The results of these studies indicated that time from fertilization to the first cleavage (2 cell) stage was markedly temperature dependent. From 10°C to 23°C time of fertilization ranged from 255 minutes to 73 minutes respectively. The kinetic relationship between ambient temperature and time to first fertilization was non-linear and indirect. For 23°C time to first cleavage was 73 minutes (range 80-65) for 19°C, 80 minutes (range 75-90), 16.5°C, 96 minutes (range 90-105), 11.5°C, 170 minutes (range 145-190). Although there was variation between experiments on different days in six studies these relationships were qualitatively similar. When these studies were evaluated with regard to time required to reach second, third, and fourth cleavage stages (4 cell, 8 cell, 16 cell, respectively) a similar temperature effect was seen. Decreasing temperature prolonged the time required for successive fertilizations. When a doubling time for cleavage was derived for the proliferation rate from the 2 to the 16 cell stage, the following figures were obtained: 23°C, 30 minutes; 19°C, 38 minutes; 16.5°C, 47 minutes; 14°C, 60 minutes; 11°C, 76 minutes. These data indicate that temperature effects the proliferating fertilized sand dollar embryo in two important ways: 1) to prolong the duration of the one cell stage or the time to initial cleavage, and 2) influences the rate of subsequent cell cleavages in a reciprocal non-linear fashion.

These physical effects of temperature on proliferation rates were then evaluated to assess the influences of such changes on the cytotoxicity by the agents cyclophosphamide and methotrexate. At drug dosages of 0.1 to 10 gamma per ml of incubation mixture no drug effects on proliferation rate or on direct cytotoxicity were visualized on the formalin fixed specimens at any temperature. These studies, which confirm and extend an early observation by Rieck, et al (Bull. Mt. Des. Is. Biol. Lab., 4:75, 1959) indicate that in a suitably slow proliferating system, ambient temperature variations from approximately 5°C below normal growth temperatures to 10°C above normal growth temperature exert important and discernible differences in rates of cell division. These differences can be evaluated with regard to drug incorporation and cytotoxicity using appropriate systems, and will form the basis of future studies.

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THE BILIARY EXCRETION OF THE ORGANIC ANIONS, SULFOBROMOPHTHALEIN (BSP) AND PHENOLDIBROMOPHTHALEIN (DBSP) BY *Squalus acanthias*

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The excretion of many organic anions in mammals is dependent on hepatic uptake from plasma, conjugation to water soluble metabolites and transport into bile against a high concentration gradient. Previous studies indicate that the liver of the dogfish shark is also capable of excreting organic anions such as BSP into bile (Bradley, et al. Bull. MDIBL 5:3, 1965). However, the importance of the bile as the major route of excretion of these compounds is in doubt, not only because dog shark livers lack organic anion binding proteins (Levine, R.I. et al. Nature New Biology 231:277-279, 1971) but presumably because these compounds may be eliminated by the gills or kidneys (Brodie, B.B. Pharmacologist 6:12, 1964).

The present study was designed to determine if the bile of the dogfish shark was the major route of excretion of BSP, and to assess the role of hepatic conjugation by comparing its biliary excretion with DBSP, a BSP analog which does not require hepatic conjugation for excretion in mammals.

All studies were performed in free swimming male dog fish sharks (1.98 ± 0.39 kg body weight) in large pools since restriction to shark boards or small tanks resulted in diminished bile secretion. Serial collections of hepatic bile were obtained through cannulas externalized through an abdominal incision and attached to small balloons (Boyer, J. Bull. MDIBL - accompanying abstract). Bile collections could be made for at least 4-5 days.

Preliminary studies in 18 fish indicated that BSP (10-50 mg/Kg body weight given via caudal vessels) was highly concentrated in gallbladder bile 18 hours after injection; bile to plasma ratios ranged from 5 to 1543, and averaged 370. Sixteen studies were subsequently performed in cannulated fish using a 10 mg/Kg body weight dose of either BSP or DBSP. Bile was collected daily and analyzed for volume and BSP or DBSP concentration. Selected aliquots of bile were applied to cellulose thin layer plates and metabolites were separated according to the technique of Whelan, F.J. and Plaa, G.L., (Toxicol. Appl. Pharmacol. 5:457-463, 1963). Results indicated that free swimming dog fish sharks excrete hepatic bile at peak rates of 1.93 ± 0.88 ml/Kg body weight/24 hours, a value similar to that obtained by Burger, J.W. (in Sharks, Skates and Rays, Chapter 20, Johns Hopkins Press, Baltimore, Maryland 1967). However, bile flow declined with each day of captivity, reaching a value of 0.8 ± 0.27 on day 4. Quantitative analysis of BSP removed from thin layer plates revealed that 95% was excreted as free BSP while three ninhydrin negative metabolits comprised the remaining 5%. None of the metabolites was identified although one migrated with BSP-glutathione obtained from the rat. Qualitative assessment of DBSP metabolites indicated that approximately 50% was excreted as the native compound while the remainder was biotransformed to a compound with a mobility identical to free BSP. Comparison of BSP and DBSP excretion indicated that 67-100% of the administered dose was recovered in the hepatic bile over a period of 4 days. Total recovery of BSP and DBSP averaged 89.6% and 86% respectively. Furthermore, there was no significant difference between the daily rate of excretion of these two compounds; Day 1 (DBSP-58 ± 27%, DBSP-55 ± 14%); Day 2 (BSP-20 ± 13%, DBSP-24 ± 17%); Day 3 (BSP-7 ± 6%, DBSP-5 ± 3%); Day 4 (BSP-8 ± 4%, DBSP-3 ± 1.5%), suggesting that the lack of significant hepatic conjugation of BSP did not retard its excretion when compared to DBSP. Conclusions: 1) Free swimming dog fish sharks secrete hepatic bile at very slow rates which decline during 5 days in captivity. 2) Despite an inefficient hepatic conjugation mechanism for BSP, and a previously demonstrated lack of specific hepatic organic anion binding proteins, the bile is still the major if not exclusive route of excretion for both BSP and DBSP, as it is in higher vertebrates. 3) Although other factors such as inefficient hepatic uptake may be involved, the slow biliary clearance of these compounds in the dog fish shark appears to be related in part to a low rate of bile formation.