It is evident that exposure to alien male urine causes the failure of pseudopregnancy in 89% of females, which is very close to the % of pregnancy failure obtained in newly-mated females by exposure to the proximity[1,2] or the urine[3,4] of alien males. In the female mouse, even if mating does not lead to pregnancy, it induces pseudopregnancy, so that oestrus is held in abeyance for about 2 weeks. The failure of mating to break the 5-day rhythm is rare. Hence it is obvious that the pheromones from males cause the failure of luteotrophic activity and stimulate the gonadotrophic activity of the adenohypophysis, resulting in oestrus and ovulation[6-8]. Incidentally this also provides a further example of the accelerating influence of the male on oestrus which has been described for several species of mammals, including the unmated female mouse[9].

Zusammenfassung. Mäuseweibchen, die durch Paarung mit vasektomierten Männchen scheinträchtig gemacht worden waren, wurden dem Urin von Männchen eines anderen Stammes ausgesetzt. Dadurch wurde die Pseudogravidität unterbrochen. Diese Beobachtung gleicht der Unterbrechung der Trächtigkeit frisch begatteter Weibchen durch den Urin fremdstämmiger Männchen und bestätigt auch die Beschleunigung des Eintritts des Östrus durch die Gegenwart der Männchen.

C. J. Dominic[10]

Physiological Laboratory, University of Cambridge (England), April 11, 1966.

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[10] Present address: Department of Zoology, Banaras Hindu University, Varanasi 5, India.

Mutual Replaceability of Amide and Ester Groups in Biologically Active Peptides and Depsipeptides

As is well known, the ester group not only models the amide group in its spatial characteristics, but is quite similar to it in electron density distribution. One could therefore expect that analogues of naturally occurring biologically active peptides and depsipeptides, wherein the ester group is replaced by the amide group and vice versa, would be very similar to the original compounds in both steric structure, i.e. topologically, and the electronic nature of the functional centres. Such topochemically similar compounds should therefore possess quite similar electronic and steric complementarity to the same receptor and, as a result, the analogues should also manifest high biological activity. These premises were the starting point of our studies on the structure activity relations of peptide and depsipeptide compounds.

The first object of these studies was the depsipeptide antibiotic valinomycin (I, Table I). Analogues of this antibiotic (II–IV) in which one of the hydroxy acid residues was replaced by an amino acid of the same type and configuration were found to possess a biological activity, in a number of cases equal to or exceeding the activity of the natural compound.

On the other hand, we replaced the amide groups by ester groups in the biologically active peptides glutathione, ophthalmic acid and bradykinin[1-5]. The resultant depsipeptide analogues, namely Glyc^3-glutathione, Glyc^3-ophthalmic acid, Glyc^4-bradykinin, Glyc^6-bradykinin and Glyc^{4,6}-bradykinin, partly or wholly retain the activity of their natural counterparts. In particular, with respect to the glyoxalase system (glyoxalase I) Glyc^3-glutathione exhibits 75% of the activity of glutathione, while, with respect to the formaldehyde/NAD-oxidoreduction system, its activity equals that of glutathione. At the same time ophthalmic acid in the glyoxalase system depresses the activity of glutathione by 60% and Glyc^3-ophthalmic acid by 35%. Moreover, ophthalmic acid does not depress the activity of glutathione in the formaldehyde/NAD-oxidoreduction system, whereas its depsipeptide analogue does so to the extent of 30%. Data on the biological activity of the depsipeptide analogues of bradykinin are summarized in Table III.

Table I

No.	Compound[a]
(I)	(D-Val-L-Lac-L-Val-D-HyIv)$_3$ (Valinomycin)
(II)	D-Val-L-*Ala*-L-Val-D-HyIv-(D-Val-L-Lac-L-Val-D-HyIv)$_2$
(III)	D-Val-L-Lac-L-Val-D-*Val*-(D-Val-L-Lac-L-Val-D-HyIv)$_2$
(IV)	D-Val-L-*Val*-L-Val-D-HyIv-(D-Val-L-Lac-L-Val-D-HyIv)$_2$

[a] The synthesis of compounds (II–IV) will be described in another paper.

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Table II

Compound	Minimal growth inhibiting concentration (γ/ml)									
	Staph. aureus 209 *P*	*Staph. aureus* UV-3	*Sarcina lutea*	*B. micoides*	*B. subtilis*	*E. coli*	*Micob. phlei*	*Cand. albicans*	*Sacch. cereviseae*	*Sclerot. libert.*
(I)	> 50	0.8	1.5	> 50	> 50	> 50	0.3	0.8	0.8	0.8
(II)	4.5	1	2	> 50	> 50	> 50	0.4	4.5	4	2
(III)	> 25	1	1.5	> 25	> 50	> 25	1	3	3	3
(IV)	> 50	0.7	1.5	> 50	> 50	> 50	0.4	12[a]	12[a]	2

[a] Depression.

The mutual replaceability of amide and ester groups in biologically active peptides and depsipeptides is especially striking[6] in enantio-enniatin B, the antipode of enniatin B. From a formal point of view, one should not expect activity on the part of this antipode, just as there is no basis to expect it in the antipodes of oxytocin[7], bradykinin[8] or angiotensin[9]. However, owing to the peculiar circumstance that enantio-enniatin B differs from enniatin B only by having the N-methylamide groups replaced by ester groups and vice versa, these two topochemically similar molecules could therefore have closely approaching activities. Indeed, enantio-enniatin B turned out to be of identical activity with enniatin B over the entire range of the antimicrobial spectrum[6].

Discovery of the exchangeability of amide and ester groups has opened the way for a rational approach to synthesis of natural peptide and depsipeptide analogues that are of theoretical and applied interest. On the one hand, such analogues, retaining the topochemical specificities of the natural compound, may greatly facilitate not only elucidation of the conformational states of peptide and protein systems, but of the mode of their interaction with the corresponding receptor in terms of the forces (dipole-dipole interaction, hydrophobic and hydrophylic interactions, hydrogen bonding etc.) acting in a given biochemical process. On the other hand, it is well known that the main obstacle to the practical use of certain polypeptides (hypertensin, bradykinin etc.) is their too brief period of action, due to rapid inactivation by the corresponding proteolytic enzymes of the organism. In such cases, replacement of the amide groups that undergo enzymatic attack, or are situated in direct proximity to the fissionable centre, by ester groups may lead to longer-acting substances, not so prone to undergo proteolysis. The results we have obtained lately confirm this assumption. Indeed, it turned out that the depsipeptide analogue of Gly^6-bradykinin[10], wherein an L-phenylalanine residue is replaced by the corresponding hydroxy acid (PhLac^8-Gly^6-bradykinin), not only lowers the blood pressure in rats to a greater extent than the hormone itself (Table III) but has a more prolonged effect. This is in accord with the data on the relative rate of human blood serum carboxypeptidase N induced splitting of bradykinin and PhLac^8-Gly^6-bradykinin. It is to be noted that in this case the amide-ester exchange in the bradykinin molecule did not involve the amide group that was the first to succumb to the action of the proteolytic enzymes (Phe-Arg), but the neighbouring amide group (Pro-Phe), and nevertheless the effect proved to be quite marked. Probably still more dramatic results would follow on replacement of the amide group directly undergoing enzymatic attack; and this is to be the subject of a forthcoming investigation[13].

Выводы. Исходя из топохимических предпосылок, показана возможность взаимной замены амидных и сложноэфирных связей в биологически активных пептидах и депсипептидах.

M. M. SHEMYAKIN, L. A. SHCHUKINA,
E. I. VINOGRADOVA, G. A. RAVDEL,
and YU. A. OVCHINNIKOV

Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow (USSR),
March 29, 1966.

Table III

Compound	Threshold doses		
	Isolated rat uterus contraction (g/ml)	Increase in rabbit skin capillary permeability (g/ml)	Decrease in rat blood pressure (g/kg)
Bradykinin	$1 \cdot 10^{-11}$	$1 \cdot 10^{-9}$	$4 \cdot 10^{-7}$
Glyc^4-bradykinin	$2 \cdot 10^{-6}$	$1.5 \cdot 10^{-5}$	$0.55 \cdot 10^{-3}$
Glyc^6-bradykinin	$5 \cdot 10^{-11}$	$3.5 \cdot 10^{-9}$	$3.4 \cdot 10^{-6}$
Glyc^{4,6}-bradykinin	$3.2 \cdot 10^{-5}$	$2.5 \cdot 10^{-5}$	$2 \cdot 10^{-3}$
PhLac^8-Gly^6-bradykinin[a]	$2 \cdot 10^{-11}$	$2.5 \cdot 10^{-8}$	$1 \cdot 10^{-7}$

[a] The synthesis of this compound will be described in another paper.

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