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RENAL AND HEPATIC EXCRETION OF FOREIGN COMPOUNDS BY *Squalus acanthias*

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The excretion of foreign compounds by marine vertebrates occurs, via the kidneys, bile, and gills (Comp. Biochem. Physiol. 42A: 171-182, 1972). Because of the proposed congruence of renal and hepatic excretory functions in mammals (Am. J. Physiol. 210, 760, 1966; ibid 220: 1755, 1971) we wish to report on these functions for several foreign compounds of diverse structure and pharmacologic activity. By studying the amounts of xenobiotics in terms of percentage of administered dose in a given tissue or body fluid at a given time, one can compare the relative importance of the renal and hepatic compartments (Bulletin MDIBL 11, 24, 1971).

Standard radiometric techniques were employed in these studies and the results appear in Table 1. This table is setup in the following manner: the five body compartments studied, plasma, liver,

TABLE 1

Distribution of Xenobiotics in Plasma, Hepatic and Renal Compartments in the Dogfish*

Compound	Dose Mg/kg	Plasma 4 hr.	Plasma 24 hr.	Liver 4 hr.	Liver 24 hr.	Bile 4 hr.	Bile 24 hr.	Kidney 4 hr.	Kidney 24 hr.	Urine 4 hr.	Urine 24 hr.
BCNU, ^{14}C	10	1.17	0.58	1.49	0	0.06	0.31	0.08	0.07	0.43	1.67
CCNU, Ring-^{14}C	10	0.56	0.30	3.13	0.51	0.02	0.20	0.40	0.07	0.38	0.82
CCNU, Side chain-^{14}C	10	1.55	0.99	7.78	3.88	0.05	0.39	8.66	0.17	0.36	1.95
BSP, ^{35}S	10	------	0.90	------	29.27	----	8.97	------	0.26	------	3.28
Salicylic acid, ^{14}C	10	9.41	5.06	3.04	1.32	0.01	0.05	0.71	0.58	2.63	22.82
Penicillin, ^{14}C	10	6.40	1.15	15.70	5.55	6.05	23.96	2.96	0.34	10.41	27.46
Antipyrine, ^{14}C	10	4.34	1.23	0.89	0	0.11	0.15	0.26	0.12	0.66	1.99
Aniline, ^{14}C	10	0	0	0	0	0.01	0.02	0.01	0.01	0.14	0.18
Sulfanilamide, ^{35}S	10	6.48	4.30	0.74	0.81	0.01	0.15	0.35	0.33	0.77	5.05
Decamethonium, ^{14}C	1	16.50	3.52	2.67	6.07	0.01	0.08	11.12	28.42	17.65	35.85
Brombenzene, ^{14}C	.05	0.28	0.14	22.20	20.60	0.03	0.04	0.05	0.02	0.16	0.11
Naphthalene, ^{14}C	.05	------	0.28	------	22.65	----	0.03	------	0.02	------	0.44
DDT, ^{14}C	0.1	4.30	1.81	34.55	48.28	0.17	0.15	1.59	0.30	0.17	0.15
Methyl mercury, ^{14}C	0.1	6.85	4.05	29.30	28.10	0.02	0.03	5.85	2.90	0.10	0.29

* Distribution in terms of percent of administered dose of compound in indicated compartment. Values are mean percent of 2-6 dogfish/time period.

bile, kidney, and urine, have their values separated into four and 24-hour segments. Consideration of these two different time points can give one some idea of the kinetics involved in the excretion of the different compounds. For example, in the case of salicylic acid the 24-hour plasma compartment contained only half as much of the drug as did the four-hour compartment. One could therefore assume that 50 percent of the drug was removed from the plasma in this interval. In contrast,

decamethonium plasma levels suggest that about 75 percent of this drug was removed from the plasma compartment in 20 hours. Similar comparisons can be made regarding salicylic acid and for example it can be seen that for the time points in question while the liver and kidney levels were declining between four and 24 hours, the biliary and urinary compartments were receiving more of the drug at these times. In comparison the fact that the hepatic levels of decamethonium between four and 24 hours increased, suggested slow uptake for this tissue and/or binding to hepatic tissue.

Considering in further detail the other drugs studied, one notes regarding the antineoplastic agent, BCNU (1,3-bis, 2 chlorethyl, 1-nitrosourea), the overall accountability in terms of administered dose is rather poor with only about 5 percent of the drug found in these five compartments. It is interesting that in 24 hours about 80 percent of the radioactivity of this drug, appears in mouse urine whereas only a few percent appear in the dogfish urine. The main reason for employing the dogfish in studies with these metabolically reactive agents was the hope that the dogfish would demonstrate "metabolism in slow motion" because of its lower body temperature compared with mammals and because of the reported poor ability of all aquatic species to metabolize drugs. Obviously the BCNU was present in other compartments not studied here. It is interesting to speculate that small radioactive molecules were formed in the metabolism of this material and may have been excreted via the gills (Maren et al. Comp. Biochem. Physiol 26: 853, 1968). The availability of two different isotopically labelled CCNU (1-2-chlorethyl-3 cycrohexyr-1-nitrosourea) molecules provided an opportunity to study the fate of these different labels. As was the case with BCNU, most of the drug was not present in the compartments under study. Whether the radioisotope was present in the ring or on the side-chain of CCNU, the plasma clearance rates were about the same and the hepatic clearance was larger for the ring labelled compound. The higher percentages of the side-chain label in the liver and in the kidney suggest that the molecule is being degraded extensively on the side-chain and that the degradation products are rapidly appearing in these two organs. The main reason for including BSP in this study was to see how much of this material, well-known to be excreted via hepatic mechanisms, would appear in these two different compartments. As is the case with mammals the hepatic routes dominate, but it was of interest to see that three percent of the BSP does appear in the urine of the dogfish.

Salicylic acid and penicillin were studied as two typical acids. It can be seen that penicillin leaves the plasma compartment more rapidly than does salicylic acid and it would appear that the reason for this is that 16 percent of the administered dose of this drug appears in the liver in four hours. The clearance from the liver over 24 hours appears to proceed at about the same rate for these two acids. Large amounts of penicillin are transported into the bile and it is seen that even at four hours, six percent of the drug has reached this compartment while 24 percent of the dose is excreted into the bile in 24 hours. About the same amount of each of the acids appears in the urine after 24 hours. Regarding the renal versus hepatic handling of these compounds, it would appear that although they are both acids, the biliary route is quite important for penicillin, whereas virtually none of the drug is excreted by this route in the case of salicylic acid. Antipyrine, an indicator of body water, appears to be excreted only to a small extent by both excretory mechanisms and hence, its excretion pattern appears to resemble that of the nitrosoureas (BCNU and CCNU) as discussed above. Excretory pathways indicated that only about six percent of administered sulfanilamide is removed by the bile and the urine, again suggesting that small molecules as the nitrosoureas, salicylic acid, antipyrine, and aniline have pathways of excretion other than via the bile and the

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.

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

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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