

were curtailed because of the high frequency of overripe eggs. Both eggs and sperm were obtained by the conventional stripping technique. In most experiments, pricking the micropyle with a fine glass needle (15μ) was the method used for inducing blastodisc formation.

Observational and analytical aids employed were a Sage model 500 cinephotomicrographic apparatus and a Fairchild television microscope system.

A total of 35 pricking experiments were performed involving 976 eggs. Experimental groups were allowed to remain in a filtered seawater solution of 10^{-3} M mersalyl for intervals which ranged from 30 minutes to three hours before pricking; the percentage of eggs which had formed a blastodisc at one hour after pricking decreased from 86% to 24% over the aforementioned time-range. In control groups which had remained in filtered seawater for similar periods before pricking, the percentage of blastodisc formation decreased from 96% to 75%.

Measurements of the mean height of blastodiscs formed one hour after pricking in experimental groups of 10 eggs each which had been in 10^{-3} M mersalyl solution for 1.0, 1.5, 2.0, and 3.0 hours were .144, .127, .115, and .096 mm, respectively; corresponding values for seawater control eggs were .204, .160, .137, and .106 mm, respectively. The smaller blastodiscs of mersalyl-treated eggs were a result of an inhibition of the migration of cortical cytoplasm to the animal pole, as well as a failure of the gelation process which normally gives the terminal form to the blastodisc.

Time-lapse films and television microscope observations confirmed that mersalyl inhibits contractility in the Fundulus egg cortex. The amplitude of cortical contractile waves was markedly reduced in mersalyl-treated eggs.

The inhibition of blastodisc formation by mersalyl suggests that SH-groups play important roles in key aspects of this process. Two major aspects of blastodisc formation with which sulfhydryl groups appear to be associated are the cortical contractions which provide the motive force for cytoplasmic streaming and the gelation into the definitive blastodisc. The results support the view that cortical contractility is a sine qua non for the formation of the teleost blastodisc.

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NEGATIVE EVIDENCE FOR EXCHANGE OF CHLORPHENOL RED AND COMPETITOR ANIONS ACROSS THE RENAL TUBULAR EPITHELIUM OF WINTER FLOUNDER (Pseudopleuronectes americanus)

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Kinter and Cline (Am. J. Physiol. 201:209-17, 1961) originally proposed that competitor enhancement of anion efflux from renal tissue in vitro might involve a carrier mediated exchange or counter-transport interposed at some point between the medium and the site of anion accumulation. I have now tested this hypothesis using a recording microspectrophotometer adapted for measurement of chlorphenol red flux into or out of the luminal fluid of single flounder tubules exposed to oxygenated saline medium with or without the dye (Kinter, Am. J. Physiol., in press). First, low medium concentrations of another anionic dye, bromocresol green, enhanced efflux of

chlorphenol red from tubular lumens, yet no blue-green color was detected in luminal fluid during these experiments. Bromocresol green is a competitor which, by itself, rarely accumulates in luminal fluid (Forster *et al.*, J. Cell Comp. Physiol. 44:1-4, 1954). Second, a classical procedure for demonstrating exchange is to preload tissue with one molecular species and, thereby, enhance the influx velocity for a second by supplementing normal influx with exchange. Of sixteen influx measurements with 2.5×10^{-5} M chlorphenol red in the medium, seven were obtained from tubules exposed for the preceding 20-30 min to medium containing a colorless competitor, 10^{-3} M PAH (p-aminohippurate) or Diodrast (iodopyracet). Yet, the mean value for initial velocity of chlorphenol red influx was, if anything, lower for the seven preloaded tubules, 3.7 ± 0.9 SD as compared to $4.7 \pm 0.9 \times 10^{-12}$ μ moles/min $\times \mu^2$ luminal surface ($P < 0.5$). It is of note that Ross and Farah (J. Pharm. Exptl. Therap. 151:159-67, 1966) were likewise unable to demonstrate increased influx of PAH- C^{14} in cortical slices of dog kidney preloaded with unlabeled PAH or Diodrast. Third, if anion exchange occurs, it should be possible to use the concentration energy of preloaded competitor to produce a transient accumulation of chlorphenol red in metabolically inhibited tubules. In a typical attempt to demonstrate exchange coupling between PAH and the dye, a tubule was treated as follows: 20-30 min in oxygenated medium with PAH to preload, 1-5 min in N_2 equilibrated medium without dye to block the influx pump by O_2 lack, and 10-30 min in N_2 equilibrated medium with dye to look for accumulation as result of exchange. In all, 37 such experiments were performed using a variety of competitors and conditions (10^{-4} to 10^{-3} M PAH, Diodrast, or probenecid in preloading medium; 99% N_2 or 0.1 to 1 mM cyanide for metabolic inhibition; and 5×10^{-5} to 10^{-3} M chlorphenol red in exchange medium). Control experiments showed that tubules were capable of normal dye uptake following removal from the metabolic inhibitors. Yet, transient accumulation of dye was never detected in either luminal fluid or cells. Thus, in so far as negative results from three different tests can be accepted as evidence, counter-transport of organic anions does not occur in flounder tubules and further work is needed to determine whether competitor enhancement of efflux derives from the intricacies of multiple transport steps across the tubular cell or from a single, heretofore unrecognized, membrane phenomenon.

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EXCRETION OF DRUGS ACROSS THE GILL OF THE DOGFISH, Squalus acanthias

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Although there is a considerable speculation concerning the practical and theoretical pharmacological problems of gill excretion, there are virtually no data on the subject. Seven drugs of varied chemical, physical and pharmacological type were studied in S. acanthias following intravenous injection at doses from 15-100 mg/kg. The plasma decay rate and urinary clearance were studied in the free swimming fish. Gill and urinary clearance were studied in a divided box arranged as follows: The entire box was 40 x 7 x 7 inches and was separated into an anterior and posterior chamber by a partition made of lucite and neoprene. The anterior chamber was 10 inches long, and was filled with 1.5 L sea water. The posterior chamber was filled with about 5 L.