Our findings confirm and extend the original observations of Burger (1962). In addition they provide a simple and reliable system to analyze the regulation of rectal gland secretion since the time course and magnitude of the effects of modifying the internal envrionments on the rate of secretion of the rectal gland can be followed with greater ease. Moreover, repeated sampling of arterial and venous blood, to analyze for hormone levels and electrolyte composition, as well as injection in different vascular beds can be carried out without difficulty. The information obtained with such measurements should permit more detailed speculation on the mechanisms regulating rectal gland secretion. This work was supported by Grants from the New York Heart Association and the NIH (AM 24064 and HLB 10384).

EFFECT OF STIMULATING SECRETION ON PURINE DERIVATIVES IN THE RECTAL GLAND AND BLOOD OF THE DOGFISH

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We have previously shown that extremely low concentrations of adenosine and some of its derivatives, such as ATP and ADP, stimulate markedly the rate of fluid and chloride secretion by the perfused rectal gland of the dogfish (Erlij, Silva and Reinach, Bull. MDIBL, 18: 92-93, 1978). As these purine derivatives are widely distributed in animal cells and fluids it is of interest to determine their concentration in the rectal gland and blood of the dogfish. It is also of interest to determine whether the increase in the rate of secretion caused by injecting saline solution can be ascribed to changes in concentrations in blood or tissue of these purine derivatives. Finally, the data on the content of high energy compounds can inform on the metabolic burden due to the high rates of secretion.

The experiments were carried out in anesthetized dogfish (Squalus acanthias). The samples of blood and glands were obtained from either animals secreting at a spontaneous resting rate or after the high rate of secretion induced by the injection of saline solution had reached a stable level. The data on the increase in secretion induced by saline solution injection in the glands used in this communication are described in an accompanying paper. (Erlij, Rubio and Silva, This Bulletin). The arterial blood was drawn from the posterior intestinal artery and venous blood from the vein of the rectal gland. The rectal glands were rapidly excised and immediately frozen and flattened with a set of Wollenberger clamps pre-equilibrated with liquid nitrogen. The glands were immersed in liquid nitrogen, pulverized, homogenized and extracted with perchloric acid. The samples of blood were also extracted following a similar procedure. After centrifugation and neutralization all the samples were analyzed for adenosine, inosine, and hypoxantine by an enzymatic method that follows uric acid production (Dobson, Rubio and Berne, Cric. Res. 29: 375-384, 1971). In addition the gland extracts were analyzed for creatine phosphate (CP), adenosine monophosphate (AMP), diphosphate (ADP) and triphosphate (ATP) following the changes in absorbance of NADH or NADPH (for details see Lamprech, Stein, Heinz and Weisser, Methods of Enzymatic Analysis U. Bergmeyer Ed. Vol. 4. pp 1777-1781, New York, Academic Press Inc., 1974, and Jaworek, D. Gruber, W. and Bergmeyer, U. in Methods in Enzymology U. Bergmeyer Ed. Vol. 4. pp 2127-2131, New York, Academic Press Inc., 1974).

So far we have carried out successful determinations in 3 non-stimulated animals and in 4 stimulated animals. The results are summarized in Tables 1 and 2. One of the interesting findings is that glands of both resting and stimulated animals contain similar levels of adenosine. These levels are an order of magnitude larger than those that activate the perfused gland. In contrast, the venous and arterial blood had no detectable concentration of adenosine in animals secreting at either low or high rates. As far as the products of adenosine metabolism - inosine and hypoxantine - are concerned they were abundantly present in both the gland and

Table 1.--Effects of Infusion of Saline on Content of Adenosine (ADO) and its Metabolites in Gland and Blood of Dogfish

	Gland (nmol/g)		Artery (nmol/ml)		Vein (nmol/ml)	
	Control	Stim.	Control	Stim	Control	Stim.
ADO	1.68 ± .37	2.03 ± .27	N.D.	N.D.	N.D.	N.D.
INO	9.89 ± 1.94	15.79 ± 3.59	4.13 ± .27	9.22 ± 3.24	3.51 ± .31	9.71 ± 2.98
HYPO	15.79 ± 3.59	25.36 ± 7.93	6.74 ± .54	15.74 ± 5.73	9.70 ± 1.68	17.89 ± 5.3

All values are X ± S.E. INO = inosine HYPO = hypoxantine N.D. = non-detectable
Control, n = 3. Stimulated, n = 4.

Table 2.--Effect of Infusion of Saline on High Energy Compounds (μmols/g) in the Rectal Gland of Dogfish

	CrP	ATP	ADP	AMP	εNUC	~P
Control (n=3)	.494 ± .137	1.513 ± .167	.567 ± .102	.174 ± .069	2.255 ± .069	3.971 ± .306
Stimulation (n=4)	.221 ± .066	.868 ± .102	.568 ± .084	.260 ±0.46	1.697 ± .183	2.593 ± .307
	.1 > P>.05	.02> P>.01	P> 1	.P > .3	.05>P>.02	P > .02

All values are $\bar{x}$ ± S.E. CrP = Creatine phosphate ATP, ADP and AMP adenosine triphosphate, diphosphate and monophosphate respectively. ε NUC, sum of all nucleotides. ~P high energy phosphate content.

the venous and arterial blood. The levels of these compounds appeared to increase in the animals stimulated to secrete at high rates, however, the differences were not significant. However, we found that the ATP concentration as well as the content of high energy bond and total phosphate nucleotides were significantly reduced in the stimulated glands. Creatine phosphate in the stimulated glands was also reduced but the change was not statistically significant probably because the number of experiments available so far is relatively small.

The first point to be considered is that the tissue concentrations of adenosine, as well as ATP, in both resting and stimulated glands are far greater than those necessary to produce maximum stimulation of secretion in the perfused gland. This finding suggests that cellular adenosine and ATP are out of reach of their receptor probably because it is localized in the outer surface of the membrane. In agreement with this suggestion is the finding that adenosine analogues that penetrate poorly through the cell membrane stimulate secretion by the gland (Erlij, Silva and Rubio; This Bulletin).

The second point is that we have not been able to detect any change in adenosine concentrations either in the tissue or in the blood associated with the stimulation of secretion that follows saline injection. Provided that adenosine concentrations either in the tissue or in the blood associated with the stimulation of secretion that follows saline injection. Provided that adenosine is not rapidly destroyed during our extraction procedures these results indicate that adenosine is not the primary mediator of the response to saline infusion.

Finally it is interesting that stimulation of secretion causes a reduction in the content of high energy phosphates in the gland. This finding indicates that the effects of saline infusion trigger an energy requiring process within the

gland and are not simply the result of an increase in the extracellular volume of the animal. It is not surprising that stimulation of secretion should result in the reduction in high energy compounds since the stimulated glands were secreting the equivalent of 9 times their own volume every hour. This work was supported by Grants from the New York Heart Association and the NIH (AM 24064 and HLB 10384).

EFFECTS OF ADENOSINE ANALOGUES ON SECRETION BY THE ISOLATED RECTAL GLAND OF THE DOGFISH, SQUALUS ACANTHIAS

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This communication describes the effects of several adenosine analogues on the rate of secretion of the isolated and perfused rectal gland of the dogfish Squalus acanthias. The interest in studying the effects of these analogues stems from our discovery (Erlij, Silva and Reinach, Bull. MDIBL. 18: 92-93, 1979) that very low concentrations of adenosine stimulate secretion by the perfused rectal gland. The study of the effects of analogues can provide evidence concerning several aspects of the mechanism of action of adenosine.

For example, the use of impermeable analogues can provide evidence useful to judge whether their action is either intracellular or extracellular. There is also evidence indicating the presence of different kinds of adenosine receptors in cell membranes; activation of one of them, Ra, leads to stimulation of adenylate cyclase while activation of the other, Ri, results in its inhibition. Comparison of relative potencies of different analogues is the usual way to determine the type of receptor involved in a given response (Londos, Cooper and Wolff, Proc. Natl. Acad. Sci. USA, 77: 2551-2554, 1980).

We perfused the isolated glands following the methods used previously (Erlij, Silva and Reinach, 1978). Figure 1

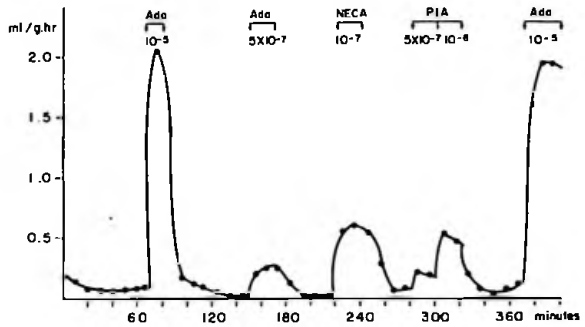

Figure 1.--Comparison of the effects of adenosine analogues on the rate of fluid secretion by the perfused rectal gland of the dogfish. Drugs were infused in periods indicated by horizontal bars. Figures are concentration in moles. "Ado" stands for adenosine; "NECA" for 5'-N-Ethylcarboxamide-adenosine and PIA for N^6-phenylisopropyladenosine.

illustrates the type of procedure used to compare the effects of adenosine and its analogues on the secretory rate of the rectal gland. First, adenosine (10^{-5}M) was perfused. This concentration produced a rapid and large increase in the rate of secretion. The increase was observed within the first 5 minutes after addition of the compound; it disappeared rapidly after perfusion with adenosine-free Ringers. The response to 5×10^{-7} adenosine was then tested, this concentration produced a smaller but still clear-cut effect on secretion. Then the effects of 5' N-ethylcarboxamide-adenosine (NECA) and N^6-phenylisopropyl-adenosine (PIA) were determined. These compounds were selected because their sequence of potency offers a sensitive indication of the receptor type involved in the response (Londos, Cooper