animals. This possibly represents a retardation of biochemical brain development of the underfed animals. It is interesting that significant differences in 5-HTPD activity appeared only on day 21. At this age the 5-HTPD activity in the malnourished brain is equivalent to the activity present in the controls in the 2nd week, representing also an immature biochemical feature. LOIZOU[10,11] reported that 5-HT content of neurone bodies and terminals in the brain of the rat attained adult values by the 3rd week. It is possible that in this period the metabolic changes induced by early malnutrition are more striking. SHOEMAKER et al.[12], studying the offspring of rats fed a low protein diet during pregnancy, found a low content of norepinephrine and dopamine in the brain on the 24th postnatal day. Our results on 5-HTPD activity also suggest a modification of the brain biogenic amines metabolism in the 3rd week of postnatal life in early malnourished rats. EAYRS et al.[13], found impoverishment of the neuropil network which normally matures from day 20th to day 25th, in the brain of early malnourished rats. The possible metabolic changes in this period could reflect alterations of synaptic maturation and function.

The concentration of the brain 5-HT was not significantly different between the 2 experimental groups at the various ages studied, although a tendency to lower values in the malnourished group on day 21 was noticed (Table II). It is possible that normal concentrations of the brain 5-HT could be supplied even by a lower activity of 5-HTPD, although low utilization or a modification in the catabolism of the amine should be also considered. On the other hand, considering the brain as a whole, real differences in 5-HT levels in the malnourished could be masked. In more discrete and specific regions of the brain, in which serotonin concentrations are normally different, more striking changes in its metabolism might be found in the developping malnourished rat. Further studies concerning 5-HTPD activity, 5-HT, and noradrenaline levels in different brain regions in normal and early underfed rats are planned.

Résumé. Dans le cerveau de rats souffrant de malnutrition depuis la naissance, on a déterminé l'activité de l'enzyme 5-hydroxytryptophane-déscarboxylase pendant le premier mois du développement. Une différence significative a été observée le 21ème jour par rapport aux contrôles. En même temps, la concentration endogène de 5-HT ne présenta qu'une tendence à diminuer.

R. J. HERNANDEZ[14]

Department of Biochemistry, Faculty of Medicine, University of Mexico, Mexico City (Mexico), 2 July 1973.

[10] L. A. LOIZOU and P. SALT, Brain Res. *20*, 467 (1970).
[11] L. LOIZOU, J. Anat. *104*, 588 (1969).
[12] W. J. SHOEMAKER and R. J. WURTMAN, Science *171*, 1017 (1971).
[13] J. EAYRS and G. HORN, Ant. Rec. *121*, 53 (1955).
[14] Present address: Institute of Physiology, Dept. of Developmental Physiology, Czechoslovak Academy of Sciences, Budějovická 1083, Praha 4, KRČ, (Czechoslovakia.)

Gramicidin S Analogs Containing N-Methylleucine in Place of Leucine

For the conformation of gramicidin S (GS), several models have been proposed. A possible model, which was first suggested by HODGKIN and OUGHTON[1], is the intramolecular antiparallel β-form with 4 hydrogen bondings between the valyl and the leucyl residues[2-4]. In order to investigate to what extent these hydrogen bondings will stabilize the conformation, we synthesized 2 analogs of GS, lacking 1 or 2 of the 4 intramolecular hydrogen bondings.

Material and method[5]. Boc-Orn(Z)-OH was coupled with H-MeLeu-OMe by the use of the water-soluble carbodiimide[6] to yield Boc-Orn(Z)-MeLeu-OMe (I) in 50% yield. Saponification of I gave the corresponding acid (II) as fine crystals. Boc-Orn(Z)-MeLeu-D-Phe-Pro-OEt (III) was obtained from II and H-D-Phe-Pro-OEt in a nearly quantitative yield using dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole[7]. After removal of the Boc group of III, the resulting tetrapeptide ester was coupled with Boc-Val-ONSu to yield Boc-Val-Orn(Z)-MeLeu-D-Phe-Pro-OEt (IV), in 80% yield, which was converted to the corresponding acid (V) by saponification and subsequently to H-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (VI) by the action of hydrogen chloride in ethyl acetate. Condensation of the azide derived from Boc-Val-Orn(Z)-Leu-D-Phe-Pro-$NHNH_2$[8] with VI gave Boc-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (VII) in 53% yield. VII was then transformed to the Boc-decapeptide N-hydroxysuccinimide ester (VIII) by the reaction of N-hydroxysuccinimide and the water-soluble carbodiimide. After removal of the Boc group of VIII with trifluoroacetic acid, the decapeptide ester

Antibacterial activity of the compounds (minimum inhibitory concentration, μg/ml)

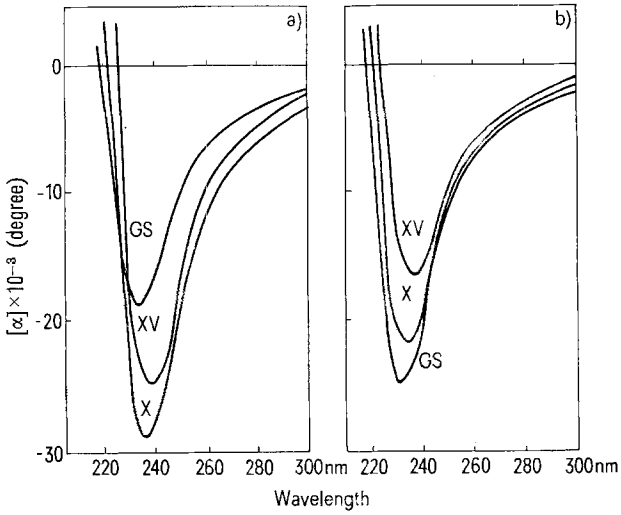
Strain	Gramicidin S	[MeLeu^3]-Gramicidin S (X)	[Di-MeLeu$^{3,3'}$]-Gramicidin S (XV)
Staphylococcus aureus	6.25	6.25	6.25
Bacillus subtilis	3.12	3.12	3.12
Escherichia coli	100	50	50
Shigella flexneri	100	100	100
Candida albicans	50	50	25

[1] D. C. HODGKIN and B. M. OUGHTON, Biochem. J. *65*, 752 (1957).
[2] R. SCHWYZER, Chimia *12*, 53 (1958). – R. SCHWYZER and U. LUDESHER, Biochemistry *7*, 2519 (1968).
[3] A. STERN, W. A. GIBBONS and L. C. CRAIG, Proc. natn. Acad. Sci. *61*, 734 (1968).
[4] M. OHNISHI and D. W. URRY, Biochem. biophys. Res. Comm. *36*, 194 (1969).
[5] The abbreviations recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [J. biol. Chem. *247*, 977 (1972)]. have been used throughout. Amino acid symbols except D-Phe denote the L-configuration.
[6] 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide.
[7] W. KÖNIG and R. GEIGER, Chem. Ber. *163*, 788 (1970).
[8] S. MATSUURA, M. WAKI, T. KATO and N. IZUMIYA, Bull. chem. Soc., Japan *46*, 977 (1973).

trifluoroacetate obtained was cyclized in pyridine. Purification through ion exchange columns followed by fractionation on silica gel gave *cyclo* (-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-) (IX) in 50% yield, mp 212–214°. Hydrogenolysis of IX afforded [MeLeu^3]-GS·2HCl·2H_2O (X), mp 237–239°, $[\alpha]^{25}$–272° (EtOH).

Boc-Val-Orn(Z)-MeLeu-D-Phe-Pro-$NHNH_2$ (XI) was prepared quantitatively by treatment of IV with hydrazine hydrate. Condensation of the azide derived from XI with VI gave Boc-Val-Orn(Z)-MeLeu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (XII), in 30% yield, which was converted to an amorphous Boc-decapeptide N-hydroxysuccinimide ester (XIII). After removal of the Boc group of XIII, the resulting decapeptide ester was treated in pyridine to yield *cyclo*(-Val-Orn(Z)-MeLeu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-) (XIV) in 67.5% yield, mp 105–115°. The hydrogenation of XIV yielded [Di-MeLeu$^{3,3'}$]-GS·2HCl·3H_2O (XV), mp 233–235°, $[\alpha]^{25}$-236° (EtOH).

Results and discussion. In the antibacterial activities against microorganisms, both of the analogs (X and XV) were almost identical with GS in potency (Table). This indicated that the hydrogens on the NH of the leucine residues are not necessary for exhibiting the biological activity.



ORD of [MeLeu^3]-gramicidin S (X), [Di-MeLeu$^{3,3'}$]-gramicidin S (XV), and gramicidin S (GS). Solvent a) ethanol and b) 8 *M* urea.

In ORD studies, the curves of X and XV determined in ethanol are shown in Figure a along with that of GS. The shapes of the 3 ORD curves are very similar to each other. In 8 *M* aqueous urea, the positions of the troughs of the 2 analogs (X and XV) remained unchanged, as well as GS (Figure b). In both solvents, the dispersions of the 3 molecules are different in their minimum rotations, and only slightly in the positions of troughs, but the shape of curves are very similar even in 8 *M* urea, suggesting that all these 3 molecules have very similar and stable conformations. These results indicate that the 2 of the 4 hydrogen bondings in the β-type structure of GS proposed by several authors would not be necessary for stabilizing the conformation. Furthermore, another model, in which NH of the leucine residues does not participate in intramolecular hydrogen bondings, should be put forward. In this respect, as is mentioned by BALASUBRAMANIAN[9], the mixed α,β-structure of HODGKIN and OUGHTON[1] and the SCHERAGA's[10] model may not be disregarded. Further studies on this are in progress[11].

Zusammenfassung. Nach Methoden der konventionellen Peptidsynthese wurden zwei Gramicidin S Analoga synthetisiert, in denen Leucin durch N-Methylleucin ersetzt ist. Beim antibakteriellen Test sind diese beiden Analoga praktisch gleich wirksam wie Gramicidin S und auch die Konformation dieser zwei Analoga ist derjenigen von Gramidicin S ähnlich.

H. SUGANO[12], H. ABE[13], M. MIYOSHI[12], T. KATO[13], and N. IZUMIYA[13]

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., Kashima-cho, Higashiyodogawa-ku, Osaka (Japan); and Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka (Japan), 29 June 1973.

[9] D. BALASUBRAMANIAN, J. Am. chem. Soc. *89*, 5445 (1967).
[10] G. VANDERKOOI, S. J. LEACH, G. NÉMETHY, R. SCOTT and H. A. SCHERAGA, Biochemistry *5*, 2991 (1966).
[11] The authors wish to express their thanks to Dr. I. CHIBATA of Tanabe Seiyaku Co., Ltd. for his encouragement in the course of this study. Thanks are also due to Associate Professor Y. MUKOHATA of Osaka University for help in ORD measurements, and to Dr. K. ENOMOTO of Tanabe Seiyaku Co., Ltd. for the biological assay.
[12] Research Laboratory of Applied Biochemistry, Tanabe Seiyaku. Co., Ltd., Kashima-cho, Higashiyodogawa-ku, Osaka (Japan).
[13] Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka (Japan).

Réversibilité de la réponse du potentiel de membrane chez *Nitella flexilis* L. à des variations de pH

Les propriétés physiologiques des cellules jeunes de Charophyceae varient avec la saison, même lorsque ces cellules sont cultivées au laboratoire en conditions normalisées[1–3]. Nous avons observé pour un lot de cellules de *Nitella* un comportement électrophysiologique différent de celui noté les mois précédents. Il s'agit de la réponse du potentiel de membrane à un changement «aller-retour» du pH de la solution extérieure. Jusqu'à présent cette réponse n'était pas réversible du moins pendant les deux premières heures qui suivaient le rétablissement de la solution extérieure initiale[4]. A partir de fin avril, le potentiel de membrane a répondu d'une manière plus ou moins réversible suivant le pH testé. Ce comportement nous a semblé assez révélateur des mécanismes de sélectivité de la membrane de *Nitella* aux ions.

Matériel et méthodes. Les essais ont porté sur les cellules internodales de l'algue d'eau douce *Nitella flexilis* L. cultivées au laboratoire depuis 10 mois (milieu II de FORSBERG[5], photopériode 18 h). Les cellules utilisées sont prétraitées pendant 15 h dans une solution de

[1] H. BRECKHEIMER-BEYRICH, Ber. dt. bot. Ges. *67*, 87 (1954).
[2] E. A. C. MACROBBIE, Aust. J. biol. Sci. *19*, 371 (1966).
[3] F. A. SMITH, J. exp. Bot. *19*, 442 (1968).
[4] C. GILLET and J. LEFEBVRE, *Ion Transport in Plants* (Ed. W. P. ANDERSON, Academic Press Inc., London 1973), p. 101.