1963 #15

EQUALITY OF H^+ AND CL^- TRANSPORT BY GASTRIC MUCOSA OF Squalus acanthius

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A distinctive feature of the isolated gastric mucosa of the dogfish is the absence of a significant transmucosal electrical potential difference. Previous study had demonstrated that the Cl^- ion is actively transported during H^+ secretion but the possibility of another abherent ion transport canceling a transport of Cl^- ion in excess of H^+ had not been excluded. For paired gastric mucosae from 12 fish values in μEq. cm^{-2}. hr^{-1} were obtained: Cl^- flux serosa to mucosa 5.8 ± 0.4, mucosa to serosa 4.5 ± 0.2 and H^+ secretion 1.3 ± 0.1. Consequently the dogfish does differ from teleosts and other vertibrates in failing to actively transport Cl^- in excess of H^+ and thus generate a short-circuit current which would give rise to an epithelial potential.

Elasmobranchs differ from other vertebrates; in having a higher interstitial $[Cl^-]$ concentration and have in common with other fish a low arterial pCO_2 + higher pH. Exposure of 8 mucosae to solutions with either a $[Cl^-]$ of 82 mEq/1 (but made iso-osmotic with sucrose) or 1% CO_2 (with 30 mEq/1 HCO_3^-) had no significant influence on the mucosal potential.

Substitution of Cl^- by SO_4^- or the isethionate ion did not induce a "reversed" potential. In confirmation of previous work, the mucosa secreted H^+ against an adverse potential difference of 75 ml and the spontaneous potential was not materially changed by carbachol stimulation or SCN inhibition.

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ISOLATED DOGFISH RECTAL GLAND: ELECTRICAL PARAMETERS, SODIUM AND CHLORIDE FLUX

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Because of the secretion of a remarkably concentrated solution of sodium chloride by the rectal gland of Squalus acanthius in vivo, the following observations on the isolated gland are reported even though secretion was not elicited in vitro.

The fish received 10 ml of 6% NaCl subcutaneously 2 hours before the experiment. After being split longitudinally, each of two 0.5 cm^2 portions of the gland were mounted between chambers with both surfaces exposed to 4 ml of saline (Na 252, K 10, Ca 10, Mg 4, Cl 240, HCO_3 30, HPO_4 2, SO_4 4 and glucose 25 mEq/1; 5% CO_2, 95% O_2) at 21.1 ± 1.4°C. Wet weight 0.35 gms cm^{-2}. Values are given as means and standard errors of paired observations on blands obtained from 6 fish.

The spontaneous transmural potential difference was insignificant; 0.27 ± .44 mV. with the mucosal surface positive to serosal surface. The D.C. electrical conductance was 1.56 + .23 millimhos . cm^{-2} and increased 30% over 5 hours.

By double-labelling experiments with Na^{22} and Cl^{33}, flux was determined, after 4 hours to attain an isotopic steady state, over 4 hourly periods. One portion of the gland was used for the serosa to mucosa and the other for the mucosa to serosa flux. The fluxes in uEq.cm^{-2}.hr^{-1} were for Na 0.53 ± .07, 0.53 ± .31 and for Cl 0.68 ± .15, 0.70 ± .12 serosa to mucosa and mucosa to

serosa respectively. Assuming that these fluxes are passive, since 1 uEq.hr^{-1} approximates 1 millimho partial conductance, Na and Cl account for about 80% of the total conductance.

In 3 of 6 preliminary experiments (without prior stimulation) not reported here, the sum of the Na and Cl fluxes were substantially less than the total conductance. A difficulty encountered in mounting the gland as a flat sheet was a tendency of the thick gland wall to crack when compressed between the flux chambers.

1963 #17

THE EFFECTS OF ANTIMETABOLITES ON THE SAND-DOLLAR EMBRYO (Echinarachnius parma)

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During the summer of 1963, we have continued our investigations on the effects of various drugs on the developing sand-dollar embryo.

(1) The analysis of the halogenated pyrimidines, 5-fluorodeoxyuridine (FUDR), and the 5-bromo-, iodo-, and chlorodeoxyuridines, and the trifluoromethyl analogues of thymidine has been completed. These drugs all have separate, definite, and consistent effects on the embryo. The protective effects of thymidine specifically, as well as non-specific protection of the purine and pyrimidine ribosides against 5-B-I-Cl CF_3-UDR, have been analyzed. They were found to protect for up to 30 minutes after the halogenated pyrimidines were added to the fertilized eggs at the time of fertilization.

(2) Studies on purine analogues which inhibit cleavage. Adenine inhibited cleavage and more than 100 adenine analogues were examined for this activity. Of a series of related purines, made available by Burroughs Wellcome Company, (a) 8-mercapto-2-piperidino-6-aminopurine proved to be an extraordinarily effective inhibitor of cleavage and was active in a concentration of 0.6 micrograms/10cc of sea-water, (b) a 6-hydroxy analogue of the above drug was not active, and (c) the simplest analogue, 6-mercapto-2-dimethylamino-6-aminopurine, was as active as the piperidino-analogues. Certain analogues with other substitutions in the 2-position wer ineffective, and the CH_3S analogue caused a marked decrease in activity. The mechanism of action of these cleavage inhibitors is under investigation. Some of our data suggest that the active compounds do not inhibit fertilization or thymidine incorporation into the pronuclei, but prevent fusion of pronuclei.

(3) Cytosine arabinoside, a pyrimidine analogue, was found to delay the first cleavage about 30 minutes, and then the second cleavage occurred at the usual interval and the embryo recovered. Microscopic sections of the embryos showed that the pronuclei fused on schedule, but the appearance of the metaphase was delayed. The embryos were exposed to pulses of tritiated thymidine and it is now being determined if the delay was associated with a disturbance in thymidine uptake.