indicated the detailed chemical structure of the grouping by which the compounds in a given Table differ among themselves. We should emphasise the fact that the Tables include only chemically pure peptides, which have been demonstrated by counter-current distribution, paper chromatography and paper electrophoresis to be homogenous. They have been assayed by pharmacological methods generally used in quantitative studies on neurohypophysial hormones: contraction of the isolated rat uterus[1] and of the cat uterus *in situ*[2]; fall in chicken blood pressure[3,4]; pressure increase in the lactating mammary gland of the rabbit[5–7]; rise in blood pressure of the rat[8] and spinal cat[9]; and inhibition of diuresis in the rat[10–12]. The quantitative results of these assays are reported in international units (IU) in the Tables. When available, the standard deviations of the assays are also indicated.

In the right-hand column of the Tables, references to papers on the chemistry and pharmacology of the synthetic analogues are cited. Most of these analogues have been synthesized and tested in our own laboratories. However, we have also included the analogues synthesized by DU VIGNEAUD or his co-workers and tested in the laboratories of DU VIGNEAUD or of VAN DYKE[13], since for these compounds extensive and accurate chemical and pharmacological data are available. Some other analogues have been synthesized by other research groups, but the lack of adequate chemical or pharmacological data has prevented us from including them in our Tables.

Examination of the data reported in the Tables calls for the following comments and conclusions.

Variations in Position 3. The natural posterior pituitary hormones differ among themselves by variations in positions 3 and 8 (Figure and Table I).

Systematic variation of the amino acid in position 3 was relatively easy to accomplish by following the general pattern of synthesis used for our synthesis of oxytocin[14]. Replacement of isoleucine by valine yielded the first analogue, *Val³-oxytocin*. The latter differs from oxytocin only by the absence of a $-CH_2-$ group from one of the six side-chains of the molecule (Table II). Pharmacological assays showed that the biological qualities characteristic of the oxytocin molecule are not equally affected by this small structural modification. On the isolated rat uterus and on the blood pressure of the chicken, Val³-oxytocin is only about one-eighth as potent as oxytocin. However, on

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13 See e.g. H. B. VAN DYKE: Vasopressins. XXIst Internat. Congr. of Physiol. Sci., Buenos Aires 9.–15. 8. 1959. Symposia and Special Lectures, p. 61–70.

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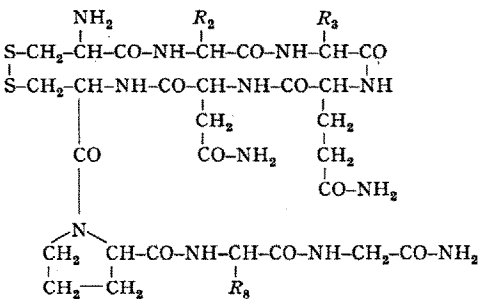
Tab. I. Posterior pituitary hormones occurring in nature

Name and chemical formula	$R_2 = -CH_2-C_6H_4-OH$	
H-CyS-Tyr-*Ileu*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Leu*-Gly-NH_2 Oxytocin	$R_3 = -CH(CH_3)-CH_2-CH_3$	$R_8 = -CH_2-CH(CH_3)-CH_3$
H-CyS-Tyr-*Phe*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Lys*-Gly-NH_2 Lysine-vasopressin	$R_3 = -CH_2-C_6H_5$	$R_8 = -CH_2-CH_2-CH_2-CH_2-NH_2$
H-CyS-Tyr-*Phe*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Arg*-Gly-NH_2 Arginine-vasopressin	$R_3 = -CH_2-C_6H_5$	$R_8 = -CH_2-CH_2-CH_2-NH-C(=NH)-NH_2$
H-CyS-Tyr-*Ileu*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Arg*-Gly-NH_2 Arginine-vasotocin	$R_3 = -CH(CH_3)-CH_2-CH_3$	$R_8 = -CH_2-CH_2-CH_2-NH-C(=NH)-NH_2$



General chemical formula of the posterior pituitary hormones.

the rabbit mammary gland and on the cat uterus *in situ*, it is still roughly half as active as oxytocin, and a similar level of activity has been observed in clinical tests in man[15,16]. Furthermore, this slight modification of structure reduces the low pressor and antidiuretic activities, inherent in the oxytocin molecule, to about one-tenth of their former values. Thus Val^3-oxytocin, while having a lower specific activity (i.e. activity per mg) than oxytocin, is actually a more selective oxytocic agent, since for a given uterine response, the clinically undesirable pressor and antidiuretic activities are less. In a manner of speaking, therefore, Val^3-oxytocin is an improvement on its natural prototype.

By replacing the isoleucine of oxytocin by leucine, we obtained *Leu^3-oxytocin* which differs from the natural hormone only in that the methyl group has been shifted along the side-chain at position 3. This change reduced the oxytocic activity to less than a quarter of that of the natural hormone.

The replacement of isoleucine by phenylalanine, which is present in the vasopressins in position 3, was investigated independently by our group and by KATSOYANNIS in DU VIGNEAUD'S laboratory. This modification results in an analogue (*Phe^3-oxytocin* or *oxypressin*) exhibiting biological properties both of oxytocin and of the vasopressins. Strangely enough the antidiuretic activity of this compound is about ten times greater than its pressor activity. This shows that modification of the structure of the vasopressins (replacement of the basic residue in position 8 by leucine) affects pressor potency more strongly than antidiuretic potency. Although this analogue still possesses oxytocin-like activity, it may nevertheless be regarded as an artificial antidiuretic hormone with low pressor activity.

The above findings seemed to show that the side-chain in position 3 of the oxytocin molecule can be changed from an aliphatic to an aromatic structure without drastically affecting the oxytocic activity. This conclusion, however, proved to be fallacious when we examined an analogue with tyrosine in position 3. This aromatic analogue, *Tyr^3-oxytocin*, differing from Phe^3-oxytocin only by an additional phenolic group, is almost devoid of biological activity. We also investigated the influence of another aromatic residue by introducing tryptophan in position 3. The oxytocic activities of this compound, *Try^3-oxytocin*, are again negligible. Furthermore no pressor activity can be detected; indeed, this compound showed a certain depressor effect on the blood pressure of the rat and the spinal cat.

Thus, structural tolerance is much more restricted than might have been suspected from an examination of the first members of this series of analogues.

When the analogous modifications were effected at position 3 of lysine-vasopressin (Table III), similar

[15] C. N. SMYTH, Brit. med. J. *1*, 856 (1958).
[16] B. BERDE and K. SAAMELI, Acta endocrin. *32*, 391 (1959).

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Occurs in the posterior pituitary of
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	vertebrates
5 ± 0.5	–	40 ± 5	60 ± 10	270 ± 20	306 ± 13	~250	pig, hippopotamus
~20	–	~60	~70	~400	~ 400	~400	most mammals
~75	–	~150	~100	~125	–	–	non-mammalian vertebrates

observations were made. Replacement of phenylalanine by tyrosine—*Tyr*^{3}-*Lys*^{8}-*vasopressin*—or tryptophane—*Try*^{3}-*Lys*^{8}-*vasopressin*—greatly attenuates biological activity. Introduction of serine in the same position—*Ser*^{3}-*Lys*^{8}-*vasopressin*—has the same result. On the other hand, introduction of isoleucine in this position gives a compound *Ileu*^{3}-*Lys*^{8}-*vasopressin* or *Lys*^{8}-*oxytocin* or *lysine-vasotocin* which, like Phe^3-oxytocin discussed above, is structurally and biologically intermediate between lysine-vasopressin and oxytocin. However, the pressor activity of lysine-vasotocin is more pronounced than its antidiuretic activity.

From these studies on systematic variations in position 3 of oxytocin and lysine-vasopressin, it can be concluded that only a limited number of modifications lead to compounds which retain any noteworthy biological properties.

Variations in Position 8. It is interesting to investigate the effect of modifying the molecule at position 8 (i.e. outside of the disulphide ring) for, in the natural neurohypophysial hormones, variations also occur in this position (Table I).

In order to study the influence of relatively small changes, we replaced the leucine of the oxytocin molecule by isoleucine and by valine (Table IV). It is hardly surprising that these small changes should have only a slight influence on biological activity. Nevertheless, it is noteworthy that *Ileu*^{8}-*oxytocin* exerts an even stronger effect than oxytocin itself on the cat uterus *in situ*. This is the first synthetic analogue of oxytocin possessing a higher activity than the natural hormone.

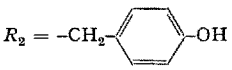
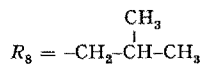
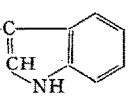
The replacement of leucine by valine in position 8 of oxytocin gives *Val*^{8}-*oxytocin* and corresponds to the removal of a $-CH_2-$ group from one of the side-chains. This slight modification has almost no influence on the activity as measured on the cat uterus *in situ*, but roughly halves the activity as determined on the isolated rat uterus.

By replacing leucine in oxytocin by the basic amino acid lysine, we obtained *Lys*^{8}-*oxytocin* (*lysine-vasotocin*), which is a close analogue of *arginine-vasotocin* recently found in non-mammalian vertebrates. Both compounds have properties intermediate between those of oxytocin and those of the vasopressins. Hence introduction of a basic group in position 8 of oxytocin enhances the vasopressin-like qualities of the oxytocin molecule and attenuates its oxytocin-like properties.

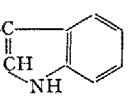
Examples of variations in position 8 of the vasopressins are found in nature: *lysine-vasopressin* occurs in the pig and the hippopotamus, *arginine-vasopressin* in all other mammalian vertebrates investigated so far (Table I). Arginine-vasopressin is somewhat more potent than lysine-vasopressin in every respect. DU VIGNEAUD and KATSOYANNIS have introduced a histidine residue in position 8 thus obtaining *His*^{8}-*vasopressin* (Table V). This modification greatly attenuates all the biological activities, the vasopressin-like qualities being particularly affected. Leucine was introduced in the same position by our group and by KATSOYANNIS of DU VIGNEAUD's group independently. This modification yields *oxypressin* (*Phe*^{3}-*oxytocin* = *Leu*^{8}-*vasopressin*), a compound discussed above, which has properties intermediate between those of oxytocin and the vasopressins.

It would appear from the data in Table V that replacement of the basic amino acid in position 8 of the vasopressins by a neutral amino acid greatly weakens the vasopressin-like activities without much affecting the oxytocin-like properties. It might therefore be expected that introduction of the slightly basic histidine

Tab. II. Variations in position 3 of oxytocin

Name and chemical formula	H-CyS-Tyr-R_3-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2	R_3 = 	R_8 = 
Oxytocin	*Ileu*		R_8 = –CH_2–$CH(CH_3)$–CH_3
Val^3-oxytocin	*Val*		R_8 = –$CH(CH_3)$–CH_3
Leu^3-oxytocin	*Leu*		R_8 = –CH_2–$CH(CH_3)$–CH_3
Phe^3-oxytocin = Oxypressin	*Phe*	R_3 = –CH_2–C_6H_5	
Tyr^3-oxytocin	*Tyr*	R_3 = –CH_2–C_6H_4–OH	
Try^3-oxytocin	*Try*	R_3 = –CH_2– 	

Tab. III. Variations in position 3 of lysine-vasopressin

Name and chemical formula	H-CyS-Tyr-R_3-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2	R_3 = –CH_2–C_6H_4–OH	R_8 = –CH_2–CH_2–CH_2–CH_2–NH_2
Lysine-vasopressin	*Phe*	R_3 = –CH_2–C_6H_5	
Tyr^3-Lys^8-vasopressin	*Tyr*	R_3 = –CH_2–C_6H_4–OH	
Try^3-Lys^8-vasopressin	*Try*	R_3 = –CH_2– 	
Ileu^3-Lys^8-vasopressin = Lysine-vasotocin	*Ileu*	R_3 = –$CH(CH_3)$–CH_2–CH_3	
Ser^3-Lys^8-vasopressin	*Ser*	R_3 = –CH_2–OH	

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	Occurs in nature
59 ± 8	226 ± 17	58 ± 4	207 ± 14	~0.2	~0.5	~0.8	*S* Boissonnas, Guttmann, Jaquenoud, Waller, Helv. chim. Acta *39*, 1421 (1956) *P* Berde, Doepfner, Konzett, Brit. J. Pharmacol. Chemotherapy *12*, 209 (1957); Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
45 ± 7	61 ± 0.7	42 ± 1	101 ± 13	~0.3	–	~0.2	*S* Boissonnas, Guttmann, Jaquenoud, Waller, Helv. chim. Acta *39*, 1421 (1956) *P* Berde, Doepfner, Konzett, Brit. J. Pharmacol. Chemotherapy *12*, 209 (1957); Berde, Stürmer (pers. comm.)
~20	~25	~30	~60	~3	~5	~30	*S* Boissonnas, Guttmann, Jaquenoud, Waller, Helv. chim. Acta *39*, 1421 (1956); Katsoyannis, J. Amer. chem. Soc. *79*, 109 (1957)* *P* Berde, Doepfner, Konzett, Brit. J. Pharmacol. Chemotherapy *12*, 209 (1957)
0.10 ± 0.03	–	~0.03	1.5 ± 0.3	~0.01	~0.01	–	*S* Boissonnas, Guttmann, Helv. chim. Acta *43*, 190 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
0.04 ± 0.01	–	~0.1	0.10 ± 0.06	< 0.01	< 0.01	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
5 ± 0.5	–	40 ± 5	60 ± 10	270 ± 20	306 ± 13	~250	Occurs in nature
~0.01	–	~0.1	~0.2	1.6 ± 0.2	2.6 ± 0.7	0.18 ± 0.08	*S* Boissonnas, Guttmann, Helv. chim. Acta *43*, 190 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	~0.08	< 0.01	~0.07	~0.3	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
78 ± 10	–	210 ± 3	180 ± 25	130 ± 13	–	24 ± 3	*S* Boissonnas, Huguenin, Helv. chim. Acta *43*, 182 (1960)*, and pers. comm.; Kimbrough, du Vigneaud, J. biol. Chem. *236*, 778 (1961)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959); Berde, Stürmer (pers. comm.)
< 0.01	~0.04	< 0.01	~0.04	< 0.01	–	~0.02	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)

Tab. IV. Variations in position 8 of oxytocin

Name and chemical formula		$R_2 = -CH_2-C_6H_4-OH$	$R_3 = -CH(CH_3)-CH_2-CH_3$
Oxytocin	H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Leu*-Gly-NH_2		$R_8 = -CH_2-CH(CH_3)-CH_3$
Ileu^8-oxytocin	H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Ileu*-Gly-NH_2		$R_8 = -CH(CH_3)-CH_2-CH_3$
Val^8-oxytocin	H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Val*-Gly-NH_2		$R_8 = -CH(CH_3)-CH_3$
Lys^8-oxytocin = Lysine-vasotocin	H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Lys*-Gly-NH_2		$R_8 = -CH_2-CH_2-CH_2-CH_2-NH_2$
Arg^8-oxytocin = Arginine-vasotocin	H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Arg*-Gly-NH_2		$R_8 = -CH_2-CH_2-CH_2-NH-C(=NH)-NH_2$

Tab. V. Variations in position 8 of lysine-vasopressin

Name and chemical formula		$R_2 = -CH_2-C_6H_4-OH$	$R_3 = -CH_2-C_6H_5$
Lysine-vasopressin	H-CyS-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Lys*-Gly-NH_2		$R_8 = -CH_2-CH_2-CH_2-CH_2-NH_2$
Arginine-vasopressin	H-CyS-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Arg*-Gly-NH_2		$R_8 = -CH_2-CH_2-CH_2-NH-C(=NH)-NH_2$
His^8-vasopressin	H-CyS-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*His*-Gly-NH_2		$R_8 = -CH_2-C(=CH-NH)-CH$
Phe^3-oxytocin = Oxypressin = Leu^8-vasopressin	H-CyS-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-*Leu*-Gly-NH_2		$R_8 = -CH_2-CH(CH_3)-CH_3$

in this position would furnish a compound with properties intermediate between those of the vasopressins and those of Leu^8-vasopressin. However, as we have seen above, this modification decreases all activities to a level much below that which might have been expected. This example serves to emphasise how misleading it is to generalise from a study of a limited number of analogues.

Variations in Position 2. The replacement of tyrosine in oxytocin by phenylalanine, i.e. the suppression of the phenolic group, was accomplished independently by our group and by DU VIGNEAUD's team yielding *Phe*^{2}-

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	Occurs in nature
289 ± 21	563 ± 74	498 ± 37	328 ± 21	6.3 ± 0.8	–	1.1 ± 0.1	*S* JAQUENOUD, BOISSONNAS, Helv. chim. Acta *44*, 113 (1961)* *P* BERDE, KONZETT, Medicina exp. *2*, 317 (1960)
200 ± 15	380 ± 40	280 ± 17	310 ± 20	9 ± 1	–	0.8 ± 0.1	*S* JAQUENOUD, BOISSONNAS, Helv. chim. Acta *44*, 113 (1961)* *P* KONZETT, BERDE, STÜRMER (pers. comm.)
78 ± 10	–	210 ± 3	180 ± 25	130 ± 13	–	24 ± 3	*S* BOISSONNAS, HUGUENIN, Helv. chim. Acta *43*, 182 (1960)* and pers. comm.; KIMBROUGH, DU VIGNEAUD, J. biol. Chem. *236*, 778 (1961)* *P* BERDE, CERLETTI, KONZETT, Symposium on oxytocin, Montevideo (1959); BERDE, STÜRMER (pers. comm.)
~75	–	~150	~100	~125	–	–	Occurs in nature

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
5 ± 0.5	–	40 ± 5	60 ± 10	270 ± 20	306 ± 13	~250	Occurs in nature
~20	–	~60	~70	~400	~400	~400	Occurs in nature
~1.5	–	~4.6	–	~1.5	–	–	*S* KATSOYANNIS, DU VIGNEAUD, Arch. Biochem. Biophys. *78*, 555 (1958)*
~20	~25	~30	~60	~3	~5	~30	*S* BOISSONNAS, GUTTMANN, JAQUENOUD, WALLER, Helv. chim. Acta *39*, 1421 (1956); KATSOYANNIS, J. Amer. chem. Soc. *79*, 109 (1957)* *P* BERDE, DOEPFNER, KONZETT, Brit. J. Pharmacol. Chemotherapy *12*, 209 (1957).

oxytocin. It can be seen from the data in Table VI that some types of activities are more affected than others by this variation. Nevertheless, appreciable oxytocin-like effects are still in evidence. This illustrates that the hydroxyl group is not vital for the oxytocic activity. The effect of methylating the phenolic group of tyrosine was investigated by DU VIGNEAUD's group. They found that this analogue—*(O-Me)Tyr*^{2}*-oxytocin*—has an unexpectedly low activity, indeed, that it has a certain antagonistic action on vasopressin. Methylation of the amino-group of the tyrosine in position 2, which is linked to the half-cystine residue occupying position

Tab. VI. Variations in position 2 of oxytocin

Name and chemical formula		$R_3 = -\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_2-CH_3$	$R_8 = -CH_2-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_3$
Oxytocin	H-CyS-*Tyr*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		$R_2 = -CH_2-C_6H_4-OH$
Phe^2-oxytocin (= Desoxy-oxytocin)	H-CyS-*Phe*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		$R_2 = -CH_2-C_6H_5$
(N-Me)Tyr^2-oxytocin	H-CyS-(*N-Me*)*Tyr*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		$R_2 = -CH_2-C_6H_4-OH$ (–CH_3 instead of –H on the nitrogen of tyrosine)
(O-Me)Tyr^2-oxytocin = = (*p*-methoxy) Phe^2-oxytocin	H-CyS-(*O-Me*)*Tyr*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		$R_2 = -CH_2-C_6H_4-OCH_3$
Ser^2-oxytocin	H-CyS-*Ser*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		$R_2 = -CH_2-OH$

Tab. VII. Variations in position 2 of lysine-vasopressin

Name and chemical formula		$R_3 = -CH_2-C_6H_5$	$R_8 = -CH_2-CH_2-CH_2-CH_2-NH_2$
Lysine-vasopressin	H-CyS-*Tyr*-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2		$R_2 = -CH_2-C_6H_4-OH$
Phe^2-Lys^8-vasopressin	H-CyS-*Phe*-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2		$R_2 = -CH_2-C_6H_5$
His^2-Lys^8-vasopressin	H-CyS-*His*-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2		$R_2 = -CH_2-C(=NH)-CH=N-CH$

1, was accomplished by our group. This modification also gives a compound—(*N-Me*)*Tyr²-oxytocin*—with an unexpectedly low activity.

To investigate the effect of substituting an alcoholic hydroxyl group for a phenolic hydroxyl group, we replaced tyrosine by serine. The compound obtained—*Ser²-oxytocin*—is almost devoid of biological activity.

Replacement of the tyrosine in position 2 of lysine-vasopressin (Table VII) by phenylalanine yielded a compound—*Phe²-Lys⁸-vasopressin*—with interesting properties. The inherent oxytocin-like qualities of lysine-vasopressin are reduced to a very low level by this modification, but the pressor activity is still considerable, viz. one-fifth of its original value, whereas

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	Occurs in nature
32 ± 2	168 ± 12	63 ± 9	141 ± 21	~0.4	~3	~0.5	*S* Jaquenoud, Boissonnas, Helv. chim. Acta *42*, 788 (1959)*; Bodanszky, du Vigneaud, J. Amer. chem. Soc. *81*, 1258, 6072 (1959)* *P* Konzett, Berde, Brit. J. Pharmacol. Chemotherapy *14*, 333 (1959); Berde, Konzett (pers. comm.)
1.2 ± 0.4	–	0.32 ± 0.05	3.1 ± 0.7	–	–	< 0.01	*S* Huguenin, Boissonnas, Helv. chim. Acta *44*, 213 (1961)* *P* Konzett, Berde, Störmer (pers. comm.)
~5	–	~5	–	Anti (3000:1)	–	–	*S* Law, du Vigneaud, J. Amer. chem. Soc. *82*, 4579 (1960)*
< 0.01	–	< 0.01	< 0.01	< 0.01	–	< 0.01	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
5 ± 0.5	–	40 ± 5	60 ± 10	270 ± 20	306 ± 13	~250	Occurs in nature
~0.3	–	~0.15	~2.5	55 ± 7	–	20 ± 2	*S* Boissonnas, Guttmann, Helv. chim. Acta *43*, 190 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	< 0.01	< 0.01	< 0.01	~0.08	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)

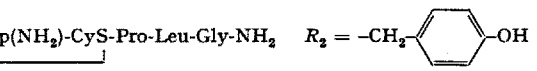
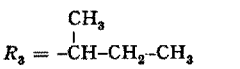
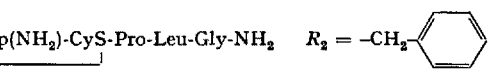
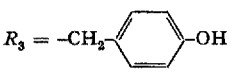
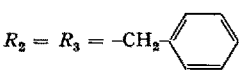
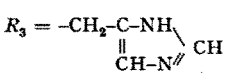
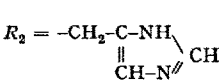
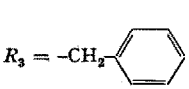
the antidiuretic potency is one-twelfth of its former value. This analogue is therefore much more selectively pressor than vasopressin. This has been confirmed in clinical experiments[17].

Introduction of histidine in position 2 gave *His²-Lys⁸-vasopressin*. As might be expected, this modification annihilates all biological activity.

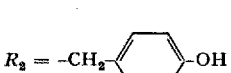
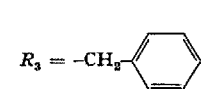
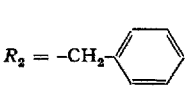
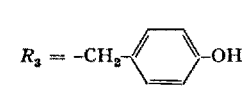
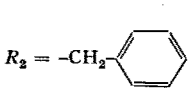
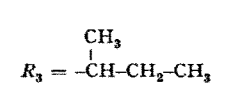
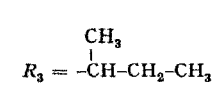
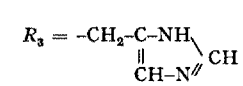
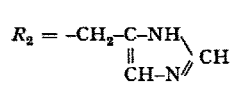
Simultaneous variations in positions 2 and 3. From the data in Tables VIII and IX, it is evident that simultaneous modifications in positions 2 and 3 generally lead to complete loss of biological activity. There are, however, two exceptions: *Phe²-Phe³-oxytocin* (= *desoxy-*

[17] U. Guhl, Schweiz. med. Wschr. *91*, 798 (1961).

Tab. VIII. Variations in positions 2 and 3 of oxytocin

Name and chemical formula		R_2 = 	R_3 = 
Oxytocin	H-CyS-*Tyr-Ileu*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		
Phe^2-Tyr^3-oxytocin	H-CyS-*Phe-Tyr*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		
Phe^2-Phe^3-oxytocin	H-CyS-*Phe-Phe*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		
Ser^2-His^3-oxytocin	H-CyS-*Ser-His*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2	R_2 = $-CH_2-OH$	
His^2-Phe^3-oxytocin	H-CyS-*His-Phe*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2		

Tab. IX. Variations in positions 2 and 3 of lysine-vasopressin

Name and chemical formula	R_2 = 	R_3 = 
H-CyS-*Tyr-Phe*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2 Lysine-vasopressin		
H-CyS-*Phe-Tyr*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2 Phe^2-Tyr^3-Lys^8-vasopressin		
H CyS-*Phe-Ileu*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2 Phe^2-$Ileu^3$-Lys^8-vasopressin = Desoxy-lysine-vasotocin		
H-CyS-*Ser-Ileu*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2 Ser^2-$Ileu^3$-Lys^8-vasopressin	R_2 = $-CH_2-OH$	
H-CyS-*Ser-His*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2 Ser^2-His^3-Lys^8-vasopressin	R_2 = $-CH_2-OH$	
H-CyS-*His-Ser*-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Lys-Gly-NH_2 His^2-Ser^3-Lys^8-vasopressin		R_3 = $-CH_2-OH$

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	Occurs in nature
< 0.01	–	< 0.01	–	< 0.01	–	~ 0.001	*S* Boissonnas, Guttmann, Helv. chim. Acta *43*, 190 (1960)* *P* Konzett, Berde (pers. comm.)
3.3 ± 0.3	–	5.4 ± 0.9	24.6 ± 9.0	~ 0.9	–	5.7 ± 0.6	*S* Boissonnas, Guttmann, Helv. chim. Acta *43*, 190 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	~ 0.03	–	–	< 0.01	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	< 0.01	–	< 0.01	< 0.01	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
5 ± 0.5	–	40 ± 5	60 ± 10	270 ± 20	306 ± 13	~ 250	Occurs in nature
< 0.01	–	< 0.01	–	0.14 ± 0.01	–	0.013 ± 0.002	*S* Boissonnas, Guttmann, Helv. chim. Acta *43*, 190 (1960)* *P* Konzett, Berde (pers. comm.)
1.0 ± 0.1	–	~ 8	12 ± 3	32 ± 6	–	1.0 ± 0.1	*S* Jaquenoud, Boissonnas (to be published) *P* Berde, Konzett, Stürmer (pers. comm).
< 0.01	–	< 0.01	~ 0.01	~ 0.1	–	~ 0.06	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	< 0.02	–	< 0.02	~ 0.04	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	–	< 0.01	–	< 0.01	–	*S* Guttmann, Boissonnas, Helv. chim. Acta *43*, 200 (1960)* *P* Berde, Cerletti, Konzett, Symposium on oxytocin, Montevideo (1959)

oxypressin) and *Phe²-Ileu³-Lys⁸-vasopressin* (= *desoxy-lysine-vasotocin*). Here again the removal of the phenolic hydroxyl of the two analogous compounds, oxypressin and lysine-vasotocin, described above, yields new substances having a lower, but still considerable activity which is rather selective. It can therefore be concluded that the removal of the hydroxyl group of the tyrosine residue lowers the biological activity but increases the selectivity of the compounds. The same is true of desoxy-oxytocin (= Phe²-oxytocin) and desoxy-lysine-vasopressin (= Phe²-lysine-vasopressin), as was shown above.

Another interesting observation in this series is that, merely by interchanging tyrosine and phenylalanine in lysine-vasopressin, a compound (*Phe²-Tyr³-Lys⁸-vasopressin*) is obtained which is almost devoid of biological activity.

Shortening of the peptide chain of oxytocin. Some years ago, a hexapeptide amide, representing the amidified ring of the oxytocin molecule deprived of its tripeptidic side-chain (Table X), was prepared by RESSLER in DU VIGNEAUD's laboratory. This lower homologue of oxytocin—*Des-(Pro⁷-Leu⁸-Gly⁹)-oxytocin*—retains about 1/150 of the activity of the natural hormone on the rat uterus and 1/400 on the rabbit mammary gland.

We have recently synthesized two other lower homologues of oxytocin from which the proline of position 7 or the glycine of position 9 was omitted. These two lower homologues (*Des-Pro⁷-oxytocin* and *Des-Gly⁹-oxytocin*) exhibit a residual activity similar to that of the cyclic hexapeptide mentioned above.

It is noteworthy that none of these three compounds affects the chicken blood pressure. In fact the two compounds with shortened side-chains actually inhibit the response to a subsequent dose of oxytocin, albeit only slightly. Shortening the side-chain of oxytocin seems to have less effect on the typical oxytocic effects on the uterus and the mammary gland than on the avian depressor activity.

Recently, in a preliminary communication, DU VIGNEAUD's group[18] announced that the omission of the amino group of the first half-cystine residue of the oxytocin molecule yields an analogue (des-amino-oxytocin) which possesses a very high avian depressor action and an appreciable effect on the rat uterus and on the mammary gland. The absence of the N-terminal amino group of the oxytocin molecule would thus appear to have no detrimental influence on the biological activities of the molecule. In our opinion, it cannot be excluded that this modification may perhaps increase the resistance of the molecule to oxytocin-inactivating enzymes.

Lengthening of the peptide chain of oxytocin. As the above-mentioned results show, the terminal amino group of oxytocin does not seem to be necessary for

[18] V. DU VIGNEAUD, G. WINESTOCK, V. V. S. MURTI, D. B. HOPE, R. D. KIMBROUGH, J. biol. Chem. *235*, PC 64 (1960).

Tab. X. Shortening of the peptide chain of oxytocin

Name and chemical formula
H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-*Pro-Leu-Gly-NH_2*
Oxytocin
H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-*NH_2*
Des-(Pro⁷-Leu⁸-Gly⁹)-oxytocin
H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-*Leu-Gly-NH_2*
Des-Pro⁷-oxytocin
H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-*Pro-Leu-NH_2*
Des-Gly⁹-oxytocin

Tab. XI. Lengthening of the peptide chain of oxytocin

Name and chemical formula
H-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2
Oxytocin
CH_3-CO-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2
N-Acetyl-oxytocin
H-Gly-CyS-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2
N-Glycyl-oxytocin
H-CyS-Tyr-*Tyr*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2
Homo-Tyr²,³-oxytocin
H-CyS-Tyr-*Ileu*-Ileu-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2
Homo-Ileu²,³-oxytocin
H-CyS-Tyr-Ileu-*Glu*-NH_2 ; Asp(NH_2)-CyS-Pro-Leu-Gly-NH_2
Isoglutamine⁴-oxytocin
H-CyS-Tyr-Ileu-Glu(NH_2)-*Asp*-NH_2 ; CyS-Pro-Leu-Gly-NH_2
Isoasparagine⁵-oxytocin

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	Occurs in nature
~3.3	–	< 0.03	~1.1	< 0.01	–	–	*S* RESSLER, Proc. Soc. exp. Biol. Med. *92*, 725 (1956)*
3.8 ± 0.9	~0.4	Anti (1000:1)	1.6 ± 0.2	~0.01	–	~0.01	*S* JAQUENOUD, BOISSONNAS, Helv. chim. Acta (in preparation)* *P* BERDE, KONZETT, STÜRMER (pers. comm.)
4.2 ± 0.7	–	Anti (10000:1)	~3	Anti (10000:1)	–	~0.02	*S* JAQUENOUD, BOISSONNAS, Helv. chim. Acta (in preparation)* *P* BERDE, KONZETT, STÜRMER (pers. comm.)

Oxytocin-like activities (in international units per mg)				Vasopressin-like activities (in international units per mg)			Bibliography
rat uterus (isolated)	cat uterus (*in situ*)	chicken blood pressure	rabbit mammary gland	rat blood pressure	cat blood pressure	rat antidiuresis	*S* synthesis *P* pharmacology * contains also pharmacological data
450 ± 30	450 ± 30	450 ± 30	450 ± 30	5 ± 1	4 ± 1	5 ± 1	Occurs in nature
1.7 ± 0.2	–	Anti (5000:1)	2.1 ± 0.2	–	–	–	*S* BOISSONNAS, PECHÈRE, GUTTMANN (pers. comm.) *P* KONZETT, BERDE (pers. comm.)
~1	–	~1 Anti (20:1)	–	~0.05	–	–	*S* DU VIGNEAUD, FITT, BODANSZKY, O'CONNELL, Proc. Soc. exp. Biol. Med. *104*, 653 (1960)*
Anti (500:1)	–	Anti (500:1)	~0.5	–	–	–	*S* GUTTMANN, JAQUENOUD, BOISSONNAS, KONZETT, BERDE, Naturwiss. *44*, 632 (1957)* *P* KONZETT, Helv. Physiol. Acta *15*, 419 (1957)
~0.1	–	~0.05	~0.2	–	< 0.01	< 0.01	*S* JAQUENOUD, BOISSONNAS (pers. comm.) *P* BERDE, CERLETTI, KONZETT, Symposium on oxytocin, Montevideo (1959)
< 0.01	–	< 0.05	–	Anti (500:1)	–	–	*S* RESSLER, DU VIGNEAUD, J. Amer. chem. Soc. *79*, 4511 (1957)* *P* RESSLER, RACHELE, Proc. Soc. exp. Biol. Med. *98*, 170 (1958)
< 0.01	–	< 0.05	–	< 0.01	–	–	*S* LUTZ, RESSLER, NETTLETON, DU VIGNEAUD, J. Amer. chem. Soc. *81*, 167 (1959)*

biological activity; it is interesting to consider the consequences of acetylating this amino group. We prepared *N-acetyl-oxytocin* (Table XI) and found it to be only about 1/250 as active as oxytocin on the rat uterus and on the rabbit mammary gland. Furthermore, instead of the high avian depressor activity exhibited by the above-mentioned des-amino-oxytocin, N-acetyl-oxytocin shows a slight antagonist effect to oxytocin in the same test. These apparently contradictory results can nevertheless be reconciled if we assume that steric effects play a preponderant rôle in the mechanism of action of oxytocin and that any additional group is detrimental to biological activity.

DU VIGNEAUD's group recently prepared *N-glycyl-oxytocin*, which differs from our N-acetyl-oxytocin only by possessing an additional terminal amino group. This compound also possesses a low oxytocic activity on the rat uterus. It has low avian depressor potency and markedly antagonises simultaneous or subsequent doses of oxytocin in the same test.

Some years ago, our group prepared a homologue of oxytocin containing two tyrosine residues instead of one (*Homo-Tyr*^{2,3}*-oxytocin*). This homologue displayed slight but definite antagonism to oxytocin on the rat uterus as well as on the chicken blood pressure. This was the first observation of an oxytocin-antagonistic effect by an oxytocin-like compound and prompted us to synthesize another homologue of oxytocin containing two isoleucine residues instead of one (*Homo-Ileu*^{2,3}*-oxytocin*). However, this compound has hardly any oxytocic or anti-oxytocic activity.

Investigating the influence of the relative positions of the amide group and of the peptide chain linked to the carboxyl group of the glutamic or aspartic residues of the oxytocin molecule, DU VIGNEAUD's team prepared *isoglutamine*^{4}*-oxytocin* and *isoasparagine*^{5}*-oxytocin*. Both are devoid of oxytocic activity. The first compound was also shown to be slightly antagonistic to the pressor effect of vasopressin.

Conclusions. This review shows that relationships which have so far emerged from a study of the chemical structures and the biological activities of the posterior pituitary hormones and their synthetic analogues are in most cases limited in application. When the first members of a series of analogues are investigated, it frequently seems to be easy to correlate biological properties and chemical peculiarities. However, when a greater number of compounds are investigated, the picture often becomes more complex. Furthermore, it is misleading to draw conclusions from only one type of biological assay, since different tests may give rather divergent quantitative results.

Valid conclusions can only be obtained by systematically comparing a fairly large number of compounds of the same chemical family in a battery of biological tests—as has been done in the present review.

Résumé. Afin de mettre en évidence l'influence de faibles modifications de structure sur les propriétés biologiques, les auteurs comparent, à l'aide d'une série de tableaux systématiques, les structures chimiques et les propriétés pharmacologiques des hormones post-hypophysaires avec celles de près de quarante de leurs analogues synthétiques, qui ont été étudiés d'une manière approfondie. Cette confrontation montre qu'il est possible de découvrir quelques relations limitées entre la spécificité biologique de ces corps et certaines particularités de leur structure.

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The Effect of Sea Water on Cytochrome Oxidase and Oxidative Phosphorylation

In the course of our work on the inhibition of cytochrome oxidase caused by a low molecular substance in the unfertilized sea urchin egg[1], it appeared important to check the influence that sea water might have on the oxidase activity. In fact, unless the eggs are thoroughly washed with some artificial solution, which did not seem to us to be advisable, it is practically impossible to avoid a contamination with sea water. As is shown in the diagram, 0.2 cm^3 of sea water added to the reaction mixture (3.0 cm^3 total) in the Warburg flask cause a 7% decrease of the oxygen consumption which reaches 30% when 0.4 cm^3 of sea water are added. On the other hand, the influence on oxidative phosphorylation is very strong indeed. In fact, as is shown in the Figure, a 30% decrease of the P/O ratio is caused by as little as 0.1 cm^3 of sea water and 0.3 cm^3 depress the ratio by 80%. A series of experiments with artificial sea water from which individual ions were removed and then with each ion separately have proved that Ca^{++} is responsible for the uncoupling effect. This is not surprising in view of the well known effect of Ca^{++} as an uncoupler of oxidative phosphorylation[2].

The possibility that the effect of sea water might have been an apparent one, i.e. due to an inhibition of hexo-

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