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Human Retention Studies with ^{74}As

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Human Retention Studies with ^{74}As. POMROY, C., CHARBONNEAU, S. M., MCCULLOUGH, R. S., AND TAM, G. K. H. (1980). *Toxicol. Appl. Pharmacol.* 53, 550–556. An experiment is described in which the human metabolism of inorganic arsenic was investigated. Six volunteers took oral doses of ^{74}As, and were measured in a whole body counter, for periods up to 103 days, with up to 39 separate measurements. Complete collections of their excreta were made for up to 7 days, and the ^{74}As content was measured. The results indicate that the data are best represented by a three-component exponential function, the values of the coefficients for the pooled data being 65.9% with half-life of 2.09 days, 30.4% with a half-life of 9.5 days, and 3.7% with a half-life of 38.4 days.

The toxicity of arsenic to humans following acute and chronic incidents of arsenic exposure has been well documented in the literature. In addition, there is now strong epidemiological evidence that inorganic arsenic is a skin and lung carcinogen in humans. However, results of numerous carcinogenicity studies in the rat, mouse, and hamster have failed to reproduce these observations (Committee, 1977).

Data on the fate of arsenic in humans are unclear. Mealey *et al.* (1959) reported that 57–99% of the dose was recovered in 10 days, following a single intravenous injection of inorganic arsenic to humans. Following a single oral dose of inorganic arsenic to humans, Bettley and O'Shea (1975) recovered 25–56% of the dose in 10 days, whereas Coulson *et al.* (1935) reported recoveries of 75–100% in a similar time period. In these studies, excretion of arsenic was via the kidney, less than 5% of the dose being recovered in the feces, indicating that orally administered arsenic is essentially all absorbed from the gastrointestinal tract. Some of this 5% may even have been excreted via the bile following initial absorption.

Results of pharmacokinetic studies in animals suggest that differences between humans and animal species may exist in the fate of arsenic which may explain the lack of carcinogenicity observed in animal studies. The need to identify an appropriate animal model to test the biological effects of arsenic as they are observed in humans became apparent. Because the data in humans were contradictory, a study in human volunteers was undertaken to define the whole body retention, excretion, and metabolism of inorganic arsenic using radiolabeled arsenic acid.

METHODS

Counting Equipment

The Radiation Protection Bureau whole body counter consists of five large NaI detectors each 12.5 × 10 cm, arranged above and below a stretcher in a shielded room with walls of 8-in. steel and 0.25-in. lead. The counter is described in more detail in the IAEA Directory of Whole Body Monitors (1970).

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For a counting time of 60 min, a human body burden of about 1 nCi ^{74}As can be measured in this counter, using the 0.511-, 0.596-, and 0.635-MeV peaks. This minimum activity, combined with the physical half-life of ^{74}As, was used to calculate the initial activity required to continue the measurements for 10 to 12 weeks. Assuming that only 1% of the original dose remained at 12 weeks, which was decaying with a 17.9-day half-life, the initial dose required was 6.4 μCi. The absorbed radiation dose from this amount was calculated.

Radiation Dosimetry

Using physical data for ^{74}As from MIRD pamphlet 11 (Snyder *et al.*, 1975), and such biological data as was available from the articles of Mealey *et al.* (1959) and Bettley and O'Shea (1975), the absorbed radiation dose was calculated. The biological data were interpreted conservatively, and Table 1 shows the values obtained. These were used as the basis of the proposal for the study made to the Health Protection Branch Human Studies Committee. For comparison it should be noted that the annual total body absorbed dose from natural radiation is about 120 mrem. On completion of the study, the new biological data were used to recalculate the absorbed doses, which are also shown in Table 1. These are significantly lower than the original estimates, confirming the conservative approach taken.

Experimental Protocol

The six male subjects, ranging in age from 28 to 60 years, and weight from 64 to 84 kg were all in normal health. They were asked to sign a consent form, which described the experiment and the possible hazards. This consent form had been approved by the Health Protection Branch Human Studies Committee, along with the proposal for the study itself. All the volunteers were familiar with scientific terms and able to understand the possible hazards. Volunteers whose working relationship to the experimenters could imply any degree of obligation to participate were rejected. After fasting from the previous midnight they took the ^{74}As (carrier-free) the following morning in the form of arsenic acid in a gelatin capsule, followed by a glass of water. The 6.4 μCi of carrier-free ^{74}As was equivalent to about 0.06 ng of arsenic. The fast was then continued until midday in order to allow absorption from the gut to proceed as quickly as possible. At midday the first whole body count was made and further counts were made each day for about 2 weeks and then at decreasing frequency until the counts were not significantly different from background. Complete 24-hr samples of urine and feces were collected in separate plastic containers for up to 7 days after the start of the experiment. These samples were counted in the whole body counter and compared to a standard prepared from an aliquot of the dose solution, and made up to a volume of 1 liter in a similar plastic container. The count from each sample was corrected for volume.

Analysis of Whole Body Counting Results

If first order compartment kinetics apply, then a model of the form

$$E_n = \sum_{i=1}^{n} a_i \cdot e^{-b_i \cdot t}$$

will describe the whole body retention (Létourneau *et al.*, 1972). Here t is time in days, a_i and b_i are the unknown parameters of the model, and n is the number of exponential components. Usually a_i is converted to percentage form, so that:

$$A_i = \frac{a_i}{\sum_{j=1}^{n} a_j} \times 100\%.$$

In order to fit mathematical functions to the whole body retention data, the nonlinear curve-fitting procedure in the computer program package BMDP (Dixon, 1975) was used. Fits for $n = 2$, 3, and 4 were carried out for various cases, as described below. A model consisting of ln (E_n) was also fitted to the individual subjects, but since it did not lead to any im-

TABLE 1

RADIATION DOSIMETRY OF ^{74}As AND ^{73}As[a]

	Absorbed radiation dose (mrem/6.4 μCi)	
Organ	Estimate before study	Estimate based on model derived from study
Total body	64	20
Kidney	120	72
Liver	112	66
Testes	70	26
Stomach	91	46
Large intestine	85	46

[a] The ^{74}As as received from the supplier contained about 20% ^{73}As. The additional radiation dose from this was calculated to be about 10% of that due to ^{74}As. The ^{73}As, being of much lower energy than ^{74}As, does not contribute any counts to the region of interest used for the latter.

TABLE 2

PARAMETERS ESTIMATED FROM FITS OF THREE-EXPONENTIAL (E3) MODELS TO ^{74}As WHOLE BODY HUMAN RETENTION DATA—INDIVIDUALS AND COMBINED

Subject	Parameter	First term	Second term	Third term
1	A (%)	74.0	26.0	0[a]
25 Observations	b (day^{-1})	0.271	0.0476	0[a]
during 94 days	$t½$ (day)	2.56	14.6	—
2	A (%)	63.1	35.8	1.1
29 Observations	b (day^{-1})	0.397	0.0775	0
during 103 days	$t½$ (day)	1.75	9.0	—
3	A (%)	56.2	42.0	1.8
21 Observations	b (day^{-1})	0.384	0.0637	0
during 92 days	$t½$ (day)	1.81	10.9	—
4	A (%)	57.4	37.6	5.0
39 Observations	b (day^{-1})	0.670	0.1013	0.0193
during 93 days	$t½$ (day)	1.03	6.8	36.0
5	A (%)	66.8	22.8	10.4
25 Observations	b (day^{-1})	0.339	0.0900	0.0328
during 81 days	$t½$ (day)	2.04	7.7	21.1
6	A (%)	71.9	26.9	1.2
19 Observations	b (day^{-1})	0.223	0.0562	0
during 74 days	$t½$ (day)	3.11	12.3	—
Averages	A (%)	64.9	31.9	3.2
(unweighted)	b (day^{-1})	0.381	0.0727	0.0087
	$t½$ (day)[b]	1.82	9.5	79.7
Combined	A (%)	65.9	30.4	3.7
158 Observations	b (day^{-1})	0.332	0.0728	0.0180
	$t½$ (day)	2.09	9.5	38.4

[a] In effect, no third term could be fitted. Zero values are used for the unweighted averages.
[b] $t½$ here is calculated from the corresponding b value.

provement in the fit or the parameter estimates, it is not reported further here.

In fitting mathematical functions to data, weighting factors are commonly used to provide desirable statistical properties by compensating for a change of variance over the range of observation. In the work described here, weights proportional to the inverse of the counts provided significantly improved fits over unweighted procedures, especially for times greater than 8 days.

RESULTS

Number of Measurements

The total number of whole body count measurements varied from 19 to 39 and the total time of the experiment varied from 74 to 103 days. Details for each subject are listed in Table 2.

Efficiency of Excreta Collection

In this type of experiment it is important to account for all the administered dose, and Lathrop *et al.* (1976) have stated that a radioactivity "budget" should be determined for each subject. Table 3 shows the budget for one subject, which is similar to that for the other subjects. Apart from Day 1, the total ^{74}As accounted for it within ±3% which shows that the collection of urine and

TABLE 3

^{74}As BUDGET FOR SUBJECT NO. 3[a]

Day	Whole body content	Cumulative urine content	Cumulative feces content	Total
1	5.84			5.84
2	5.49	1.16		6.65
3	4.01	2.16	0.54	6.71
4	3.23	2.68	0.66	6.57
5	2.71	2.97	0.70	6.38
8	1.92	3.59	0.70	6.21

[a] All amounts in μCi. For Days 2 to 8 mean = 6.50 ± 0.21 (SD) μCi.

feces was complete. For Day 1, which was based only on the whole body count made 2 h after dosing, the total was significantly lower. For the first subject, a series of blood samples were taken at 1-hr intervals, and the ^{74}As concentration peaked at 4 hr. This suggests that at 2 hr, the time of the first whole body count, the ^{74}As distribution was changing rapidly, and thus the response of the counter would not be the same as for later measurements.

For one subject, excretion via the sweat was checked, by means of measuring his sports clothing in the whole body counter after a vigorous squash game on Day 2 of the experiment. No ^{74}As was detected on the clothing.

Retention Curves

The data points for each subject and the retention curve for the combined data are shown in Fig. 1. The data are normalized to 100% on Day 8. Day 1 was not used as a base due to uncertainty as to whether the ^{74}As distribution was changing at that time. This procedure allowed the long-term components, which are of more interest, to be more accurately compared. It can be seen that the data sets for the different subjects did not differ to any great extent after 8 days.

The three-exponential function E3 gave significantly better fits than E2 to all subjects, except subject No. 1. The results of the best fits for individuals, the average of the individuals, and the combined data, are shown in Table 2. All subjects showed similar first and second compartments, averaging 1.8 and 9.5 days half-life, respectively. Three subjects had flat third compartments, indicating "permanent" retention of

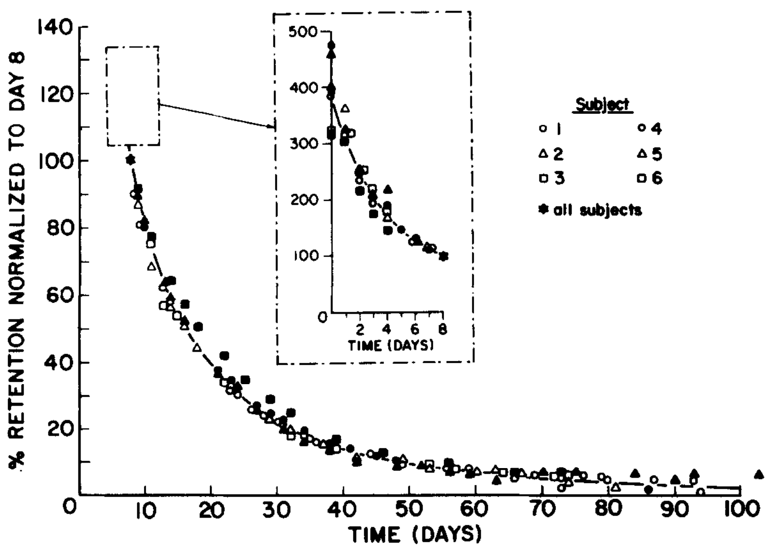

FIG. 1. Arsenic retention in six human subjects.

TABLE 4

PARAMETERS ESTIMATED FROM FITS OF FOUR-EXPONENTIAL (E4) MODELS TO ^{74}As WHOLE BODY HUMAN RETENTION DATA—ONE INDIVIDUAL ONLY

Subject	Parameter	First term	Second term	Third term	Fourth term
4	A (%)	12.5	49.8	34.0	3.7
39 Observations	b (day^{-1})	40.3	0.488	0.0900	0.0157
	$t½$ (day)	0.017	1.42	7.70	44.1

a fraction of the dose; two subjects had third-compartment half-lives of 21 and 36 days, respectively; while subject No. 1 had no third compartment. The combined data had half-lives of 2.1, 9.5, and 38.4 days for the three compartments.

The four-exponential function E4 showed an improved fit only for subject No. 4. These results are shown in Table 4. The pattern is similar to E3 except that a very short-lived compartment (half-life of 0.017 day = 24 min) was identified. No meaningful fourth compartment could be identified for the combined data.

Analysis of Excreta

Table 5 shows the percentage of the dose of arsenic excreted in the urine and feces during the first 7 days following dosing. Thereafter, the levels of radioactivity were below detection levels. Essentially all of the arsenic was absorbed; 62% was recovered in the urine and 6% in the feces. Whether the arsenic in the feces was nonabsorbed, or was excreted via the bile, cannot be determined from this study.

DISCUSSION

The long period during which whole body counting was continued and the efficiency of excreta collection, together with the fact that variations between subjects were small, form a sound basis for the statistical analysis of the data. The triexponential model generally gave the best fit to the observed data, and identification of the three compartments may be attempted by reference to the data of Mealey *et al.* (1959). Analysis of their data shows that the three most important compartments, in decreasing order of magnitude and increasing order of half-life, are kidney, liver, and muscle, respectively. It must be emphasized that this identification is very speculative, as the present study was not designed to obtain such conclusions.

Comparing the results to animal data, the closest resemblance is found in the monkey. Charbonneau *et al.* (1978) reported that in Cynomologus monkeys, 75% of the dose of inorganic arsenic was recovered in urine in 10 days. Our results for humans show 62% in 7 days, while in dogs 90% was recovered in urine in 4 days, (Hollins *et al.*, 1979). Following a single oral dose to hamsters, 90% is excreted in 2 days, 60% of the dose being recovered in the feces, (Charbonneau *et al.*, 1979a). In the rat the binding of arsenic to the red blood cells, a phenomenon not seen in man or other species, makes the rat an inappropriate animal model (Committee, 1977). As reported elsewhere (Tam *et al.* 1979), following a single oral dose of inorganic arsenic (^{74}As) to humans, monomethylarsenic acid, dimethylarsenic acid, and inorganic arsenic were identified in the urine. However, only dimethylarsenic acid and inorganic arsenic were found in the urine of the dog, hamster (Charbonneau *et al.*, 1979a,b), pig, mouse, or monkey (G. K. H. Tam, unpublished observation), following oral dosing with inorganic arsenic. Such results indicate that monomethyl-

TABLE 5

PERCENTAGE OF DOSE EXCRETED FOLLOWING A SINGLE ORAL DOSE OF ^{74}As (ARSENIC ACID) TO HUMANS

	Subject																	
	1 % Excreted			2 % Excreted			3 % Excreted			4 % Excreted			5 % Excreted			6 % Excreted		
Time	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
1	23.8	2.0	25.8	26.6	3.4	30.0	17.9	—	17.9	24.1	2.0	26.1	21.4	0.7	22.1	20.9	0.3	21.2
2	15.9	1.6	17.5	14.4	0.4	14.8	15.8	8.3	23.9	15.7	4.8	20.5	15.0	1.9	16.9	14.6	1.3	15.9
3	6.1	0.8	6.9	8.8	2.0	10.8	8.0	1.9	9.9	9.1	0.3	9.4	9.7	0.1	9.8	10.0	0.6	10.6
4	5.9	0.2	6.1	4.9	0.5	5.4	4.6	0.7	5.3	7.7	0.2	7.9	6.1	0.5	6.6	7.0	1.3	8.3
4	3.3	—	3.3	3.8	—	3.8	4.5	—	4.5	4.5	0.1	4.6	4.9	0.2	5.1	4.9	—	4.9
6	4.4	—	4.4	3.1	—	3.1	2.7	—	2.7	4.0	0.1	4.1	3.4	0.2	3.6	1.3	—	1.3
7	2.1	—	2.1	2.4	0.1	2.5	2.4	—	2.4	3.0	—	3.0	2.3	—	2.3	3.0	—	3.0
Total	61.5	4.6	66.1	64.0	6.4	70.4	55.7	10.9	66.6	68.1	7.5	75.6	62.8	3.6	66.4	61.7	3.5	65.2

Average total excretion after 7 days (%)

	Urine	Feces	Total
Mean ± SD	62.3 ± 4.0	6.1 ± 2.8	68.4 ± 4.0

arsenic acid may be a metabolite unique to man.

This study has shown that inorganic arsenic metabolism in humans is well represented by a triexponential model. It has also shown that the selection of an appropriate animal model for man is not a straightforward matter.

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