Protective Effects of Freeze Dried Swordfish on Methylmercury Chloride Toxicity in Rats

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Several environmental disasters as a result of human consumption of organic mercury derivates have led to stepped up surveilance of various foodstuffs for their mercury content (CLARKSON, 1972).

Fish are the single food source responsible for concentrating environmental organic mercury and the major source of environmental mercury in human diets (D'ITRI, 1972). As a result of pharmacokinetic analysis of the individuals poisoned in the Minamata Bay area, an action level of 0.5 ppm on fish was established by the USFDA (CLARKSON, 1972). The single fish industry most seriously effected by this action level has been the swordfish industry as most other ocean fish have mercury levels in the range of 0.1-0.4 ppm.

A considerable literature has begun to appear on the protective effect of factors in ocean fish on methylmercury toxicity (GANTHER et al, 1972). Due to its economic importance, tuna stands out as the fish most frequently tested for its protective effect on methylmercury toxicity (GANTHER et al, 1972) (STOEWSAND et al, 1974) (POTTER et al, 1974) (IWATA et al, 1973) (OHI et al, 1976) (STILLINGS et al, 1974). This protective factor appears to be a seleneum compound as sodium selenite also protects against mercury toxicity (GANTHER et al, 1972) (STOEWSAND et al, 1974) (POTTER et al, 1974) (IWATA et al, 1973) (OHI et al, 1976) (STILLINGS et al, 1974).

It is the purpose of the present communication to quantify the seleneum levels in commercial swordfish. In addition, determinations have been made on whether there is a relationship between the size of swordfish and their mercury or seleneum content. Finally, data is presented on whether swordfish, like tuna, will protect against methylmercury toxicity.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing from 90-110 gms were used in all studies and were purchased from Flow Research Laboratories. These rats were housed 2 per cage in HEPA filtered cages equipped with automatic watering and flushing devices. Each

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experimental group contained 9-10 rats. Rats were fed the semisynthetic diet shown in Table I ad libitum. Samples of swordfish steak were freeze dried and added to the diet as 15% of the diet (w/w) replacing casein. Methylmercury chloride for feeding studies was dissolved in DMSO and mixed with casein as a carrier. Rats were weighed weekly. Methylmercury neurotoxicity was determined as crossed rear legs (IWATA et al, 1973) and was recorded on the day when the rats were weighed.

TABLE I
TEST DIET COMPOSITION

Component	g/Kg
Corn Starch	576
Dextrose, Technical, Monohydrate	121
Corn Oil	60
Non-Nutritive Fiber (Cellulose)	36
Mineral Mix, Williams-Briggs Mod.	43
Vitamin Mix, Teklad	12
Casein (Vitamin Free)	150
or	
Freeze Dried Swordfish	

The mercury content of swordfish was determined by flameless atomic absorption spectrophotometry (OHI et al, 1976). Swordfish seleneum content was analyzed on freeze dried samples by reaction with diaminonaphthalene (OLSON, 1969). The % hydration of fish samples was determined by quantitating the change in weight of fish samples before and after freeze drying.

RESULTS

The body weights of rats fed either a 15% casein diet or a 15% freeze dried swordfish diet both in the presence and absence of 200 ppm methylmercury chloride is shown in Figure 1. As can be seen, there was no consistent difference between the control groups fed 15% casein or 15% freeze dried swordfish. This is consistent with similar food consumption in both groups. Both groups fed 20 ppm methylmercury chloride gained weight less rapidly than their corresponding controls. Through the first nine weeks of this study there was no significant difference between these two groups. However, it is noteworthy that in the last week of this study there was a precipitous loss in mean body weight in the casein group of 20 grams per rat.

The lethal effects of methylmercury chloride to groups fed 15% casein or 15% dried swordfish is shown in Figure 2. There was noteworthy protection from toxicity observed in groups fed the 15% freeze dried swordfish in contrast to the 15% casein. However, when mean survival time was calculated, the 15% casein - 40 ppm methylmercury chloride rats survived 4.6 weeks while the 15% freeze dried swordfish groups survived 5.3 weeks. Although

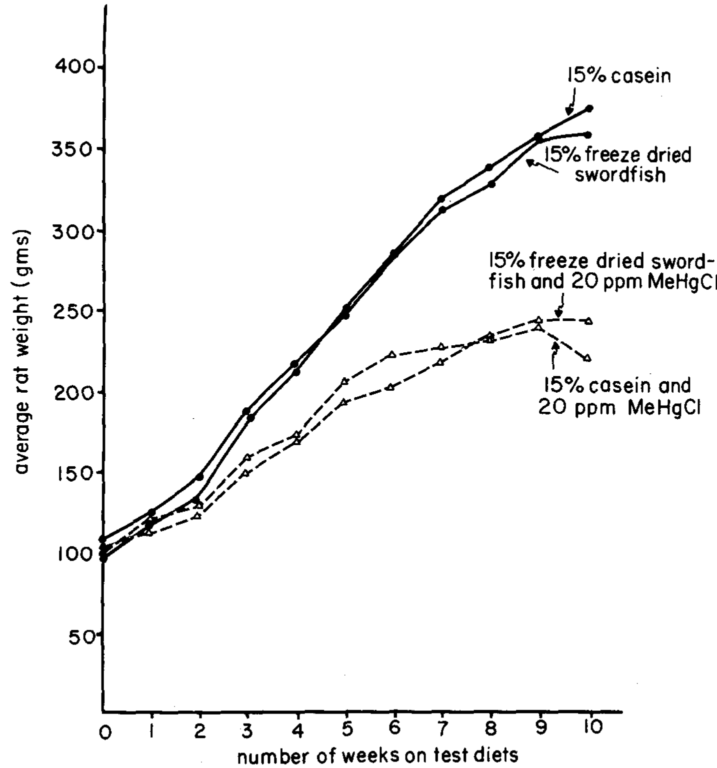

FIGURE 1. AVERAGE BODY WEIGHT IN RATS FED FREEZE DRIED SWORDFISH AND METHYLMERCURY CHLORIDE

this represents only a 15% increase in survival, if the data were expressed in terms of daily survival in contrast to weekly survival one would observe a greater than 30% increase in survival. No mortality was observed in the group fed 15% freeze dried swordfish in combination with 20 ppm methylmercury chloride. In contrast, one of the 10 rats fed 15% casein in combination with 20 ppm methylmercury chloride died.

Figure 3 shows the neurotoxicity of the dietary methylmercury chloride in each of the experimental groups. As can be seen, there was no difference in the number of animals showing neurotoxicity in the 15% casein and 15% freeze dried swordfish fed rats in response to 40 ppm methylmercury chloride although the serverity of the response was greater in the casein group. In contrast there was a marked difference in the case of the responses to the 20 ppm methylmercury chloride. The most striking difference

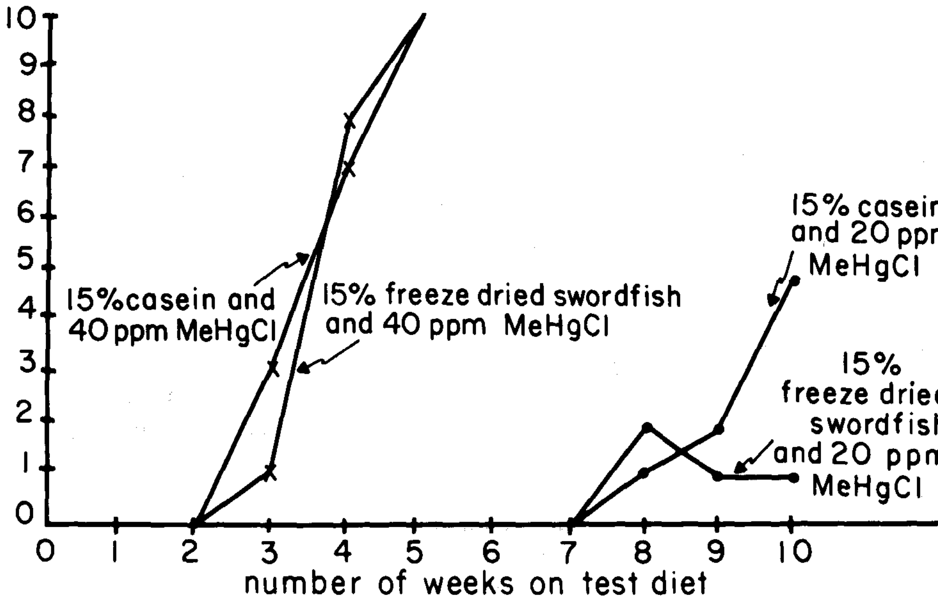

FIGURE 2. MORTALITY IN ANIMALS FED FREEZE DRIED SWORDFISH AND METHLYERMCURY CHLORIDE

was in the tenth week when 50% of the 15% casein fed rats showed neurotoxicities while only 1 of the 10 freeze dried swordfish fed rats showed any sign of toxicity. There was 1 freeze dried swordfish fed rat which showed the crossed leg behavior in week 7 but did not demonstrate this manifestation in any later examination.

The relationship between original fish weight and the water content, total mercury and seleneum content of the steaks is shown in Table II. Twenty-one steaks were submitted for analysis coming from fish weighing between 22 lbs. and 333 lbs. Inspection of these data suggests that fish weighing from 225 lbs. and 300 lbs. have more mercury than their larger or smaller counterparts. This was analyzed by linear regression using the formula: fish weight = $B_o + B_1$ (mercury content in ppm). This provided an excellant fit with B_o (which would represent the minimum mercury content) at 0.72 ppm and B_1 (which represents the increase of ppm mercury per pound of fish) at 0.00263. The correlation coefficient was 0.517. There was also a significant relationship between % solids in the steaks and fish weight with a correlation coefficient of 0.640.

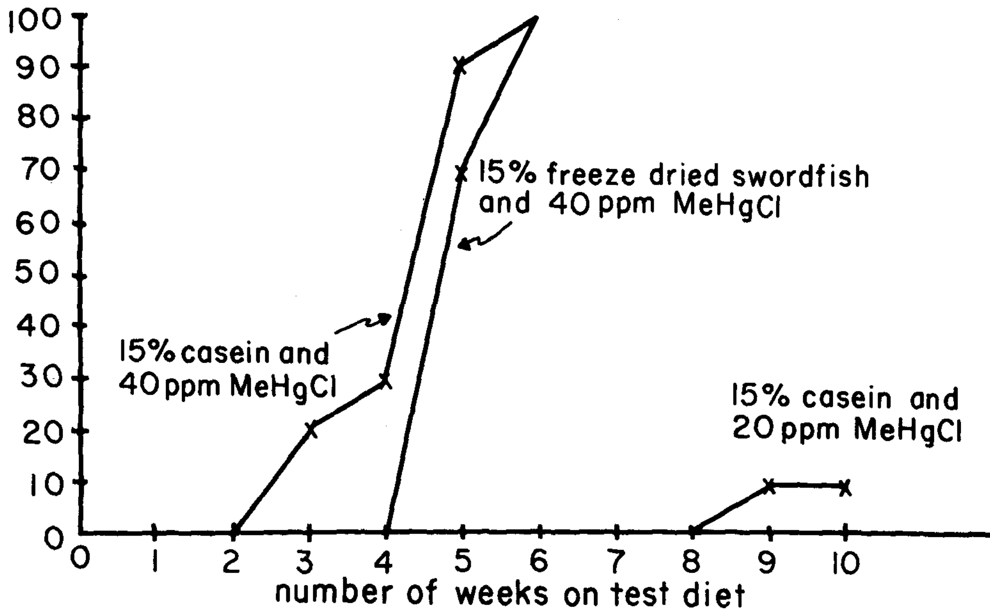

FIGURE 3. NEUROTOXICITY IN RATS FED FREEZE DRIED SWORDFISH AND METHYLMERCURY CHLORIDE

DISCUSSION

Two questions were raised and answered by this research. First, does swordfish consumption protect against methylmercury chloride toxicity? From Figures 1, 2, and 3 the answer appears to be that there is (are) protective agent (s) in swordfish. Secondly, is there a relationship between fish size and mercury content? From Table II one can see a relationship between fish size and mercury content which parallels but does not correlate to content of solids in the fish.

The presence of these protective factor (s) in swordfish is neither unique nor unexpected. Ganther et al originally showed that tuna protected against methylmercury toxicity (GANTHER et al, 1972) which has been confirmed by many investigators (IWATA et al, 1973) (OHI et al, 1976) (STILLINGS et al, 1974). Many of these communications tested sodium selenite for its protective activity and indicated that seleneum was the active factor. The seleneum concentration in swordfish (Table II) compares favorably with that found in tuna.

Although the protection against methylmercury toxicity was probably due to the seleneum in swordfish, there were several fundmental differences in the two diets tested here.

TABLE II
RELATIONSHIP BETWEEN FISH SIZE, SOLIDS CONTENT
MERCURY CONTENT AND SELENEUM CONTENT

Fish Wt.	% Hydration	ppm Hg.	ppm Se
22 lbs	31.6%	0.40	1.96
49 lbs	30.7%	0.89	1.40
55 lbs	25.5%	0.77	2.20
68 lbs	32.4%	0.42	3.52
73 lbs	36.2%	0.30	2.17
88 lbs	34.8%	0.84	1.83
96 lbs	32.0%	0.98	2.67
108 lbs	36.2%	1.15	0.79
112 lbs	37.4%	1.27	1.91
140 lbs	26.7%	1.67	2.81
140 lbs	32.4%	0.79	0.86
227 lbs	32.5%	1.30	1.56
254 lbs	37.4%	1.71	Lost
260 lbs	38.8%	1.27	0.91
268 lbs	34.3%	1.65	1.85
279 lbs	38.7%	1.35	1.68
294 lbs	36.4%	1.61	4.49
294 lbs	34.4%	0.91	2.97
311 lbs	39.2%	0.83	1.50
316 lbs	36.7%	0.98	4.84
333 lbs	42.4%	0.97	1.58

% Hydration = Freeze wt/Frozen wt x 100%
ppm Se = ug Se x (100%) ÷ % Hydration

The casein supplemented diet contained 15% protein and the rest of the components listed in Table I. Freeze dried swordfish, although rich in protein, (approximately 70%), contained fatty acids and triglycerides, and small amounts of other normal meat constituents. Although diet consumption in the casein and freeze dried swordfish were very similar, the diets were probably not isocaloric. The significance of these differences is currently not clear.

The mercury content of the swordfish presented here is consistent with other published values for mercury in swordfish (KAMPS et al, 1972). Furthermore, the increase in mercury content with fish size has been shown in Pacific blue marlin (RIVERS et al, 1972). The mean mercury and seleneum values in swordfish tested were 1.05 $\pm$ 0.41 and 2.18 $\pm$ 1.19, respectively. There is, therefore, at least twice as much seleneum as mercury in these samples. In fresh water fish there is 2.1 --3.4 times more mercury than seleneum (ROSSI et al, 1976). In aquatic mammals, which consume large quantities of salt water fish, there is 2.5 times more seleneum than mercury in their livers. The action level for mercury in fish was set on people consuming fresh water fish which have low seleneum contents in contrast to ocean fish with high seleneum levels.

The practical relevance of these data to the establishment of guidelines on swordfish consumption is readily apparent. The observations bearing on this are as follows (1) Swordfish has twice as much seleneum as mercury. (2) Freeze dried swordfish protected rats from methylmercury toxicity. (3) Rats fed a diet containing 15% freeze dried swordfish for 70 days showed no toxic effects. In light of the above observations, supported by considerable published observations, one may want to raise the question of the harmful effects of dietary swordfish.

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