urine (Maren et al. Comp. Biochem. Physiol. 26:853, 1968). Decamethonium, an organic quarterary base was most interesting from several points of view. Of all the drugs under study it achieved the highest levels in the four-hour plasma compartment and readily cleared this compartment within 24 hours. The rapid clearance of the plasma seems to coincide with increases in hepatic storage of this compound but very little appeared in the bile. The kidney tissue levels of this compound were about 10 percent in four hours and 30 percent in 24 hours, suggesting both uptake and storage by renal tissue. After 24 hours about 36 percent of the drug had been excreted into the urine.

Brombenzene and naphthalene were studied because of the reported irreversible binding which occurs concurrent with the metabolism of these materials (Am. N.Y. Acad. Sci. 179, 11-18, 1971). Although these binding studies have been previously conducted only in mammals, both of these materials may be metabolized via glutathione pathways and it therefore was of interest to study them in the dogfish. While very little of these materials appeared in plasma, kidney, or urine, about 20 percent was present in the liver at the time points studied. Further studies are underway to identify the nature of these materials apparently stored in the liver of the dogfish. It is noted that, like DDT, these compounds are very lipid soluble and are likely stored in the fatty liver of the dogfish.

Finally, similar compartmental analysis studies were conducted on two pollutants, DDT and methyl mercury. About 50 percent of the DDT is cleared from the plasma between four and 24 hours whereas only about 30 percent of methyl mercury is so cleared. The prolonged storage in the liver for this chlorinated hydrocarbon had been reported previously (Bulletin MDIBL, 10: 12-15, 1970). Comparatively small amounts of this material were found in the compartments. Methyl mercury was found to accumulate, although not to quite the extent of DDT in the liver. There was also some significant amount, about six percent at four hours and about three percent at 24 hours, in the kidneys of the dogfish. While such studies in dogfish have not been recorded before, it has been reported (Bulletin MDIBL, 11, 26-28, 1971) that the hepatopancreas of the lobster contained large amounts of methyl mercury after 24 hours. These studies on such a wide variety of xenobiotic compounds show that the dogfish is an excellent model for pharmacokinetic studies where one can very rapidly identify the dominant compartment by which a drug is handled.

1972 #21

COMPARATIVE ASPECTS OF RENAL AND HEPATIC HANDLING OF PHENOL RED AND INDOCYANINE GREEN IN THE DOGFISH, FLOUNDER, AND HAGFISH

A.M. Guarino, J.B. Pritchard, P.M. Briley, J.B. Anderson, M.A. Kinter, and D.P. Rall, Laboratory of Toxicology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, and National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina

These studies were designed to extend the earlier work of Rall and Burger (Am. J. Physiol. 212, 354-356, 1967) on the hepatic and renal excretions of model compounds by various marine species. In the present work the capacities of the dogfish, flounder, and hagfish to handle two model compounds, phenol red and indocyanine green (ICG), were examined. The method for analyzing these

materials in fish tissues was described previously (Comp. Biochem. Physiol. 42A, 171-182, 1972) as was the method for multiple compartment analysis (Bulletin MDIBL, 11, 24-26, 1971).

TABLE 1

Renal and Hepatic Handling of Phenol Red and Indocyanine Green in the Dogfish, Flounder, and Hagfish

	Percent of Administered Dose of Compounds in Indicated Compartment*										Fluid Ratios			
	Plasma		Liver		Bile		Kidney		Urine		Bile/Plasma		Urine/Plasma	
	4 hr.	24 hr.	4 hr.	24 hr.	4 hr.	24 hr.	4 hr.	24 hr.	4 hr.	24 hr.	4 hr.	24 hr.	4 hr.	24 hr.
Dogfish, *Squalus acanthias*														
Phenol Red, 10 mg/kg	6.10	<2.00	41.5	<5.00	2.60	28.0	0.2	0.1	13.3	23.80	17,500	>525.0	47,400	>47,700
Phenol Red Glucuronide	<1.00	<1.00	<5.0	<5.00	1.10	10.0	<0.1	<0.1	1.4	6.80	———	———	———	———
Indocyanine Green, 2 mg/kg	3.80	0.90	76.4	33.70	0.60	33.4	0.0	0.0	0.0	0.00	7,100	1589.0	———	———
Flounder, *Pseudopleuronectes americanus*														
Phenol Red, 10 mg/kg	1.20	1.00	3.0	0.55	33.40	38.8	2.5	1.1	28.2	37.50	442.000	423.0	183.000	160.000
Indocyanine Green, 1.5 mg/kg	10.60	11.70	62.3	70.60	0.50	17.0	3.2	4.6	0.0	0.04	.052	107.0	.004	.043
Hagfish, *Myxine glutinosa*														
Phenol Red, 40 mg/kg	0.36	0.09	31.2	5.70	7.10	59.7	—	—	—	—	65.000	2747.0	———	800**
Indocyanine Green, 4 mg/kg	5.40	3.20	25.6	45.20	0.17	1.5	—	—	—	—	0.770	9.0	———	———

* Mean values for six fish at each time period.
** From: Am. J. Physiol. 212: 354-356, 1967.

In Table 1 it can be seen that the dogfish rapidly cleared phenol red from plasma and excreted 28 percent of the administered dose as the parent compound into the bile in 24 hours; an additional 10 percent appeared in this compartment at the same time as a glucuronide. This species also excreted phenol red both as the free drug and the glucuronide into the urine. The bile to plasma and urine to plasma ratios at both time points under question are considerably greater than unity suggesting active transport into bile and urine. ICG was cleared rapidly from dogfish plasma but in contrast to phenol red the 24-hour levels of ICG still were considerably elevated. The amount of ICG excreted in 24 hours into the bile was about the same as for phenol red. Detectable levels of ICG were not observed in either the renal or urinary compartments. In the flounder phenol red was as readily excreted into bile and urine as in the dogfish even though the plasma levels did not clear as rapidly. The high fluid to plasma ratios suggest active transport of this material into the biliary and urinary systems of this species. The flounder did not exhibit any significant clearance of ICG from the plasma between four and 24 hours after injection. Liver appeared to rapidly take up ICG at four hours and continued to do so at 24 hours. Only about one-half as much ICG appeared in the bile of the flounder as appeared in the bile of the dogfish. It is interesting to note that ICG did appear in small quantities in renal tissue of the flounder and a small amount did occur in the 24-hour urine of this species. ICG reportedly occurs only in vascular, hepatic, and biliary compartments and therefore the presence of this material in flounder renal compartments is unexpected. The fluid ratios indicate active transport into the biliary system but not into the urine of the flounder.

The hagfish, after subcutaneous injection with phenol red, showed rapid uptake of this material into the liver from the vascular compartment. A greater percentage of the administered dose of phenol red occurred in the bile, and higher bile to plasma ratios were seen in the hagfish than for either of the other species under study. Rall and Burger (reference above) reported U/P ratios for phenol red of about unity suggesting that there is no secretory function in the hagfish for this material. Neither the flounder nor the hagfish demonstrated any phenol red glucuronide in any of the tissues or fluids under study. ICG clearance from hagfish plasma was slow while uptake by liver was obvious at four hours and continued after 24 hours, about doubling over the 20-hour period. Only small amounts

of this dye occurred in the bile of the hagfish even after 24 hours. Further study is needed on this latter point, but it is interesting to speculate that there may be a molecular weight cut-off in the hagfish biliary system, because both phenol red and ICG are sulfonic acids but the molecular weight of the latter is more than two times that of the former. The bile to plasma ratio does suggest active transport of this material although this ratio is the smallest seen in 24 hours compared with the other two species. ICG was not looked for in the urine but careful inspection of the urinary ducts of the hagfish showed only clear fluid with no suggestion of a green tint.

These three species served very well to study a compound which can be metabolized like phenol red and a non-metabolized one like ICG. It is interesting that the dogfish, an elasobranch, has the capacity to convert phenol red to its glucuronide. It is not surprising that the primitive hagfish apparently does not have this capacity. Further studies on other species such as the skate are in progress.

1972 #22

STUDY OF THE RECTAL GLAND OF *Squalus acanthias* IN VITRO

John P. Hayslett, Donald Schon, and Mark Epstein, Yale University School of Medicine, New Haven, Connecticut

Burger and Hess (Science 131; 670, 1960) established that the rectal gland in *Squalus acanthias* is a salt-secreting organ which produces a hypertonic sodium chloride solution with a concentration nearly twice that of plasma. In order to examine the characteristics of ion transport in this unique tissue, studies were conducted on the *in vitro* perfused gland. Within five minutes after removal from freshly killed fish the solitary artery of glands weighing 1.5 and 2.5 gm was cannulated with polyethelene tubing (PE90) while similar tubing was secured into the duct. After rinsing with heparinized saline, a constant perfusion was begun using a Harvard peristaltic pump (Model 1201) with fluid containing in mM/L, NaCl 280, KCl 6, $CaCl_2$ 2.5, $MgCl_2$ 3, Na_2SO_4 0.5, NaH_2PO_4 1, Urea 350, glucose 5 and $NaHCO_3$ 8. The perfusion fluid was gassed with 95 percent O_2 and five percent CO_2 and the temperature of the gland maintained at 16 - 18°C. A bubble chamber and mercury manometer were placed on line between the pump and perfused gland. The secreted fluid was collected in timed aliquots under mineral oil for analysis. Perfusion rates varied between 2.0 and 4.0 ml/min, approximately four to eight times the estimated flow rate of whole blood *in vivo* (0.22 ± 0.15 ml/gm/min) (MDIBL Bulletin 11; 53, 1971). The perfusion pressure at 2.0 ml/min was 10.3 ± 1.7 mm Hg (mean ± SE) and 30.4 ± 3.2 at the rate of 4.0 ml/min.

The average Na and K concentrations in the rectal gland fluid from 12 glands were 405.7 ± 0.7 mEq/L and 11.5 ± 0.7, respectively, compared to the values in the perfusion fluid which averaged 281.2 ± 2.9, Na and 5.9 ± 0.4, K. These data indicated a gland fluid to plasma ratio of 1.45 ± 0.04 for Na^+ and 1.97 ± 0.14 for K^+ under free flow conditions. A steeper concentration gradient for sodium across the cell membrane facing the ductal system was demonstrated from measurements of intracellular ion concentration. The Na^+ concentration in 10 glands, expressed as mEq/L tissue water was 167.5 ± 17.3, while that of K^+ was 106.8 ± 10.6. From the estimated value of 0.24 for