surprisingly low, especially if the possibility that the zooxanthellae supply the coral host with food materials is considered. But if this were indeed the case, then the rate of transfer of labeled products from algae to coral should have been far greater, and a much higher level of tissue radioactivity than was actually found would have been expected. The activity observed in the tissues of our normal corals, which were kept in the light for 50 hours at 27°C, was much lower than that in the anemone section shown in Fig. 3 of Muscatine and Hand's paper (*1*, p. 1262), which came from a specimen run for only 18 hours at 14°C. It should be pointed out that the specific activities, tissue-section thickness, and exposure times of Muscatine's experiments were roughly the same as ours.

Under the conditions of our experiments, the results suggest that there is some movement of labeled photosynthetic products from the zooxanthellae to the coral, but that this occurs at a very low level. Work is now in progress to determine the quantitative relations of this process. It appears probable that the amount transferred from the algae to the animal host can at best satisfy only a very small proportion of the coral's total nutritional requirement. This preliminary conclusion would be in agreement with the observations of Yonge *et al.* (*5*), who demonstrated that reef corals starved of animal food, but still containing zooxanthellae, could not use their algal commensals as a substitute source of food.

If the corals cannot utilize the zooxanthellae as a source of food, what then is the role of the zooxanthellae in the bioeconomy of the coral host, particularly in relation to the algal products which do reach the coral tissues and are incorporated there in small amounts? Elsewhere we have shown that the zooxanthellae exert an enormous potentiating influence on the calcium deposition rate of those hermatypic corals we have tested (*8*). A possible mechanism for this has been suggested, and evidence has been presented that the zooxanthellae may have a general stimulating action on the coral host's metabolism, possibly mediated through vitamin or hormone-like trace factors which are secreted in small amounts by the algae but which by themselves do not contribute significantly to the nutrition of the coral (*7*, *11*).

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Antibacterial Activity of Acyclic Decapeptide Analogs of Gramicidin S

Abstract. Three acyclic decapeptide analogs of gramicidin S, although found to possess antibacterial activity, apparently have modes of action which differ from that of the naturally occurring cyclic antibiotic. In contrast to the immediate action of gramicidin S, one of the decapeptides produced complete bacteriostasis only after several cell divisions had occurred. Furthermore, mixtures of gramicidin S with either of two of the acyclic peptides were synergistic. Some implications of these findings are discussed.

In an earlier communication (*1*), the antibacterial activity of a decapeptide analog of gramicidin S was described. The compound (Fig. 1, decapeptide I) (*2*), possessed all the amino acids of the naturally occurring antibiotic in their proper sequence but lacked the cyclic structure. It was found to be approximately 1/10 as active as gramicidin S against *Escherichia coli* and 1/40 as active against *Staphylococcus aureus*.

The synthesis of two additional decapeptide analogs has recently been completed (*3*). Their structures and that of gramicidin S and decapeptide I are shown in Fig. 1. In one of them (decapeptide II), D-tyrosine residues replace the D-phenylalanine residues of the natural product. In the other (decapeptide III), L-lysine residues replace L-ornithine residues. All the synthetic analogs are acyclic.

The antibacterial activities of gramicidin S and the synthetic analogs against a strain of *E. coli* B are shown in the first four rows of Table 1. The conditions of the assay are described in the legend. Neither decapeptide II nor decapeptide III is as active as decapeptide I, which, in the earlier experiments (*1*), was shown to be about 1/10 as active as gramicidin S. Apparently both the L-ornithine and D-phenylalanine residues contribute to the activity of acyclic decapeptide I. This finding would appear to conflict with that of Schwyzer and Sieber (*4*), who reported that the *cyclic* lysine analog of gramicidin S (that is, the cyclic analog of decapeptide III) was as active as gramicidin S. We now have evidence, however, that the mode of action of the acyclic analogs differs from that of gramicidin S, a possibility which was mentioned but not considered seriously in our earlier paper (*1*).

First of all, it was observed that decapeptide I and gramicidin S differ in the manner in which they inhibit *E. coli* B. Gramicidin S, at all effective concentrations, produces immediate bacteriostasis; decapeptide I, on the other hand, at concentrations between 12 and 25 μg/ml, inhibits growth only after the organism has undergone several cell divisions.

Second, it was possible to show that mixtures of gramicidin S with either decapeptide I or decapeptide III exhibit a synergistic effect which is lacking when the two decapeptides are combined. The experimental data are shown in the last three rows of Table 1.

We may infer, therefore, that the mode of action of gramicidin S differs from that of either decapeptide and that presumably it is dependent upon the unique cyclic structure of the antibiotic.

The original purpose of this program (*5*) was to determine the influence of the cyclic structure of gramicidin S upon its activity. The finding that deca-

Table 1. Minimal concentration of peptides required to inhibit *E. coli* B (24 hours at 37°C). Medium: 1 liter contains 7 gm of K_2HPO_4, 3 gm of KH_2PO_4, 0.5 gm of sodium citrate, 0.1 gm of $MgSO_4 \cdot 7\ H_2O$, 1 gm of $(NH_4)_2SO_4$, and 2 gm of glucose (*p*H adjusted to 7.0). Inoculum: 300,000 organisms per milliliter (stationary phase).

Compound	Concn. (μg/ml)
Gramicidin S	1.85
Decapeptide I	15
Decapeptide II	107
Decapeptide III	66.7
Gramicidin S + I	0.5 + 3.8
Gramicidin S + III	0.5 + 12.8
I + III	(7.6 + 33)*

* Only slight inhibition occurred with this combination, as determined by nephelometric measurements during the first 10 hours of the run.

Gramicidin S

Val-Orn-Leu-Phe-Pro-Val-Orn-Leu-Phe-Pro
(L-L-L-D-L)$_2$

Decapeptide I

H·Val-Orn-Leu-Phe-Pro-Val-Orn-Leu-
Phe-Pro·OH
(L-L-L-D-L)$_2$

Decapeptide II

H·Val-Orn-Leu-Tyr-Pro-Val-Orn-Leu-
Tyr-Pro·OH
(L-L-L-D-L)$_2$

Decapeptide III

H·Val-Lys-Leu-Phe-Pro-Val-Lys-Leu-
Phe-Pro·OH
(L-L-L-D-L)$_2$

Fig. 1. Formulas for gramicidin S and synthetic decapeptide analogs.

peptide I possessed antibacterial activity had led to the conclusion that the cyclic structure was not essential, although it served to enhance the antibacterial properties of the antibiotic (*1*). It has now been shown that, despite marked similarities in the chemical structure of these compounds, the mode of action of the acyclic peptides is different from that of the naturally occurring antibiotic, and hence the earlier conclusion was not valid. Another example of an instance where similarity in chemical structure was not paralleled in biological function is described by Sorm and his colleagues (*6*), who reported that D- and L-cycloserine do not have the same mode of action and that, indeed, the racemic mixture was more active than either isomer.

The experimental approach employed in this study—namely, the synthesis and bioassay of analog of naturally occurring substances for the purpose of elucidating the relationship of structure to biological activity—requires that the analogs and the naturally occurring substance have the same mode of action. Where this is not definitely established, the experimental data, as we have found, can lead to erroneous conclusions. The interesting study of Katchalski *et al.* (*7*) of the antibacterial activities of polyamino acids containing leucine, valine, ornithine, and D- and L-phenylalanine may be another case in point. These authors found that a number of the polymers exhibited considerable antibacterial activity, and on the basis of their results they drew inferences about the relationship of structure to the activity of gramicidin S. That gramicidin S and the polymers have the same mode of action certainly is open to question.

The foregoing comments apply, as well, to any study which attempts to use synthetic analogs as a means of analyzing structure-activity relationships in biologically active materials and are, perhaps, especially pertinent when the activities of the analogs are found to be of a low order.

The manner in which decapeptide I acts upon *Escherichia coli* deserves some comment. It was reported by Rose and Fox (*8*) that, in the presence of sulfonamides, *E. coli* are able to undergo a number of cell divisions before growth is inhibited. They suggested that the sulfonamide prevented the synthesis of a growth factor (now known to be folic acid). The organism was therefore forced to distribute its original supply to its progeny, and, after a certain number of divisions, the quantity of the growth factor became insufficient to permit further multiplication. An extrapolation of this explanation to our system would lead to the inference that *E. coli* requires an essential substance (cofactor ?) whose synthesis or activity can be inhibited by decapeptide I, perhaps competitively.

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Function of the Rectal Gland in the Spiny Dogfish

Abstract. The rectal gland of the spiny dogfish, *Squalus acanthias,* secretes a fluid which is essentially a sodium chloride solution with a concentration about twice that of the plasma and greater than that of sea water. Observed volumes of flow are sufficiently large to make it clear that the rectal gland can remove from the blood relatively large amounts of sodium chloride, and presumably this is its function.

The conspicuous rectal or digitiform gland (appendix digitiformis, processus digitiformis), lying in the dorsal mesentery and opening by a duct into the intestine behind the spiral valve, has been observed by thousands of students in the dissection of various laboratory elasmobranch fishes. While anatomical descriptions are available (*1*), and some chemical tests have been applied (*1, 2*), the function of the gland is obscure (*3*).

The secretion of the gland and data on the rate of flow were secured as follows. The body wall and intestine immediately anterior to the pelvic girdle were opened in the ventral mid-line. The tip of several hundred centimeters of polyethylene catheter tubing was bent to fit the angle at which the duct enters the intestine and was pushed through the anus into the intestine. The tip was inserted into the duct and secured by two ligatures with additional stitches holding the tubing to the intestinal wall and the ventral tail skin behind the anus. It was determined by the injection of colored fluid that this was a leakproof arrangement. The intestine and body wall were sutured, and the fish was placed unrestrained in a tank of running sea water. The free end of the tubing was secured to a graduated tube placed below the level of the tank. The tubing between its ends was free to move with the fish. This arrangement necessitated that the gland develop and maintain a secretion pressure of about 31 cm-H_2O in order to collect progressively the secreted fluid. In retrospect, we see that an arrangement which does not require the maintenance of this hydrostatic pressure could have been devised.

The chemical composition (*4, 5*) of the collected fluid is given in Table 1. The fluid is colorless, nearly neutral, containing relatively small amounts of urea, magnesium, calcium, potassium, bicarbonate, and sulfate (*5*), but containing sodium chloride at about twice the plasma concentration and at a concentration higher than that of the external sea water. The data on osmolarity indicate that probably no other substance was present in high concentration. The fluid is watery and certainly contains little mucus, as has been suggested (*6*).

The above chemical data point to the possibility that the rectal gland is another "salt gland" concerned with the removal of sodium chloride from the blood. For this to be true, a volume of fluid must be formed sufficient to have more than a negligible effect on plasma salt.

Some flow from the rectal gland was secured from each of nine dogfish tested. The flow in two fish was dramatic. Fish 3 gave a continuous flow of 0.85 ml/kg per hour for a first 24-hour period and 0.72 ml/kg per hour for a succeeding 24-hour period. Urine flow for these 48 hours averaged 0.82 ml/kg hr. Fish 8 gave a flow of 1.3 ml/kg hr for a 12-hour period with a