gold K electron x-ray peak at 67 to 69 kev and the gold K electron x-ray and gamma-ray peak at 77 to 80 kev—both of which result from the electron-capture decay of 64-hour ^{197}Hg. Samples of "modern" tuna and swordfish, that is, fish caught during 1970–71, were activated and counted in the same fashion. The lower limit of the sensitivity of our instrumental NAA technique, as used in these experiments, was about 0.02 ppm for mercury in a 1-g sample.

A summary of the mercury levels observed in these fish samples is given in Table 1. After the activation analysis, the loss of weight on drying for 16 hours at 100°C was determined for each specimen, so that the mercury results could be expressed on a dry-weight basis (last column of Table 1). The volatile content (such as water or alcohol) varies appreciably among such samples. In fresh tuna and swordfish the mercury content (ppm) on a wet-weight basis is typically between 3.3 and 4.0 times lower than the mercury content (ppm) on a dry-weight basis; this corresponds to a moisture content of 70 to 75 percent.

The mean mercury concentration (dry-weight basis) in the seven museum tuna samples listed in Table 1 is 0.95 ± 0.33 ppm (the values range from 0.53 to 1.51 ppm). The corresponding mean mercury concentration for the five recent tuna samples is 0.91 ± 0.47 ppm (the values range from 0.44 to 1.53 ppm). It is evident that there is no significant difference in mercury concentration between the museum tuna samples, caught 62 to 93 years ago, and the samples of tuna caught recently. The average mercury value (1.36 ± 0.31 ppm on a dry-weight basis) for the single specimen of swordfish caught 25 years ago falls within the range of values (0.94 to 5.1 ppm) found for "modern" swordfish. The data for both tuna and swordfish lend support to the contention that the mercury levels now being found in wide-ranging ocean fish are not primarily the consequence of man-made pollution but are of natural origin.

There is no reason to believe that the seven museum specimens of tuna were contaminated with any additional mercury during the time they were preserved in formaldehyde, and then alcohol, in the Smithsonian Institution, although this possibility cannot be rigorously excluded. For the swordfish, samples of the original preservative solution were also available. In addition, "background" samples were obtained, consisting of a smaller fish—the pipefish (*Syngnathus griseolineatus leptorhyncus*)—caught in 1946 off Baja California, and its preservative solution, the same brand of 40 percent isopropanol. No mercury was detected in the isopropanol used for preserving the swordfish or the pipefish, and only a minute concentration of mercury, about 0.17 ± 0.15 ppm (dry-weight basis), was detected in the pipefish sample (whole fish). The absence of any significant mercury content in either the pipefish or its preservative solution is evidence that there was no mercury contamination from (i) the original preservative solution, (ii) subsequent external contamination during storage, or (iii) external contamination during our measurement procedures.

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4. The estimated mercury production is several times 10^5 metric tons, plus about 10^5 metric tons released by the burning of coal. In the world's oceans there are about 1.3×10^{18} metric tons of water, with an average concentration of mercury of about 0.1 ppb.
5. This broadbill swordfish (*Xiphias gladius*) specimen was not a typical specimen, although its abnormality presumably is not related to its mercury content. It was caught in August 1946 off the west coast of Baja California, and was found to have its bill bent around and embedded in its head. It weighed between 160 and 165 pounds (73 to 75 kg). The samples analyzed for mercury were flesh samples taken from regions near the head.
6. All tuna samples were homogenized portions of the light muscle taken from the center of the right dorsal side.
7. Loss of mercury from samples sealed in polyethylene vials has been reported by L. C. Bate [*Radiochem. Radioanal. Lett.* **6**, 139 (1971)] for much more intense neutron-irradiation conditions than ours. We have also observed a small loss of mercury from aqueous standard solutions in polyethylene vials, but not from fish samples, under our irradiation conditions.
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Selenium: Relation to Decreased Toxicity of Methylmercury Added to Diets Containing Tuna

Abstract. *Japanese quail given 20 parts per million of mercury as methylmercury in diets containing 17 percent (by weight) tuna survived longer than quail given this concentration of methylmercury in a corn-soya diet. Tuna has a relatively high content of selenium and tends to accumulate additional selenium when mercury is present. A content of selenium in the diet comparable to that supplied by tuna decreased methylmercury toxicity in rats. Selenium in tuna, far from being a hazard in itself, may lessen the danger to man of mercury in tuna.*

Tuna and swordfish have a tendency to accumulate Hg in excess of 0.5 part per million (ppm), the content established by the U.S. Food and Drug Administration as the maximum allowable concentration. This fact raises the following important questions: (i) How toxic is a low concentration of Hg, as ingested in the form of tuna? and (ii) What factors present in tuna might cause Hg to accumulate or might modify its toxicity? We have been engaged in a study of the long-term effects of various concentrations of Hg in diets containing tuna. We here describe the surprising finding that tuna in the diet decreases the toxicity produced with high concentrations of methylmercury. Evidence is presented that Se present in tuna may be responsible for this effect.

Approximately 2000 pounds (906 kg) of canned tuna having an Hg content of 0.7 to 1.0 ppm (total contents of the can, fresh basis) was obtained from the processors. The cans were opened and drained, and the solids were lyophilized at ambient temperature to a moisture content of approximately 2 percent (by weight) and then packed in 5-gallon (18.9-liter) cans under nitrogen ("high-Hg tuna"). A smaller quantity of tuna with an Hg content of 0.09 to 0.14 ppm was obtained from the same sources and dried in the same way ("low-Hg tuna"). Preliminary experi-

ments had shown that lyophilization produced a form of tuna suitable for incorporation into diets, without significant loss of Hg. The tuna was analyzed for Hg by the flameless atomic absorption method (*1*). A small portion of the high-Hg tuna was sprayed with an aqueous solution of methylmercury hydroxide (Alfa Inorganics, Beverly, Mass.), aired in a hood for 2 days, and then mixed thoroughly to give a "premix" with an Hg content of 850 ppm.

A total of 360 1-day-old Japanese quail (*Coturnix coturnix japonica*) were divided into eight groups of 45 each. Two groups received a corn-soya diet (*2*) containing either 0 or 20 ppm of Hg added as the methylmercury premix. This premix added only 2.4 percent of tuna to the diet. The other groups were fed diets containing 17 percent tuna, from the low-Hg tuna, high-Hg tuna, or mixtures to achieve Hg concentrations of approximately 0.075, 0.25, and 0.5 ppm. Mercury concentrations of 1, 10, and 20 ppm were obtained by adding the Hg premix to high-Hg tuna. The contents of soybean meal and corn in the tuna diets were decreased and the content of oats was increased to keep crude protein content and energy level approximately equal to those of the corn-soya diet; adjustments were also made for calcium-phosphorus balance and salt intake. All diets were prepared weekly and replaced daily to minimize deterioration. At the end of week 4 approximately 25 birds from each group were retained for breeding, and the remaining birds were killed so that their tissues could be analyzed for Hg. The rate of growth and feed intake (Table 1) for all diets were comparable through week 4, thereafter declining somewhat in groups 7 and 8 which were receiving 20 ppm of Hg. At approximately 4 weeks, incoordination and other signs of Hg poisoning began to appear in group 8 receiving the corn-soya diet; this group had a high mortality rate, reaching a maximum on day 42. Between week 4 and week 6, 52 percent of the quail in this group died. In contrast, the quail in group 7, receiving the same amount of Hg in a tuna diet, were free of signs of poisoning for a longer time and only 7 percent died between week 4 and week 6. By week 7, however, deaths in this group were frequent as well, reaching a maximum rate on day 48. It is apparent that something in the tuna diet prolonged survival in quail given high concentrations of Hg.

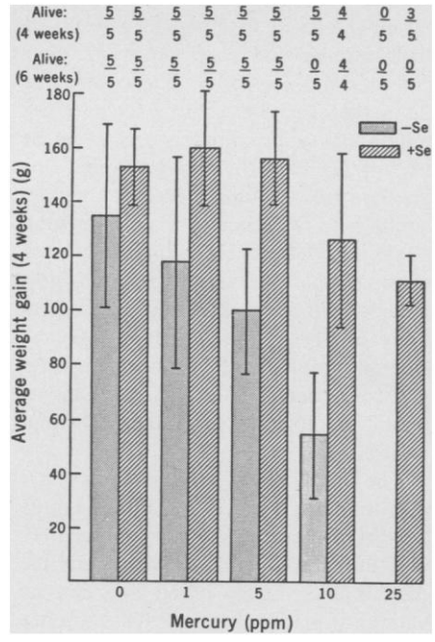

Fig. 1. Effect of the dietary content of Se on the toxicity of methylmercury added to the drinking water of rats. Fifty weanling male Holtzman rats weighing 40 to 60 g each were divided into ten groups of five each and housed in individual wire cages. All rats were depleted of vitamin E on a basal purified diet containing (in percentages): vitamin-free casein, 20; glucose monohydrate, 66.9; stripped lard, 6; cod liver oil, 2; salts (*6*), 4.8; vitamins (*6*), 0.25; and choline chloride, 0.05. After a 2-week depletion, five groups were given the basal diet supplemented with 0.5 ppm of Se as Na_2SeO_3, and five groups continued to receive the unsupplemented basal diet. Simultaneously, Hg was administered for 6 weeks in the drinking water in the form of methylmercury hydroxide at 0, 1, 5, 10, and 25 ppm of Hg. Growth during weeks 1 through 4 after Hg administration is shown by the bar for each group (mean ± standard deviation). The average weight gain at week 2 for the group receiving 25 ppm of Hg but no Se (four out of five surviving) was 24 ± 5 g, as compared to 37 ± 10 g for the group receiving Se plus 25 ppm of Hg.

Fish meals and marine foods in general have a higher content of Se than products from animals of terrestrial origin (*3*). The Se content of tuna is comparable to that of fish meals and always exceeds the content of Hg. We analyzed several multicase lots of either low-Hg or high-Hg tuna for Hg and Se (*4*) after lyophilization. Surprisingly, we found that the low-Hg tuna (0.32 ppm of Hg on the average) contained less Se (1.71 to 2.16 ppm; average, 1.91 ppm) than the high-Hg tuna (2.87 ppm of Hg, 2.43 to 3.40 ppm of Se average, 2.91 ppm). On a molar basis, the Hg/Se ratio approached 0.5 in the case of the high-Hg tuna. Moreover, the increment in Hg content between low- and high-Hg tuna was in an approximate 1 : 1 molar ratio with the increment in Se. It thus appears that Hg and Se tend

Table 1. Comparison of methylmercury toxicity in Japanese quail fed tuna or corn-soya diets. The total number of birds in each group was 45; data for the period between week 4 and week 6 are for approximately 15 females (F) and 10 males (M) retained for breeding.

Group No.	Diet*	Mercury (ppm)		Feed consumed (g per chick per day)		Average weight gain (g)				Mortality‡ (%)	
						0–4 weeks		0–6 weeks			
		Added as methylmercury	Total†	2–4 weeks	4–6 weeks	M	F	M	F	0–4 weeks	4–6 weeks
1	Corn-soya	0		10.4	14.3	83	87	101	116	0	0
2	Tuna	0	0.048–0.075	9.4	13.5	82	82	102	113	2	0
3	Tuna	0	0.22 –0.26	9.2	12.4	78	80	100	106	2	0
4	Tuna	0	0.46 –0.51	9.6	13.4	84	82	106	108	13	0
5	Tuna	0.5	0.83 –1.01	9.2	12.6	79	80	107	105	7	0
6	Tuna	9.5	10	9.1	13.1	78	83	104	104	4	0
7	Tuna	19.5	20	9.0	12.8	78	83	97	102	7	7
8	Corn-soya	20.0	20	9.9	§	82	85	95	94	9	52

* See (*2*) for composition of diets. The total Se content (in parts per million) of the analyzed diets was as follows: group 1, 0.23; group 7, 0.67; and group 8, 0.35. † Calculated from the Hg content of tuna (various lots) plus the Hg added as methylmercury. The Hg content of the corn-soya diet (group 1) was that of the reagent blank. ‡ Excluding accidental deaths. § Not calculated because of high mortality during this period.

to be accumulated together in tuna.

Since tuna has a substantial content of Se, the question arises of whether this amount of Se is in itself a hazard, or whether its presence might reduce the toxicity of Hg. The content of tuna added to the diet in these experiments supplied approximately 0.3 to 0.6 ppm of Se. This is a desirable nutritional content and is roughly an order of magnitude less than the content at which signs of toxicity appear (*5*). Since the growth of quail on the tuna diets was comparable to that on corn-soya diets (Table 1), no evidence exists that this content of Se was harmful.

Direct evidence that dietary Se can decrease the toxicity of methylmercury was obtained in a study in which rats received a purified basal diet containing 20 percent casein or the same diet supplemented with 0.5 ppm of Se, with various concentrations of methylmercury added to the drinking water (Fig. 1). The basal diet was quite low in Se content (approximately 0.02 ppm) and also lacked vitamin E, since the experiment was designed to test whether Hg might induce signs of Se–vitamin E deficiency in a manner similar to that observed with Ag (*6*). Although Se produced a slight growth response when added to the basal diet, no signs of Se or vitamin E deficiency were observed in any of the groups tested. Mercury depressed growth at all concentrations, and Se improved growth at all concentrations of Hg, the effect being most evident at the higher concentrations of Hg. The most interesting finding, however, was that all the rats fed 10 ppm of Hg without Se had died, but those fed the same diet with Se were still alive at the end of week 6. In the groups receiving 25 ppm of Hg, all animals were dead by week 6, but the addition of Se increased the survival at week 4.

The suggestion that Se in tuna may reduce the toxicity of Hg is supported by studies from Parizek's laboratory showing that Se reduces the acute toxicity of $HgCl_2$ injected into rats, and may complex with Hg in the blood to decrease the availability of each element (*7*). The biological availability of Se in tuna is only 50 percent of that found for other sources of Se, even though comparable concentrations of Se in the blood are attained regardless of the source (*8*); it may be no coincidence that molar ratio of Hg to Se in tuna approaches 0.5.

Selenium, like S, readily complexes with Hg, and both Se and Hg tend to be associated with S in proteins. It is thus reasonable to expect that Se and Hg might occur together, as a result of their affinity for each other or their common affinity for S. The content of Se in the oceans is about 0.09 part per billion (*9*), approximately the same as that of Hg (*9, 10*); thus both elements are strongly accumulated by tuna. In addition to Se, other substances in tuna might also affect Hg accumulation or toxicity. The observation (*10*) that individuals can have elevated concentrations of Hg in the blood and yet be free of symptoms of Hg poisoning suggests, as one possibility, that agents modifying toxicity may indeed be operative.

The implications of this study are that the danger for man of Hg in tuna may be somewhat less than anticipated, and that the total Hg contents in the diet or even in the blood may not be valid criteria because of the presence of modifying factors. Clearly, further research on these matters is needed.

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Chalcocite Oxidation and Coupled Carbon Dioxide Fixation by Thiobacillus ferrooxidans

Abstract. *The reaction of cell suspensions of* Thiobacillus ferrooxidans *with pulverized chalcocite (Cu_2S) in a Warburg manometric apparatus resulted in oxygen uptake accompanied by increased solubilization of copper and fixation of carbon dioxide. Since the only detectable oxidized products were cupric ions and the more oxidized form of the sulfide mineral, that is, digenite or covellite, the apparent source of energy for the carbon dioxide fixation was provided by the oxidation of the cuprous copper of the chalcocite.*

Thiobacillus ferrooxidans, a chemolithoautotrophic bacterium, obtains energy for CO_2 fixation and growth by oxidizing certain reduced forms of sulfur or Fe^{2+} (*1*). Furthermore, the oxidation of certain sulfide-containing minerals yields energy for CO_2 fixation (*1*) and usually produces SO_4^{2-} accompanied by a lowering of the *p*H of the reaction mixture. Fox (*2*) noted an increased *p*H when growing *Ferrobacillus (Thiobacillus) ferrooxidans* on chalcocite (Cu_2S) and speculated therefore that SO_4^{2-} was not a product of chalcocite oxidation and that the small amount of growth observed resulted from the energy made available by the oxidation of Cu^+ to Cu^{2+}. We pre-