from .143 to 0.082 μmoles/gxhr. A similar effect of medium Na^+ concentration was observed on the rate of BALA outflux from RBC (Table 1); outflux rate dropped from 0.145 μmoles/gxhr in 300 mEq Na^+/L to 0.085 μmoles/gxhr in 200 mEq Na^+/L.

Incubating skate RBC in hypoosmotic (780 mOsm) saline medium (Table 2) had no significant effect on the rate of BALA influx, but BALA outflux was increased 67%; 0.075 μmoles/gxhr in saline medium containing 200 mM NaCl + 100 mM LiCl compared to 0.125 μmoles/gxhr in medium containing 200 mM NaCl + no LiCl.

The effects of short term (2 days) and long term (7 days) adaptation of skates to ½ SW on BALA influx into RBC is shown in Table 3. Short-term adaptation had no effect on the rate of BALA influx. However, gradual adaptation (10%/day for 5 days) to ½ SW reduced BALA influx to nearly 50 percent; the rate of BALA influx was .082 μmoles/gxhr in cells from control (SW) skates incubated in medium containing 200 mM NaCl + 100 mM LiCl and 0.038 μmoles/gxhr in cells from skates adapted to ½ SW and incubated in medium containing 200 mM NaCl with no LiCl.

The BALA concentration of RBC in skates kept in SW is approximately 50 μmoles/g, while in RBC in skates adapted to ½ SW for 7 days the BALA concentration is 23 μmoles/g (Boyd et al., J. Exp. Zool., in press). The present study has shown that extracellular Na^+ concentration and osmolarity as well as acclimation to ½ SW can all affect the rates of BALA influx and outflux in skate RBC. The effect of physiological change in external Na^+ concentration on BALA influx and outflux are of simialr magnitude and, therefore, cancel each other. In contrast lowering medium osmolarity to that found in the extracellular fluid (ECF) of skates adapted to ½ SW increases the rate of BALA outflux by 0.05 μmoles/gxhr. This increased rate of outflux, in the absence of an effect of medium osmolarity on the rate of BALA influx, would lower the BALA concentration in RBC. Assuming that lowering medium osmolarity causes an elevation in BALA outflux by increasing the passive leak of the amino acid from the cells, then the BALA concentration inside the cells would fall in a first order fashion ($k = .001\ hr^{-1}$) to a value of 43 μmoles/g in seven days. This concentration is significantly above the level found in RBC of skates adapted to ½ SW for 7 days. However, the adaptive decrease in BALA influx in skates acclimated to ½ SW will also tend to lower the RBC BALA concentration. The magnitude of this effect is difficult to estimate since the time course of the adaptation in BALA influx is not known. Thus, it is not known whether the effects of ECF osmolarity on BALA outflux and the adaptive decrease in BALA influx can account for the total drop in BALA concentration seen in the RBC of skates acclimated to ½ SW or whether other effects (e.g., adaptive increase in BALA influx) come into play as well.

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THE BLOOD-BRAIN BARRIER OF THE SPINY DOGFISH, *Squalus acanthias*; APPLICATION AND CORRELATION WITH PUBLISHED ANTINEOPLASTIC DRUG TESTING IN A MOUSE BRAIN TUMOR SYSTEM

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The treatment of brain, spinal cord, and meningeal tumors by the intravascular administration of antineoplastic drugs has met with only limited success. Since the capillaries of the central nervous system (CNS) are relatively impermeable to polar materials (the so-called blood-brain barrier phenomena), the ineffectiveness of many of the drugs which have been tried may be a result of the slow or insignificant exchange of the drug from blood to CNS and cerebrospinal fluid (CSF).

The purpose of this study was to seek, in a preliminary fashion, a possible correlation between the published antitumor activity of various chemotherapeutic agents and their ability to be taken up by the brain and CSF from the blood by a lower vertebrate, the dogfish. The reason for selecting the dogfish was that this species has been shown to be a valid model for biliary and urinary transport of xenobiotics (Boyer et al., Am. J. Physiol., 230:970, 974, 1976; Guarino and Anderson, Xenobiotica 6:1, 1976; Bungay et al., J. Pharmacokinet. Biopharm. 4:377, 1976) and that the blood-brain and blood-CSF barriers in the dogfish appear to be physiologically similar to those of higher vertebrates (Fenstermacher and Patlak, Am. J. Physiol., in press, 1977).

Dogfish, weighing 5-6 kg, were given a single intravenous injection of the radiolabeled (except for cis-diamminodichlaroplatinum, CDDP, which was chemically assayed) antineoplastic agents at the doses indicated in Table 1. Four or 24 hrs after IV administration of these agents, blood

Table 1 Utilization of the Spiny Dogfish Model in Predicting Penetrance of Antineoplastic Drugs via the Blood-Brain-Barrier*

NSC No.	Compound	Dose mg/kg	Dogfish Studies Ratio Brain/Plasma 24 hrs.	Dogfish Studies Ratio CSF/Plasma 24 hrs.	Published Mouse Brain Tumor Results
143,647	DL-Alanosine, ^{14}C	10.0	43.9	2.90	+
71,795	Ellipticine, ^{14}C	10.0	33.1	0.09	+
142,982	Hycanthone, ^{3}H	10.0	10.0	0.43	+
83,265	Tritylcystine, ^{14}C	1.0	2.00	0.05	-
762	Nitrogen Mustard, ^{14}C	1.0	1.67	1.00	-
3,055	Puromycin, ^{3}H	1.0	1.15	0.21	NTA
19,893	5-Fluorouracil, ^{14}C	10.0	1.08	0.78	-
757	Colchicine, ^{14}C	1.0	1.00	0.33	NTA
154,890	Coralyn sulfoacetate, ^{14}C	10.0	1.00	0.09	NTA
79,037	CCNU, Ring, ^{14}C	10.0	0.97	1.26	+
755	6-Mercaptopurine, ^{14}C	5.0	0.94	0.70	-
45,388	Imidazole-4-carboxamide, ^{14}C	10.0	0.94	0.74	+
26,271	Cyclophosphamide, ^{14}C	10.0	0.93	0.88	+
94,100	Dibromomannitol, ^{14}C	10.0	0.87	0.84	+
3,053	Actinomycin D, ^{3}H	1.0	0.84	0.27	-
56,054	Pseudourea, ^{14}C	10.0	0.84	0.28	-
1,895	Guanazole, ^{14}C	10.0	0.83	0.83	-
63,878	Cytosine Arabinoside, ^{14}C	50.0	0.81	0.84	+
3,070	Diethylstilbesterol, ^{14}C	1.0	0.80	0.20	-

samples were drawn, the animals were sacrificed, 1 ml of CSF was withdrawn and brain tissue (olfactory lobe) was taken. Biological samples were processed for radioassay as described previously (Guarino, et al., MDIBL Bull., 12:41, 1972). Most data on the mouse ependymoblastoma

Table 1 (cont.)

NSC No.	Compound	Dose mg/kg	Dogfish Studies Ratio Brain/Plasma 24 hrs.	Dogfish Studies Ratio CSF/Plasma 24 hrs.	Published Mouse Brain Tumor Results
102,627	Propanol-iminodi dimethanesulfonate, ^{14}C	10.0	0.71	0.66	-
77,213	Procarbazine, ^{14}C	10.0	0.63	0.31	+
13,875	Hexamethylmelamine, ^{14}C	10.0	0.60	0.40	-
119,875	Cis-diamminodichloro-platinum	1.0	0.60	0.20	+
409,962	BCNU, ^{14}C	10.0	0.57	0.44	+
102,816	5-Azacytidine, ^{14}C	40.0	0.45	0.45	-
8,806	Melphalan, ^{14}C	10.0	0.41	0.17	-
740	Methotrexate, ^{3}H	1.0	0.35	0.52	-
104,801	Cytembena, ^{14}C	10.0	0.33	0.67	+
118,994	Diglycoaldehyde, ^{14}C	10.0	0.15	0.21	-

*Animals were treated as described in text.

Values are mean of 5-6 dogfish per time point. Mouse tumor data are from Geran et al., 1974.

$$+ = \frac{\text{Test Median Survival Time}}{\text{Control Median Survival Time}} \times 100 = \frac{T}{C} > 125\%$$

$$- = \frac{T}{C} < 125\%$$

NTA = Not Tested Adequately

model (Table 1) were taken from Geran et al., Cancer Chemotherap. Repts., 4 (pt. 2) 53, 1974, while the remaining values were determined in exactly the same manner. In the table note that the following agents were positive, i.e., gave increases in the life span in tumor-bearing mice of greater than 125%: alanosine, ellipticine, hycanthone, CCNU, imidazole-4-carboxamide (DTIC), cyclophosphamide, dibromomannitol (DBM), cytosine arabinoside, procarbazine, CDDP and BCNU. Of the eleven drugs, only four, procarbazine, CDDP, BCNU, and cytembena gave a brain/plasma (B/P) ratio of less than 0.8 at the 24 hr time point. Hence, if we predicted that for this group of drugs the requirement of a 0.8 ratio would be associated with good brain tumor activity, we would have been correct 64% of the time. The following agents have poor (< 125%) antitumor activity and B/P ratios of < 0.8: propanol-iminodi-dimethane sulfonanate (PIDMS), hexamethylmelamine (HMM), 5-azacytidine (5-AC), melphalan, methotrexate (MTX), and

diglycolaldehyde (DGA). For these six agents, the negative correlation between the ratio and anticancer activity was good. Of the other agents where adequate negative tumor data exist and there are B/P ratios of > 0.8, some pharmacokinetic points usually can be evoked to explain the discrepancy. For example, the high ratios for 6-mercaptopurine (6-MP), nitrogen mustard (HN_2) and 5-fluorouracil (5-FU) are known to represent radiolabeled metabolites and not parent material. Very poor correlations exist for tritylcystine, pseudourea, guanazole and diethylstilbesterol (DES), but no explanation can be offered at the present time because adequate metabolic data are not yet available for these drugs. Regarding the agents for which the experimental therapeutics data are incomplete, colchicine and coralynsulfoacetate, one could speculate, on the strength of the high B/P ratios, that the anticancer activity would be good. For the three compounds with the highest B/P ratios, alanosine, ellipticine, and hycanthone, the tumor testing was positive. Other time points for B/P ratios and other studies of CSF levels, gave essentially the same results as for 24 hr B/P ratios.

In summary, the correlation between drug concentration at 24 hrs in nonmalignant brain tissue and drug effectiveness against the model tumor system showed a fairly good correlation for the effective drugs; however, a significant number of the ineffective drugs achieved relatively high levels in the normal brain tissue and CSF, and several effective drugs occurred at relatively low levels in the normal brain tissue. Adequate delivery of the drug to the tumor is considered to be necessary for the effective treatment of brain tumors. In addition to this correlation with drug distribution to normal brain tissue, attempts should now be considered to correlate effectiveness in this solid brain tumor model with other pharmacological and biochemical factors.

DISTRIBUTION AND TOXICITY OF SELECTED WATER POLLUTANTS IN THE SPINY DOGFISH, *Squalus acanthias*

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There is inadequate information for marine fish about the pharmacologic disposition and toxicology of water pollutants, including those resulting from oil spills. We studied in detail two known components of crude oil, octane and phenol, as well as a detergent which occurs in many natural bodies of water, sodium lauryl sulfate (SLS). The general procedures used here for administration of compounds, tissue handling and pharmacokinetic modeling in the spiny dogfish were described previously (Guarino et al., MDIBL Bull., 15, in press, 1976). ^{14}C-Octane, ^{14}C-phenol and ^{35}S-SLS were available commercially and were administered in dogfish saline (3%) except that for octane, ethanol:emulfor:dogfish saline (1:1:8) was used as a suspending agent. Nonisotopic compounds were all commercially available and except where indicated were administered in the emulfor system.

First considering the data in Table 1, the shortest plasma $t_{\frac{1}{2}}$'s (time required for plasma levels to decrease to one-half the initial level; Co is designation for initial plasma levels) were for octane (37 min) and phenol (44 min) while SLS was longer (61 min). SLS had an apparent volume of distribution (V_D) closest to that of plasma indicating very little early metabolism, binding and excretion, while the higher values for the oil pollutants indicate moderate increases in V_D for octane and large increases for phenol. Plasma binding was the highest for octane (99.6%) and the lowest for SLS (25.5%). Data in Table 2 demonstrate that SLS was excreted extensively in the