chlorphenol red from tubular lumens, yet no blue-green color was detected in luminal fluid during these experiments. Bromcresol 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 x μ^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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1966 #24

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.

The fish was placed in the box so that the head lay in the anterior chamber to and including the pectoral fin. In some cases a watertight seal was maintained between the two chambers; in other cases the fish lay freely across the partition with water levels arranged so there would be no mixing. The water in the anterior chamber was aerated or oxygenated, pumped and continuously recirculated through the gills via the spiracles at the rate of 1 L/min, cooled in a condenser through which sea water ran rapidly. The anterior chamber fluid, or gill effluate was sampled at intervals, and in some cases the fluid was changed between sampling periods. This latter procedure did not generally affect the results; concentration of drug in gill effluate never reached the level of the plasma.

To place Table 1 in context, it may be recalled that glomerular filtration rate in this species (inulin clearance) is about 18 ml/hr (Forster and Berglund, J. Cell. Comp. Physiol. 49:281, 1957), and the U/P ratio of inulin is 2-4 (Cohen, ibid. 53:205, 1959). Secreted substances such as phenol red may be excreted (at low plasma concentrations) as much as 22 times as fast as inulin, but in the range of plasma concentrations used in the present study (20-50 μg/ml) the phenol red/inulin excretion ratio was about 5 (Kempton, Biol. Bulletin 130:359, 1966).

Table 1 shows that only one drug, the carbonic anhydrase inhibitor benzolamide (CL 11,366) was rapidly excreted by the kidneys. The clearance rate approached that of phenol red, as studied by W. Smith (J. Cell. Comp. Physiol. 14:357, 1959). We have previously shown that at very low plasma concentration the U/P ratio of benzolamide is about 300. Benzolamide is concentrated in the kidney tissue, and little or none is found in the gill (this Bulletin 5, #1, p. 38, 1963). Benzolamide is highly ionized, lipid insoluble, and very slowly excreted by the gill. The renal excretion of all the other substances tested is substantially less than that of inulin; these drugs are partially or completely unionized at body pH. Table 1 shows that renal drug clearance is generally somewhat less in the laboratory environment than in the ocean.

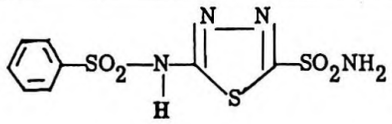
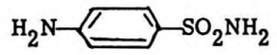
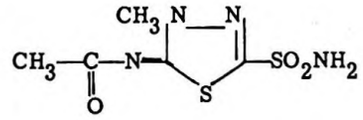
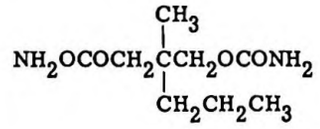
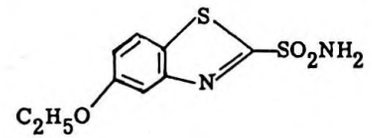
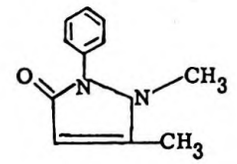
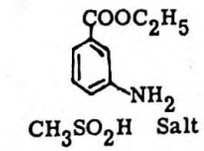
Gill excretion varies several hundred fold, and appears to correlate remarkably well with lipid solubility, as measured by the $CHCl_3$/buffer partition coefficient. Gill clearance data of Table 1 are reliable only as relative values among the several drugs; it will be shown below (Table 2) that even during the first hour that the animal is in the box, gill clearance declines, due to the artificial environment. Within this limitation Table 1 shows the entire range from virtually no gill excretion to passage rapid enough to eliminate almost all drug within an hour. Except for the case of ethoxzolamide (whose t 1/2 may reflect redistribution or metabolism) the half-lives of the drugs stand in reasonable relationship to their total gill + urinary clearance.

Table 2 shows that in the first five minutes of placing the fish in the box, 14% of the injected MS 222 is excreted across the gill. Assuming that this represents the true rate for physiological conditions, the $t\ 1/2 = \frac{0.7}{.028\ min^{-1}}$ = 25 minutes. Directly measured in the free swimming fish (Table 1), t 1/2 = 36 minutes. This good agreement appears to show that the gill clearance obtained at 0-5 minutes, 60 ml/min, is the true value. Burger and Bradley (J. Cell. Comp. Physiol. 37:389, 1951) reported cardiac output (= gill blood flow) up to 56 ml/min by direct measurement, while Robin et al. (J. Cell. Physiol. 67:93, 1966) reported average cardiac output by dye dilution at 75 ml/min. It is thus probable that MS 222 is cleared in a single passage through the gill. A survey of the literature shows that this compound has the highest $CHCl_3$/buffer ratio of any currently studied drug.

Table 2 also shows that clearance of MS 222 declines as the fish remains in the box, doubt-

Table 1

GILL AND URINARY EXCRETION OF DRUGS OF DIVERSE CHEMICAL AND PHYSICAL TYPE, IN THE DOGFISH S. acanthias

Name and (n)‡	Structure	pKa	Partition $CHCl_3$ pH 7.4 buffer	Plasma t 1/2 free swim† (hrs)	Urinary clearance Free†	Urinary clearance Box* (ml/hr)	Gill clearance first hr
Benzolamide (CL 11,366) (2)		3.2	0.0003	4	60	15	2
Sulfanilamide (2)		10.4	0.08	6	4	2	4
Methazolamide (1)		7.4	0.06	24	2	1	10
Meprobamate (1)		None	2.7	-	-	-	42
Ethoxzolamide (4)		8.1	27	1.3	3	3	52
Antipyrine		1.4	28	4	1	0.2	70
Tricain Methane Sulfonate (MS 222) (6)		~4	312	0.6	3	0.2	540

*Refers to fish in divided box, for first few hours.

†Refers to the free-swimming fish in the live-car.

‡(n) = number of experiments for each drug.

Table 2

GILL CLEARANCE OF MS 222 IN S. acanthias

Time min	Plasma μg/ml	Gill		Urine	
		Output μg	Clearance ml/min	Output μg	Clearance ml/min
0 - 5	30	9,000	60		
5 - 20	26	6,300	16		
20 - 35	22	3,200	10		
35 - 50	20	2,000	7		
50 - 170	16	5,400	3		
0 - 170 mean and cumulative values	20	26,000	8	10	.003

21 mg/kg injected at 0 time i.v. into 3 kg fish. The gill perfusion fluid (1.5 liters) was changed each period.

less due to compromise of physiological function including cardiac output, in the abnormal non-swimming environment (also applies to renal clearance, Table 1). The same effect was noted with all the drugs studied, although it was less for those drugs cleared slowly, i.e., less dependent on cardiac output. Fish were kept in the box up to eight hours; they became progressively anoxic, acidotic and lethargic. Most survived for several days when returned to the live-car in the sea, but generally did not recover normal activity.

The general conclusion is that the gill is relatively impermeable to drugs, compared with other membranes. Antipyrine for example enters the CSF, with a rate constant of 0.07 min^{-1} (Rall et al., J. Pharm. Exptl. Therap. 125:185, 1959), but from Tables 1 and 2 it may be judged that the corresponding value for gill exit (extrapolating to the free swimming fish) is about 0.003 min^{-1}. However, the relative gill clearance of drugs does reflect their lipid solubility, and at least one extremely lipid soluble compound, MS 222, is limited in its gill excretion only by gill blood flow.

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1966 #25

ACID-BASE PARAMETERS OF WILD VERSUS LABORATORY MAINTAINED DOGFISH—THE PROBLEM OF NORMAL VALUES IN MARINE BIOLOGY

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The demonstration that trawl captured dogfish, Squalus acanthias, maintained in captivity, have elevated arterial lactic acid concentrations (Bull. M.D.I.B.L., 5-2:30-31, 1965) prompted this study of acid-base parameters in wild fish. Dogfish were caught by handline in 5-10 fathoms of water, and blood samples were obtained within one minute. An 18 gauge needle was inserted into the caudal artery, 1 ml of blood was drawn in a non-heparinized syringe, then a heparinized