Exposure of Dogfish Shark Feti to Mercury

by E. A. CHILDS, J. N. GAFFKE, and D. L. CRAWFORD
Seafoods Laboratory, Department of Food Science and Technology
Oregon State University, Astoria, Ore. 97103

Introduction

Although studies concerned with movement of mercury from maternal to fetal tissue have been performed in mice (Berlin and Ullberg, 1963a, b, c; Suzuki *et al*, 1968; Nakamura and Saeki, 1967; Spyker and Smithberg, 1971; and Childs, 1973), hampsters (Gale and Ferm, 1971), and quail (Stoewsand *et al*, 1971), no investigations involving ovoviviparous fish have been published.

In this study, the relationship of maternal mercury load to fetal mercury load was examined in the Pacific spiny dogfish shark *(Squalus suckleyi)* which accumulates mercury *in situ* to concentrations of 0.3 - 1.2 ppm (Childs and Gaffke, 1973). Greater than 75% of the mercury present is in the methyl form. Dogfish shark embryology is more primitive than that of mice, hampsters, or quail because it is an anamnionic, ovoviviparous organism, without a chorioallantoic placenta. During reproduction, several eggs are initially enclosed in a thin, horny membrane formed by the oviducal gland; but this soon disappears and the developing feti lie free in the uterus. Nutrients are derived from an associated yolk sac, rather than by transfer from maternal tissue. Because the embryo has limited contact with the walls of the uterus, transfer of water, and perhaps other materials, from mother to fetus may occur. The yolk sac was initially derived from maternal tissue (Hisaw and Albert, 1947).

Materials and Methods

S. suckleyi samples were obtained from the continental shelf adjacent to the North Oregon coast. The samples were thoroughly homogenized, and 5 g

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samples placed in 250 ml flat bottom flasks connected to 45 cm cold water condensers. The samples were digested in 4 volumes of 1:1 (v/v) concentrated nitric and sulfuric acid, with 5 - 10 mg of V_2O_5[1] being used to catalyze the reaction. The digestion was completed in 10 minutes, at which time 3 - 5 drops of hydrogen peroxide solution (30% w/w) were added, and the volume of the digested samples brought to 100 ml with deionized water. The amount of mercury in the digested samples was determined by flameless atomic absorption spectroscopy (Hatch and Ott, 1968). We have determined the method to be accurate (>95% recovery) and precise (std. error of mean <5% of mean) for samples containing 6.0 µg or less mercury (i.e. based on a 5 g sample, the method is accurate for 0.01 - 1.2 ppm Hg).

The total mineral content of samples was determined by drying 5 g samples for 24 hours at 105°C, followed by dry ashing for 24 hours at 550°C. By comparing the mercury ash fraction (i.e. concentration of mercury in unashed sample/% ash) between maternal and fetal samples, it was possible to determine if mercury was uniquely absent from the fetus.

Proximate analyses were performed to determine the protein and lipid content of follicle and maternal samples.

Results and Discussion

The level of mercury in the fetus was significantly lower than in the mother. Maternal muscle samples had a mean mercury content of 0.66 ppm with a range of 0.332 - 0.999 ppm (Table I). Mercury concentrations in the uterine wall were consistently 10% the concentration of maternal muscle tissue. There was no apparent relationship between the concentrations of mercury in the fetus and mother. Regardless of the concentration of mercury in the maternal tissue, no fetal sample taken from undamaged uteri contained greater than 0.058 ppm mercury, and no follicle greater than 0.119 ppm mercury. The mean of all follicle samples was 0.048

[1]The use of V_2O_5 to catalyze the digestion was originally described by Robert Munns, Food and Drug Administration, Denver, Colorado.

TABLE 1

Mercury concentrations in maternal and fetal stages of *S. suckleyi*

Sample number	Maternal loin muscle tissue	Uteral membrane	Graafian follicles (1 cm)	Graafian follicles (3 cm)	Fetus (7.5 cm)	Yolk sac (7.5 cm fetus)	Fetus (10 cm)	Yolk sac (10 cm fetus)	Pup
1	0.774ppm[a](2)[bc]			0.067(2)					0.058(10)
2	0.847(2)			0.030(12)					
3	0.999(2)	0.093(2)		0.028(12)					0.024(15)
4	0.754(2)	0.085(4)		0.033(6)			0.038(6)	0.016(6)	
5	0.332(2)	0.028(2)	0.021(5)		0.028(6)	0.016(5)			
6	0.920(2)			0.027(2)					
7	0.772(2)	0.082(5)	0.079(2)				0.026(7)	0.003(12)	
8	0.390(2)	0.023(3)	0.005(1)		0.004(3)	0.003(7)			
9	0.460(2)	0.035(4)		0.015(3)			0.012(6)	0.025(6)	
10	0.954(2)	0.183[d](4)		0.018(3)	0.126(3)	0.054(6)			
11	0.445(2)	0.055(3)	0.119(3)				0.020(6)	0.005(6)	
12	0.360(2)	0.007(2)	0.000(3)				0.003(5)	0.000(4)	
Mean[e]	0.660	0.080	0.047	0.034	0.046	0.024	0.021	0.009	0.037
Total N	24	29	14	40	12	18	30	34	25

[a]All data are ppm wet weight.

[b]Number of samples. Two tissue samples were taken from each mother. All other samples were single, whole samples of the follicle, fetus, or uteral candle.

[c]Data on the same horizontal level are from the same mother. Single mothers did not contain all follicle and fetal stages.

[d]This placenta was bloody, indicating hemorrhaging. All fetal data in this sample were extremely high.

[e]Actual mean of all samples.

ppm mercury, and of all fetal samples 0.024 ppm mercury. These data clearly demonstrate that mercury is not concentrated in *S. suckleyi* feti *in situ*. The great difference in mercury content of maternal and fetal samples suggests that mercury may be excluded from the fetus.

Follicle and fetal stages had an equal or higher ash content than did the maternal tissue (Table 2). This indicates that there is no general inhibition of movement of minerals into the fetus. In the adult, the mercury portion of the ash (ppm mercury/% ash) was 21 times greater than in any follicle or fetal stage and 42 times greater than the uterine wall. Although the fetal samples could have contained more cartilage material than maternal samples, it would seem unlikely that this could account for the large differences in mercury content of the ash amongst samples. These data show that mercury is uniquely absent from the fetal environment, and suggest that it may be selectively excluded.

TABLE 2

Percentage ash and mercury fraction of ash in maternal and fetal samples of *S. suckleyi*

Sample type	% ash (dry wt.)		Hg fraction of ash (ppm Hg/% ash)	
	(Mean)	(Range)	(Mean)	(Range)
Maternal tissue	2.69[a]	1.90- 5.20	0.25	0.14-0.53
Uteral membrane	7.67	5.02-12.50	0.01	0.03-1.58
Follicles	2.96	2.00- 4.90	0.01	0.01-0.04
Fetal stages	6.25	5.50- 7.80	0.01	0.01-0.02

[a]All data are the mean of 12 or more random samples of each sample type.

One possible explanation for the absence of mercury in feti might be that the follicles are primarily lipid, and because mercury is normally bound to a sulfhydryl ligand (Cullen and McGuinness, 1971) it would not move into the follicle. Proximate analysis of follicles and maternal flesh samples gave a lipid/protein ratio of 0.987 in the follicle and 1.213 in flesh. These data show that the low mercury content of the feti is probably not the result of the lipid content of the follicles.

Since previous work with more advanced amnionic, chorio-allantoic and placental organisms has indicated that the methyl mercury load of the fetus is comparable to that of the dam (Childs, 1973), perhaps the evolutionary development of the embryonic membranes and placenta may have resulted in a lowering of fetal defenses to mercury exposure. A second possibility is that the musculature is a final reservoir for ingested mercury in dogfish shark, and the mercury which enters this depot cannot be readily mobilized into the fetus as in mice.

In summary, feti of the primitive anamniote *S. suckleyi* are not exposed to the high concentrations of mercury found in maternal musculature even though they have an equivalent content of other metals.

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