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Arsenic Excretion by Monkeys Dosed with Arsenic-containing Fish or with Inorganic Arsenic

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The presence of high levels of arsenic in certain edible marine fish and shellfish has been well documented (CHAPMAN, 1926; MONNIER-WILLIAMS, 1950). More recently, high levels have been reported for fish and shellfish from Canadian waters (LEBLANC and JACKSON, 1973; MUNRO, 1976). Recently, EDMONDS et al. (1977) have identified the form of arsenic in rock lobster as arsenobetaine. The chemical forms of arsenic in other species of marine life have not yet been identified. However, preliminary data suggest that the arsenic present in fish is not arsenobetaine (PENROSE et al. 1977).

Excretion studies in man by COULSON et al. (1935), WESTOO and RYDALV (1973) and CRECELIUS (1977) using arsenic present in shrimp and crab, suggest that most of the ingested arsenic is excreted in the urine within a few days. Because of its rapid elimination, the arsenic compounds in edible marine products have been considered to pose little threat to human health. However, the analytical method used by COULSON et al. (1935) may not have accounted for all of the organic arsenic compounds excreted, and in the studies by WESTOO and RYDALV (1973) and CRECELIUS (1977) urinary but not fecal, excretion of arsenic was examined. Thus it was decided to repeat the excretion studies using modern analytical techniques and to measure the extent of arsenic excretion in both the urine and feces.

Data on the elimination of inorganic arsenic following oral ingestion in man suggest that the majority of arsenic is excreted via the urine, the remainder being stored in the body (COULSON et al., 1953; MEALEY et al., 1959; RAY BETTLEY and O'SHEA, 1975). However, to critically assess the extent of arsenic storage in the body metabolic balance studies are required. Consequently, an arsenic balance study was undertaken in monkeys to determine the excretion pattern for arsenic administered orally in the form of fish.

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Methods

Four female adult Cynomologus monkeys of 2.9 to 4.4 kg. body weight were used. They were housed in individual stainless steel metabolism cages equipped with wire screen floors and urine collection trays. Food (Purina Primate Chow) and water were provided ad libitum throughout the experiment. Analysis of the monkey chow and water for total arsenic showed 0.10 ppm arsenic in the food and 0.01 ppm in the water. Food consumption was measured daily throughout the experiment. Body weights were recorded weekly and the animals were observed daily after dosing for general health. To assess background arsenic excretion, 24 hr samples of urine and feces were collected and analyzed for total arsenic for 10 days prior to dosing. Following this period of acclimatization, the monkeys were given a single test meal of high arsenic fish, Altantic grey sole (Glypocephalus cynoglossus) by stomach tube. The fish was prepared by homogenizing the edible muscle portion in a waring blender. The fish homogenate, analyzed in quadruplicate for total arsenic, contained 77 ppm As. The amount of homogenate administered to the animals provided a dose of approximately 1 mg fish-arsenic/kg bw. Following dosing with the fish homogenate, 24 hr samples of urine and feces were collected for 14 days and analyzed for total arsenic content. Average daily background excretion of arsenic in the urine and feces was substracted and the percent of the administered dose excreted per day in the urine and feces was calculated.

Following an additional two week period to ensure that the arsenic excretion values returned to baseline, the experiment was repeated on the same animals using arsenic trioxide. After a 7 day period during which 24 hr samples of urine and feces were collected to re-affirm base-line levels, arsenic trioxide (to provide a single dose of 1 mg As/kg body weight) was administered by stomach tube. One monkey (E-772; Table II) vomited a small amount after dosing. The concentration of arsenic in the vomitus was determined and the total amount of arsenic received was corrected for the amount loss due to vomiting. Twenty-four hour samples of urine and feces were collected for 14 days after dosing and analyzed for total arsenic content. Food consumption was decreased for one day following dosing with inorganic arsenic in monkeys E-772 and C-53.

All arsenic analyses were conducted using a modification of the method of MORRISON and GEORGE (1969). The samples were mixed with a magnesium oxide/magnesium nitrate slurry, dried and ashed. Hydrochloric acid, potassium iodide and stannous chloride were added to

TABLE I

Percent of Dose of Fish Arsenic Excreted by Monkeys

Animal #	E-772			C-53			E-52			C-342		
Dose Given	4463 ug			4461			3463 ug			4406 ug		
Body weight	3900 g			4120 g			3000 g			3900 g		
Time in days	% excreted			% excreted			% excreted			% excreted		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
1	39.0	-	39.0	54.0	-	54.0	36.0	0.5	36.5	30.0	-	30.0
2	16.0	0.4	16.4	19.0	-	19.0	14.0	6.6	20.6	24.0	8.3	32.3
3	7.0	14.4	21.4	6.8	0.1	6.9	3.1	1.2	4.3	4.8	6.5	11.3
4	2.0	0.2	2.2	1.2	0.4	1.6	0.9	0.1	1.0	0.9	1.0	1.9
5	0.6	-	0.6	1.2	-	1.2	1.0	0.1	1.0	0.4	-	0.4
6	0.2	-	0.2	0.4	0.3	0.7	0.5	-	0.6	0.2	-	0.2
7	0.2	-	0.2	0.2	0.2	0.4	0.4	-	0.4	-	-	-
8	0.1	-	0.1	0.1	-	0.1	0.2	-	0.2	0.1	0.1	0.2
9	-	-	-	0.2	-	0.2	0.3	-	0.3	0.1	-	0.1
10	-	-	-	0.1	-	0.1	-	-	-	-	-	-
11	-	-	-	0.1	-	0.1	0.2	-	0.2	0.1	-	0.1
12	-	-	-	0.1	-	0.1	0.3	-	0.3	0.1	-	0.1
13	-	-	-	0.1	-	0.1	-	-	-	0.1	-	0.1
14	-	-	-	0.1	-	0.1	0.3	-	0.3	-	-	-
Total	65.1	15.0	80.1	83.6	1.0	84.6	57.2	8.5	65.7	60.8	15.9	76.7

Average Total Excretion After 14 days

	Urine	Feces	Total
Mean	66.6	10.1	76.7

TABLE II

Percent of Dose Inorganic Arsenic Excreted by Monkeys

Animal #	E-772			C-53			E-52			C-342		
Dose Given	3647 ug			4000 ug			3000 ug			3646 ug		
Body Weight	3795 g			4000 g			2987 g			3795 ug		
Time in days	% Excreted			% Excreted			% Excreted			% Excreted		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
1	60.7	-	60.7	59.2	0.1	59.3	50.9	0.1	60.0	18.4	0.8	19.2
2	6.8	-	6.8	11.9	-	11.9	11.9	1.7	13.6	7.1	-	7.1
3	1.9	0.6	2.5	4.1	-	4.1	2.3	0.9	3.2	2.7	0.6	3.3
4	1.0	-	1.0	2.0	-	2.0	0.9	0.7	1.6	0.9	0.9	1.8
5	0.2	-	0.2	1.0	0.1	1.1	0.6	0.2	0.8	0.2	0.4	0.6
6	-	-	-	0.7	-	0.7	0.3	-	0.3	0.3	-	0.3
7	0.3	-	0.3	0.4	0.3	0.7	0.5	0.1	0.6	0.3	0.1	0.4
8	0.1	-	0.1	0.3	0.8	1.1	-	-	-	-	-	-
9	-	-	-	0.1	0.1	0.2	0.2	-	0.2	0.2	0.1	0.3
10	-	-	-	0.1	-	0.1	0.1	-	0.1	0.1	-	0.1
11	-	-	-	0.1	-	0.1	-	-	-	-	-	-
12	-	-	-	0.1	-	0.1	-	-	-	-	0.1	0.1
13	-	-	-	-	-	-	-	-	-	-	0.2	0.2
14	-	-	-	-	0.1	0.1	-	-	-	-	-	-
Total	71.0	0.6	71.6	80.0	1.5	81.5	67.7	3.7	80.4	30.2	3.2	33.4

Average Total excretion after 14 days (excluding C-342)

	Urine	Feces	Total
Mean	72.9	1.9	74.8

the ashed samples and the arsenic reduced to arsine with zinc. The arsine gas was then bubbled through a solution of silver diethyldithiocarbamate in pyridine and the absorbance measured at 540 nm against a reagent blank.

Results

The amount of arsenic ingested from the diet was about 7 ug per monkey per day. Background excretion of arsenic was 1 and 3 ug/day in the urine and feces respectively; the remainder was not accounted for. Some of it may have been retained in the body or excreted with hair.

Tables 1 and 2 show the excretion data for the individual monkeys following ingestion of fish arsenic or inorganic arsenic. After ingestion of fish arsenic, the majority of the dose appeared in the urine within the first four days. Except for monkey C-53, a small but significant amount was recovered in the feces on day 2 and 3. Following ingestion of inorganic arsenic, urinary excretion of arsenic was more rapid, a significant amount appearing in the urine within the first 24 hours after dosing in 3 monkeys. Monkey C-342, had a markedly reduced arsenic excretion in the urine on day 1 as compared to the other 3 monkeys. The amount of arsenic excreted via the feces was negligible following dosing with inorganic arsenic. Arsenic excretion in the urine had returned to background levels by day 11.

Discussion

The elusive nature of the toxicity and potential carcinogenity of arsenic in man has been well documented (FOWLER, 1977). No animal studies have been successful in reproducing the chronic toxicity or the carcinogenic potential of the compound as seen in man (PELFRENE, 1976).

To study the mechanism and dose-response relationship of arsenic toxicity in man, a suitable animal model must be identified in which the pharmacokinetics and metabolism of arsenic are similar to man. The recent reviews of KLEVAY (1976) and PENROSE (1975) summarize the more pertinent studies conducted regarding the biochemical effects of the various arsenic compounds in animals and man.

Following oral administration of inorganic arsenic in man less than 4% of the administered dose is recovered in the feces (COULSON et al. 1935; RAY BETTLEY and O'SHEA, 1975) suggesting that essentially all of the arsenic is absorbed from the gastrointestinal tract and that little is excreted via the bile. The excretion of both orally and parenterally administered

inorganic arsenic in man is primarily via the kidneys (COULSON et al., 1935; HUNTER et al., 1942; MEALEY et al., 1959, RAY BETTLEY and O'SHEA 1975). In the present study excretion of inorganic arsenic via the feces was essentially nil, all of the excreted arsenic being eliminated by the urine. Differences between the individuals in the various human studies are marked and as suggested by RAY BETTLEY and O'SHEA (1975) may be due to individual metabolic differences in arsenic storage and excretion. Such differences were also observed in the present study. Following ingestion of inorganic arsenic, monkey C-342 excreted only 30% of the ingested dose whereas the other three, excreted an average of 73%. GUTHMAND and GRASS (1932) reported that in pregnant and menstrating females, blood arsenic levels increase considerably. It was noted that monkey C-342 began menstration the day prior to dosing. This may have accounted for some of the decreased arsenic excretion noted in this monkey.

Recent studies by CRECELIUS (1977) have demonstrated that following ingestion of inorganic arsenic, methylarsonic and dimethylarsinic acid are present in substantial quantities in the urine. These observations reaffirm the earlier studies of BRAMAN and FOREBACK (1973) who demonstrated the presence of methylated forms of arsenic in human urine and postulated that this methylation of inorganic arsenic in the body may be a detoxification mechanism. The ability of individuals to methylate arsenic may explain in part some of the variation observed in the pattern of arsenic toxicity. Methylation of inorganic arsenic has been shown to occur in animals. Studies by LAKSO and PEOPLES (1975) indicate that following ingestion of inorganic arsenic by cows and dogs, methylated forms of arsenic are recovered in the urine, similar to the situation observed in man. Further studies to determine if this occurs in monkeys are presently underway in our laboratories.

The problem of the fate of arsenic present in marine fish and shellfish following ingestion by man is made even more difficult, because, until recently, the chemical form of this arsenic has not been identified, in spite of numerous attempts to do so. Furthermore, the form of arsenic in the various forms of marine life may not be identical (EDMONDS and FRANCESONI, 1977).

Balance studies in animals suggest that the excretion of fish-arsenic is incomplete and some of the arsenic may be retained in the body. MUNRO (1976) reported that following ingestion of fish arsenic by swine and adolescent monkeys, 90% and 63% respectively of the administered dose was recovered in the excreta, 20% of the recovered arsenic being excreted via the feces in both cases. The results of the present study

with adult monkeys confirm the findings that following ingestion of fish arsenic, some arsenic is eliminated via the feces. The reasons for the increased excretion in adult monkeys as compared to adolescent monkeys, as reported by MUNRO (1976) are not known at present and could be related to the age of the animals.

Resumé

Four adult female Cynomolagus monkeys were given single oral dose of fish arsenic. 67% of the arsenic was excreted with the urine and 10% with the feces, essentially within 5 days post exposure. The same monkeys were later dosed with inorganic arsenic and 76% of it was excreted with the urine, and practically nil with the feces.

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