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DEMONSTRATION OF ACTIVE CHLORIDE TRANSPORT BY THE ISOLATED RECTAL GLAND OF THE DOGFISH, *Squalus acanthias*, BY THE USSING-ZHERAN TECHNIQUE

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The rectal gland of the spiny dogfish secretes sodium chloride through its main duct. It has been demonstrated that the rate of sodium chloride secretion is greatly stimulated by theophylline and cyclic AMP in intact glands in vitro perfused with balanced salt solution through the vascular system and with collection of fluid through the excretory duct. (see Silva et al. 1975 and 1976, this Bulletin). In a recent report (Zadunaisky and Silva 1976, this Bulletin 16, 109) we have shown that the stimulation by theophylline and cyclic AMP can be demonstrated in glands that had been opened along the length of the central duct and placed as a thick membrane between two well stirred hemichambers utilizing the Ussing-Zheran method for epithelia (1951). Under these conditions a very small potential difference is maintained by the gland duct that is stimulated to levels of 8-12 mV after addition of both theophylline (10^{-4} M) and Db cyclic AMP (10^{-5} M) to the shark Ringer solution bathing both sides of the gland. The short-circuit current after full stimulation is of the order of 16 μ Amps/cm² and its development is extremely sensitive to the presence of glucose in the bathing solutions. Furosemide proved to inhibit dramatically the short-circuit current generated by the gland.

At this time we report results of the determination of chloride fluxes with ³⁶Cl across 27 glands that were maintained under short-circuit condition during maximal stimulation with Db cyclic AMP and theophylline. Rectal glands were dissected out as previously described (Zadunaisky and Silva 1976, this Bulletin), their ends sectioned and then opened along the main secretory duct. The resulting layer was placed as a flat sheet between lucite hemichambers used before for studies of corneal transport (Zadunaisky 1966, Amer. J. Physiol. 211:506) and bathed with shark Ringers solution of the following composition: (nmoles/liter) Na 280; K 6; Mg 3; Ca 2.5; Cl 290; Phosphate 1; Sulfate 0.5; Bicarbonate 8; Urea 350; Glucose 10. After full development of a stable value for short-circuit current, ³⁶Cl Ringer was added to the mucosal (duct) side or to the serosal (capsular) side at a final concentration of 4 μ Ci/ml. After 90 minutes, sampling was started from the cold and hot side with constriction pipettes of 250 and 50 μ l respectively, at 30-minute intervals. Samples were dissolved in liquid scintillation fluid (TT-21) Yorktown Research Co. Hackensack and counted in an automatic Nuclear Chicago Liquid Scintillation Counter. Calculations for unidirectional fluxes were made as described (J. Zadunaisky 1966, Amer. J. Physiol. 211:506) on the basis of specific activity of the radioisotope determined from counting of samples and determination of chloride concentration by amperometric titration of samples of cold shark Ringers. The electrical equipment consisted of an automatic short-circuit current unit modified for low current detection. The flux from capsular to secretory side and the opposite flux were detected simultaneously in two halves of the same rectal gland.

In some instances current-voltage curves were determined in a few glands. Detection of the stimulation by Db cyclic AMP (10^{-5} M) and theophylline (10^{-4} M) and the inhibitory effect of furosemide (10^{-3} M) were tested in decapsulated glands. Decapsulation was performed under observation with a dissecting microscope and then the tissue mounted as described for the intact glands.

The results of 27 outfluxes and 25 influxes in short-circuited stimulated glands is shown in Table II. It can be observed that the flux from serosal to mucosal (capsule \rightarrow duct or outflux) was approximately 4 times greater than the passive flux of chloride in the opposite direction. The resulting net flux of 0.579 μ Eq/h/cm² is statistically similar to the short-circuit current circulating across the preparation,

TABLE 1

Unidirectional and net flux of chloride determined with ^{36}Cl and comparison to the short-circuit current of flat sheets of rectal glands of the dogfish (*Squalus acanthias*) stimulated with cyclic AMP and theophylline

OUTFLUX (Capsule to duct) $\mu\text{Eq/h/cm}^2$	INFLUX (Duct to capsule) $\mu\text{Eq/h/cm}^2$	NET FLUX (Capsule to duct) $\mu\text{Eq/h/cm}^2$ $\mu\text{Amp/cm}^2$		NET FLUX (Short-circuit current) $\mu\text{Amp/cm}^2$
$0.813 \pm 0.253^*$	0.234 ± 0.045	0.579	15.5	13.5 ± 2.5
N = 27	N = 25			N = 52

* Standard error of the mean.

$15.5 \mu\text{Amp/cm}^2$ from the chloride flux and $13.5 \mu\text{Amp/cm}^2$ from the direct determination of the short-circuit current. The small difference indicates variability and is not statistically significant. Therefore under these experimental conditions all of the current produced by the rectal gland can be accounted for by a transport of chloride ions moving into the duct side of the chambers.

In order to determine if rectification occurred across the gland, current voltage curves were constructed by changing these parameters step wise across isolated glands. Figure 1 shows the linearity of the voltage-current plot indicating linear ohmic behavior for the isolated rectal gland of *Squalus*.

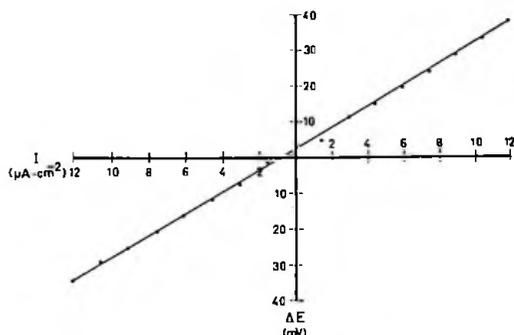


Figure 1. Voltage current plot for the isolated rectal gland of *Squalus* placed as a membrane. Note the linear characteristics of the function, reflecting good ohmic behavior.

The presence of the capsule is an important barrier for diffusion as indicated by the rapid onset of the response to the stimulating drugs as well as the quick inhibition produced by furosemide. Figure 2 shows a comparison between an intact gland and a decapsulated one in their response to these drugs. Note particularly the rapid onset of inhibition by furosemide in the decapsulated against the intact gland. The thickness of the sheet of tissue interposed between the two hemichambers poses some experimental constraints. However, the relationship of net flux of chloride to short-circuit current after appropriate equilibration of the radioactive species in the tissue is valid regardless of the thickness of the layer examined. In all experiments, equilibration of the radioisotope with the tissue was allowed for 1 to 1.5 hours before sampling, and the existence of a real steady state confirmed by the constancy of the unidirectional flux with time after equilibration. A problem of more significance is that the oxygenation of the gland, when perfused through the vasculature, is extremely high (Silva 1976, this Bulletin) and the half thickness of the sheet

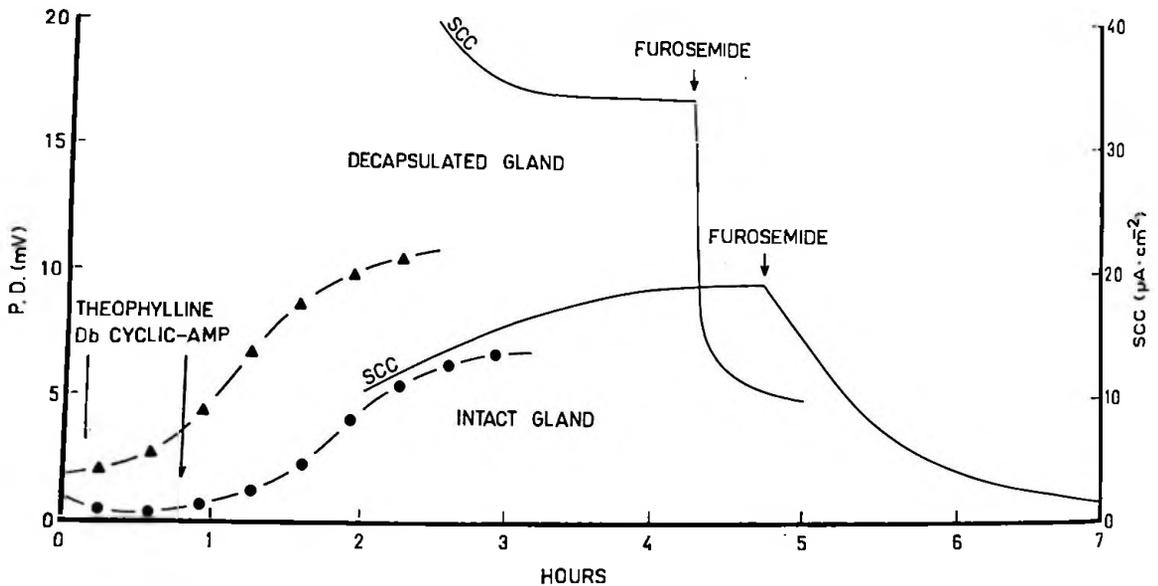


Figure 2. Time course of development of electrical properties of two rectal glands of *Squalus* stimulated first with theophylline (10^{-4} M) and Db cyclic AMP (10^{-5} M) and inhibited afterwards with furosemide (10^{-3} M). Note that the decapsulated gland develops the potential difference and short-circuit current to higher levels and more promptly while the inhibition by furosemide is immediate in comparison with the intact tissue.

of tissue utilized in our experiments does not rule out the probability that the center of the tissue is not well oxygenated during the performance of the flux determination. Calculations kindly provided by Dr. John Stephenson from the National Institutes of Health based on the known oxygen uptake and thickness of the layer of tissue utilized by us, support the notion that some degree of anoxia might exist in the tissue. However, this will have the effect of producing an overall reduction in metabolic rate that will reflect in lower rates of chloride transport. From evidence collected at this Station on the secretory characteristics of the rectal gland (see Silva 1976, this Bulletin) and the evidence presented here it can be safely stated that the driving force for the secretion of sodium chloride by the rectal gland of the shark is mainly a chloride active transport operating in the direction capsule to duct.

CENTRAL NERVOUS SYSTEM DISTRIBUTION OF BLEOMYCIN IN *Squalus acanthias*

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Little is known about the entry of Bleomycin into the central nervous system, even though it is an important anti-cancer therapeutic agent. The dogfish was used as an animal model to study Bleomycin because of the background of material available on central nervous system studies in the dogfish and the small quantity of albumin present in dogfish plasma to potentially impair interpretation of data. Three goals were pursued:

1. To compare the distributional space of Indium Bleomycin with Indium Chloride to determine the relative fastness of Indium to Bleomycin in the model being studied;