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Clearance of Arsenic Ingested by Man From Arsenic Contaminated Fish

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Certain species of commercial fish caught on the high seas, as well as those caught in some coastal areas of Canada, have levels of arsenic many times higher than the upper limit believed to be safe for food. Repeatedly investigations have shown that arsenic occurs in fish mainly as arseno-organic compounds and not in the highly toxic arsenite form (REINKE et al. 1975). This compound has been of great concern because it has not been fully characterized and its toxicity to man is unknown.

We report on the clearance of total arsenic, by urinary excretion from six volunteers who ate two consecutive meals of commercial fish containing naturally high levels of arsenic. The purpose of this study was to determine whether or not arsenic from these fish was substantially retained by man, and at what rate it was cleared, in order to obtain additional information that may be used in assessing the toxicity of this arsenic form in man.

MATERIALS AND METHODS

Six lb of flounder fillets (mixed species), purchased from a local fish market, were minced in a food chopper and blended to give a homogeneous mixture. The fish mixture was analyzed for arsenic and was found to contain 13.6µg/g (wet wt.).

Fish Eating Experiment. Six men each ate a total of 385 g of the arsenic-contaminated fish divided between two consecutive meals, one at 11:00 a.m. and the second at 3:00 p.m.. The fish consumed at each meal were in the form of fish cakes made from a homogeneous mixture of the minced flounder plus filler composed of corn flakes, hens eggs, Worstershire sauce, and finely chopped parsley. Each man ate 2 fried fish cakes/meal, which meant the participants ate 192.5 g of fish and 72.4 g of filler at each meal. There was no significant quantity of arsenic added by the filler and additives, and the total quantity of arsenic ingested per person per meal was 2.5 mg, making a total of 5 mg over

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the two consecutive meals. Each participant was asked to limit his fluid intake to approximately 2 L per day.

Determination of Arsenic. Total arsenic was determined in the fish cakes and urine by the method of FREEMAN et al. (1976) using an atomic absorption spectrophotometer. The acidified (4% HNO_3) urine samples were determined directly, after dilution with water to the required concentration. The minced fish were prepared for analysis by the wet ashing procedure of UTHE et al. (1974).

Collection of Urine for Arsenic Determination. Total urine samples were collected separately by each participant at each urination in glass bottles. Nitric acid (4%) (v/v) and ca. 2 drops of chloroform were added to each bottle of urine. The samples were kept under refrigeration until analysis. Urine samples were also collected by all participants the day prior to eating the fish for the determination of the normal arsenic concentrations in the urine.

At the beginning of the experiment, arsenic was determined in duplicate on individual urine samples, but towards the end of the experiment when little arsenic was excreted, daily collections of urine were combined for a single determination.

During the ten day period of the experiment, the participants did not eat any additional fish, other than the experimental fish, and did not drink any alcoholic beverages. This was done to help keep arsenic intake from other sources to a minimum and urine volumes constant.

RESULTS AND DISCUSSION

During the day prior to consuming the fish, very little arsenic was excreted in the urine of any of the participants (Fig. 1). The quantities of arsenic eliminated in the urine over this period ranged from 1.5 to 9.8 µg (mean 4.8 µg).

The first meal of fish was eaten at 11:00 a.m. and a significant quantity of arsenic was excreted by all participants by 1:00 p.m. on the same day with the mean quantity of arsenic cleared to that time being 296 µg or ca. 12% of that ingested in the first meal. Two days after eating the fish over 50% of the arsenic ingested had been excreted (Fig. 1, Table 1). From this time on, the rate of clearing arsenic decreased considerably and started to "level off" (Fig. 1). The collection and analysis of urine for arsenic was terminated on the eighth and ninth day, after eating the fish. At this

time little arsenic was being cleared by anyone except subject 4 (Table 1). The mean total arsenic excreted for all six men up to this time was 77±11% (Table 1) of the 5 mg of arsenic injested. The remainder of the arsenic may have been retained in the body or may have been excreted via other mechanisms such as fecal or respiratory routes.

Although six subjects is too limited a number to draw definite conclusions, the shapes of the excretion curves (Fig. 1) for all six are worth noting. While the total amounts excreted varied, the shapes of the excretion curves were quite similar in five out of six cases. Only the curve for subject 4 differs (Fig. 1) where arsenic was excreted at a faster rate and 90% of the arsenic was excreted by day 8 (Table 1).

The percentage of arsenic excreted in the urine by the men in the present study are variable as was found for four female adult cynomologus monkeys fed fish of high (77 µg/g) arsenic content (CHARBONNEAU et al. 1977). In both the men and the monkeys most of the arsenic ingested was excreted in the urine within two days after dosing with arsenic from fish.

TABLE I

Daily percentage of arsenic excreted from that ingested (5018 µg)* by six subjects.

Subject #	1	2	3	4	5	6
Time in days (from first meal)	% Excreted					
0.5	53.2	35.7	31.9	39.2	29.5	35.1
1	10.2	14.6	20.0	16.0	19.8	10.9
2	16.1	12.6	8.0	11.2	6.0	7.9
3	4.1	4.3	4.5	7.0	2.9	4.2
4	2.1	3.7	4.5	5.7	3.5	2.5
5	1.3	3.0	2.4	4.3	2.4	1.5
6	1.1	2.7	1.6	3.4	1.3	1.0
7	0.6	1.1	0.8	2.3	0.9	0.4
8	0.4	0.5	0.5	1.0	0.2	0.3
9	0.2		0.2			
TOTAL	89%	78%	74%	90%	67%	64%

$\bar{X}$ Total arsenic excreted (8-9 days) = 77±11.0%.
* In 385 g of flounder.

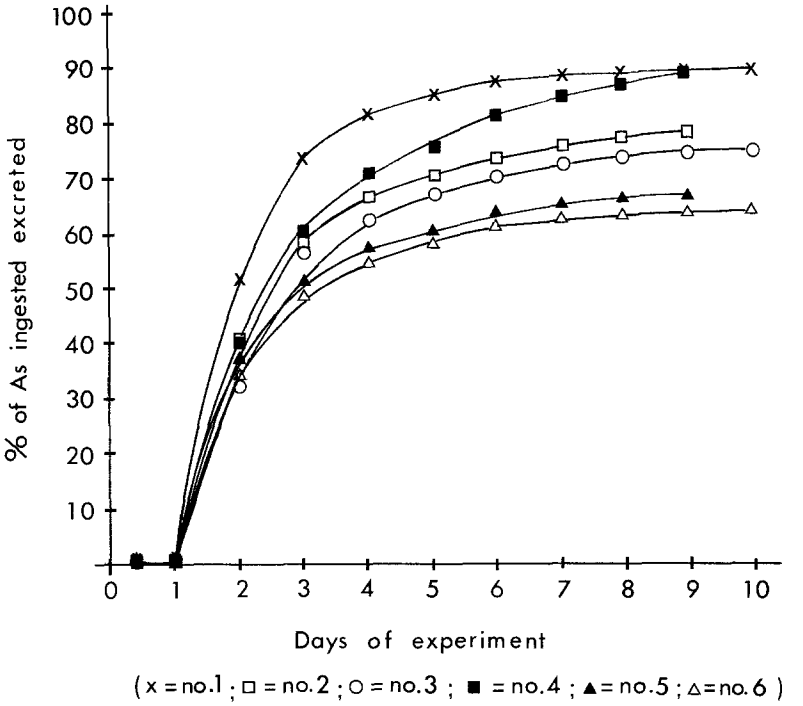

Figure 1. The Rate of Clearance of Arsenic from Fish by Six Men.

CHAPMAN (1926) fed lobster containing 33 µg of arsenic to a person and found that 24.3 µg or 74% of the arsenic ingested was cleared in the urine. This is close to the mean percentage (77%) excreted by the six men in the present study. He also reported that 0.90 µg or 3.7% of the arsenic excreted in the urine was chemically reducible without wet ashing, indicating that a very small quantity of the arsenic was excreted in the inorganic form.

CHAPMAN (1926) repeated the lobster experiment with another person who ingested less than half the arsenic consumed in the first experiment. Here again most of the arsenic was excreted in the urine in a combined form which could not be reduced directly for chemical determination without first being oxidized with nitric and sulphuric acids.

COULSON et al. (1935) carried out arsenic excretion experiments using 4 adult rats and 2 human subjects in order to determine whether or not the arsenic compound present in shrimp was absorbed from the gastrointestinal tract and to obtain some information upon the relative rate of excretion of "shrimp arsenic" as compared with inorganic arsenic. They found that

in the rats most of the arsenic ingested from the shrimp was excreted in the urine within a few days, but about 80% of the ingested inorganic arsenic was retained. It is interesting to note that about 96% of the arsenic from the shrimp was excreted in the urine of the rats whereas the inorganic arsenic was equally divided between the urine and the feces. Their results indicate that the arsenic compound from shrimp was very readily absorbed by the gastrointestinal tract of the rat, but was rapidly and almost completely eliminated. When studying clearances with 2 humans, COULSON et al. (1935) found that the arsenic from shrimp was excreted as in the rat, but the inorganic arsenic, although excreted more slowly than the "shrimp arsenic", was eliminated more completely by man than by the rat.

In other experiments where approximately equal quantities of arsenic as arsenic trioxide and arsenic in shrimp were fed to two groups of rats over a three-month period, only 0.7% of the ingested "shrimp arsenic" was accumulated in the bodies of the rat, while more than 18% of the inorganic arsenic was stored (COULSON et al. 1935).

More recently, CRECELIUS (1977) studied the clearance of arsenic from a 30 year old man after eating cooked Dungeness crab *(Cancer magister)* meat. He reported that most of the arsenic in the cooked crab meat was in the form of an arseno-organic compound, but after the tissue was digested overnight in 2N sodium hydroxide at 80 C, approximately 90% of the arsenic present was converted to dimethylarsenic acid (DMAA). His data indicated that the arseno-organic compound in crab meat was excreted in the urine within 1 to 2 days after ingestion without change in its chemical form. EDMONDS and FRANCESCONI (1977) reported some preliminary studies in which people consumed fish containing arsenic. They reported, in agreement with CRECELIUS (1977), that arsenic remained in an organically bound state in the body and was excreted in that form in the urine.

EDMONDS and FRANCESCONI (1977) were able to break down the isolated arseno-organic complex excreted from the mussel *(Mytilus edulis planulatus)*, the rock lobster *(Panulirus longipes cygnus)*, and stingray *(Dasyatis thetidis)* by sodium hydroxide digestion (10 to 40% sodium hydroxide) to dimethyl and trimethyl arsenic and a small portion of inorganic arsenic, but they did not identify the parent compound.

We have found, in agreement with CHAPMAN (1926), COULSON et al. (1933), CRECELIUS (1977), and EDMONDS and FRANCESCONI (1977), that the arsenic which occurs in fish is mainly an arseno-organic compound. We have

also found that it is necessary to break down this compound by vigorous acid digestion in order to quantify the arsenic using the reduction method where arsine is generated (UTHE et al. 1974).

Our studies also agree with those of CHAPMAN (1926), COULSON et al. (1935), and CRECELIUS (1977) in that most of the arsenic has a short residence time in man. It is factual however, that a short residence time in man is not synonymous with lack of toxicity.

We have not as yet, determined the form in which arsenic was excreted in the urine collected during our arsenic clearance studies, but this will be done shortly. We may conclude, however, that there is much to be done before a detailed toxicological assessment can be made of arsenic in marine fauna.

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