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.

BERNARD F. ERLANGER
LOUISE GOODE
Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York

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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 leak-proof 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

single hour flow of 1.9. The urine flow for this 12-hour period averaged 0.81 ml/kg hr.

Flows in other fish were lower and more spasmodic. Fish 1 averaged 0.051 ml/kg hr for a 24-hour period, but the flow rose to 0.54 during a subsequent 90-minute period. Fish 2 averaged 0.32 ml/kg hr for a 5-hour period, and fish 9 averaged 0.22 ml/kg hr for a 12-hour period. Fishes 4 and 5 averaged 0.012 ml/kg hr for 5 and 24 hours, respectively. Fishes 2, 4, and 5 had periods of an hour to hours when no flow was observed. In short, the observed flows ranged from 0 to 1.9 ml/kg hr. The over-all tendency was toward a continuous, variable flow rather than an alternation of no flow with bursts of high flow.

The above rates of flow should be regarded as preliminary parameters. While in fish 3 flow began immediately after catheterization, there was in other fish a delay up to 3 hours. In two fish not included in Table 1, fluid appeared in the catheter but stopped when it had to climb uphill. In one fish, flow stopped when a blood sample was withdrawn. Since these fish were caught by hook and line, which results in blood loss from insignificant to severe, it is not clear whether or not the low flows and the low secretion pressures observed were a natural state of affairs or were the result of capture and the experimental conditions. The data do show unmistakably that the rectal gland can sustain a high rate of flow for days with a sodium chloride concentration sufficient to remove relatively large amounts of sodium chloride from the blood.

The data secured are too meager for assessing the total water-electrolyte economy of the dogfish. The data in Table 1 agree with those of Smith (*7*) that the blood is hypertonic to sea water and the urine hypotonic. Our observations over many years agree with Smith's that the dogfish is not a continuous drinker of sea water as are marine teleosts. Laboratory fish may be engorged with sea water and survive, so that, as Smith suggests, there must be a mechanism for handling ingested sea water. Smith has postulated a normal osmotic uptake of water in the absence of ingested sea water. The problem remains as to how much water and how much salt in what concentrations are taken up by the dogfish. The net water loss can not be less than the combined rectal gland-urine loss, although our observations do not conclusively prove that all the rectal gland secretion is lost from the anus. It would seem, however, to be ridiculous for a gland to concentrate sodium chloride only to reabsorb it back into the blood in an immediately adjacent region. Similarly, the minimum net loss of electrolyte is the combined rectal gland-urine loss.

Table 1. Comparison of the composition of fluid from the rectal gland, plasma, and urine.

Fish (No.)	Wt. (kg)	Fluid	*p*H	Osmolarity (mosmole)	Concn. (mmole/liter)						
					Na	K	Cl	Ca	Mg	Urea	CO_2
1	4.3	Rectal gland	7.0	1011	522	5.6	510		>1		
		Plasma	7.55	1011							
2	3.1	Rectal gland	6.8	1018	535	6.8	549	1	>1	20	
		Plasma		1018	320	7.0	250			349	
3	4.7	Rectal gland	6.8	1036	580	8.4	562	1	>1	11	2.8
		Plasma		1036	300	4.4	247			347	
		Urine	*	754	352	2	170				
4	5.0	Rectal gland	6.9	1020	542		552			14	
		Plasma		1020							
5	4.8	Rectal gland		1001	502		490			28	
		Plasma		1001	254		252			356	
		Urine		806	339		286	10	50		
8	6.4	Rectal gland	6.7	1020	560	7.5	549	>1	>1	13	
		Plasma		1020	283		242	2.6	3.7	352	
		Urine		780	327		174	4	25		
9	3.1	Rectal gland					521	>1	>1		
		Plasma					239				
		Urine					182				
Average of		Rectal gland		1018	540	7.1	533	>1	>1	14.5	
		Plasma		1018	286		246			351	
		Urine		780	337		203				
		Sea water		925–935	440	9.1	492–500	10	51		

*W. Smith has shown that the *p*H of dogfish urine is constant at 5.75 (*8*).

In fish 8, which had an active rectal gland and a good urine flow, the combined loss of sodium was about 460 mmole/liter of a composite fluid composed of rectal gland fluid and urine, corrected for the rate of flow for each. The loss of chloride was about 405 mmole/liter. The actual volume output of urine and rectal gland fluid was about 169 ml for a 12-hour period. This loss of sodium and chloride is roughly equal to the sodium and chloride of sea water, considering the sodium and chloride obligated to each other only. The sodium loss here is too high and the chloride a bit too low. What seems important from these data is that through the combined action of the rectal gland and the kidneys, the dogfish can eliminate sodium chloride at roughly corrected sea water concentrations. This cannot be done by the kidneys alone, where the sodium and chloride loss does not approximate corrected values for sea water. Indeed, the urinary loss of chloride is usually below the plasma values. Thus, while it is clear that the rectal gland can easily make up for the renal deficiencies in chloride loss *vis a vis* plasma concentrations, the rectal gland and kidneys seem to be able roughly to handle an inflow of sea water whether through the gut or gills, or other source. It may thus turn out that the gills are not a source of in-out sodium chloride net flux.

Fish in the above series produced urine but had low flows from the rectal gland. What imbalance here results awaits further study. The above comments on total water-electrolyte balance should not obscure the basic observation of this study, that the rectal gland secretes sodium chloride at about twice the concentration found in the plasma and in sufficient volume to remove significant amounts from the plasma.

J. WENDELL BURGER
Trinity College, Hartford, Connecticut, and *Mount Desert Island Biological Laboratory, Salisbury Cove, Maine*
WALTER N. HESS
Hamilton College, Clinton, New York, and *Mount Desert Island Biological Laboratory*

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5. Analytic methods: *p*H, Beckman meter; osmolarity, Fisk osmometer; Na and K, flame photometer; Ca and Mg, ethylendiamine tetraacetic acid titration; Cl, Shales method and electroconductivity titration with $AgNO_3$; urea, Conway diffusion method; CO_2, micro-Van Slyke; SO_4, only semiquantitative comparison of $BaCl_2$ precipitates.
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