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Tissue Distribution and Subcellular Binding of Arsenic in Marmoset Monkeys After Injection of ^{74}As-Arsenite

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Abstract. The distribution and retention of arsenic in Marmoset monkeys, given ^{74}As-arsenite (0.4 mg As/kg body weight) i.p., were studied by means of whole-body autoradiography and determination of ^{74}As-levels in tissues and excreta. Only about 30% of the dose was eliminated over 4 days, mainly via the kidneys. All of the arsenic in urine and tissues was found to be in inorganic form. Tissues with highest accumulation 4 days after dosing were liver (about 20% of the dose), squamous epithelium of oral cavity and esophagus, kidney cortex, skin, testes (mainly tubuli seminiferi) and intestinal wall. As a rule the major part of the arsenic in these tissues was found to be associated with cellular organelles. In the liver about 50% of the arsenic was strongly bound to the rough microsomal membranes. In the soluble extract of tissues, arsenic was mainly associated with macromolecular constituents. The long retention time and tight binding of arsenic could partly be explained by the fact that no biotransformation into methylated arsenic occurred, in contrast to all other species studied so far.

Key words: Arsenite – Monkey – Distribution – Autoradiography – Subcellular fractionation – Biotransformation

Introduction

The metabolism of inorganic arsenic in experimental animals is dependent on the chemical form of the administered compound; arsenite causes higher tissue concentrations than arsenate (Vahter and Norin 1980). There are also marked differences in the metabolism of arsenic in different species. In human subjects, 60–70% of an ingested dose of arsenite is excreted in the urine within 48 h (Crecelius 1977; Yamauchi and Yamamura 1979). Rabbits excrete about 80% (Bertolero et al. 1981; Marafante et al. 1982) and mice as much as 90% (Vahter 1981) of an administered dose of arsenite within the same time frame. The

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retention time of arsenic in rats is considerably longer than in other animal species, mainly due to accumulation in the red blood cells (Hunter et al. 1942; Lanz et al. 1950). The elimination rate of arsenic in nonhuman primates seems to be more similar to that reported for man. Studies on Cynomologus monkeys receiving arsenic trioxide orally showed a urinary excretion of about 70% of the dose within 5 days (Charbonneau et al. 1978).

The observed differences in retention time of arsenic among various species may be due to differences in biotransformation. Inorganic arsenic is methylated in the body, and all laboratory animals studied to date excrete arsenic in the form of dimethylarsinic acid (about 60–90% of the excreted arsenic) besides inorganic arsenic (Tam et al. 1978; Charbonneau et al. 1979; Bertolero et al. 1981; Vahter 1981). In man the urinary excretion of arsenic at low dose levels consists of about 20% methylarsonic acid, 60% dimethylarsinic acid and 20% inorganic arsenic (Crecelius 1977; Smith et al. 1977; Tam et al. 1979; Yamauchi and Yamamura 1979; Buchet et al. 1981).

Another explanation for the observed differences in retention time among animal species may be differences in the interaction of arsenic with cellular constituents. Such differences have been reported for rats and rabbits (Marafante et al. 1982).

The great variation in arsenic metabolism among species makes it difficult to find a single animal model, which corresponds well with man. Thus data on the metabolism in different species have to be collected to get a picture of the underlying mechanisms. To this end we have studied the whole-body distribution of ^{74}As-arsenite in a New World monkey (Marmoset, *Callithrix jacchus*) by means of autoradiography. The results showed prominent arsenic retention in the liver compared to that reported for rodents. The study was extended to include concentrations and chemical forms of arsenic in the tissues and subcellular fractions in order to get more information on the mechanism of the binding.

Materials and Methods

Chemicals

^{74}As-arsenite was prepared by adding ^{74}As-arsenic acid (Amerhsam, England) to a standard solution of sodium arsenite (Merck, Darmstadt, FRG) and reducing the arsenate present to arsenite with sulphite as earlier described (Vahter and Norin 1980). More than 97% of the arsenic was in the trivalent form at the time of exposure, as analysed by ion exchange chromatography (Reay and Asher 1977).

Autoradiography

In the first part of the study two male Marmoset monkeys (305 and 410 g) were injected intraperitoneally with ^{74}As-arsenite (0.4 mg As/kg body weight). The animals were sacrificed 24 h and 4 days after administration, respectively, by exposure to gaseous carbon dioxide and were frozen by immersion in a mixture of n-hexane and solid carbon dioxide (−78° C). Autoradiography was performed according to Ullberg (1954 and 1977). Sections of the animals (20 µm thick) were taken on tape and freeze-dried. The sections were then pressed against X-ray films (Industrex, Kodak) and kept at −20° C during exposure. Thereafter the films were developed and selected sections were stained.

Tissue Concentrations and Subcellular Distribution

In the second part of the study one male Marmoset monkey (400 g) was given ^{74}As-arsenite by i.p. injection (0.4 mg As/kg body weight). Urine and feces were collected daily for 4 days after which the monkey was sacrificed under anesthesia (Ketalar, Parke Davis, GB). Blood was collected from the heart with a heparinized syringe and centrifuged at 20,000 rpm. Stomach and intestines were opened and rinsed thoroughly with 0.9% NaCl. Other organs were measured for total ^{74}As-content without further treatment.

Brain, epididymis, esophagus, eyes, gallbladder, heart, intestines, kidney cortex and medulla, lungs, muscle, seminal ducts, skin and testes were homogenized in 0.1 M Tris-Cl buffer (pH 8.1) containing 0.25 M sucrose. Soluble cytoplasmic fractions of tissues were obtained by centrifugation of the homogenates at 105,000 *g* for 90 min.

Separation of arsenic metabolites in soluble cytoplasmic fractions of tissues, plasma and urine was performed according to the method of Tam et al. (1978) on columns of BioRad AG 50 WX-4 resin after ultrafiltration on Centriflo membranes with a cut-off of 25,000 daltons (Amicon, B.V., Amsterdam, NL).

Chromatographic separation of the cytosols and the soluble extracts of the nuclear and mitochondrial liver fractions was carried out on columns (2.5 and 5 × 100 cm) of Sephacryl S-200 resin (Pharmacia, Uppsala, Sweden). UV transmission of the eluate was monitored continuously at 280 nm using a LKB Uvicord II instrument. Chromatographic columns were calibrated with standard proteins of known molecular weight. Horse spleen ferritin (mol. wt 400,000) was eluted at Ve/Vo = 1, bovine serum albumin (mol. wt 67,000) at Ve/Vo = 1.8, cytochrome c (mol. wt 12,500) at Ve/Vo = 2.2 and DNP-L-alanine (mol. wt 255) at Ve/Vo = 3.1.

Subcellular fractions of the liver were separated by differential centrifugation after homogenization in 0.1 M Tris-Cl buffer (pH 8.1) containing 0.25 M sucrose. The crude nuclear (10 min at 700 *g*), mitochondrial (10 min at 9,000 *g*), lysosomal (25 min at 27,000 *g*) and microsomal (90 min at 105,000 *g*) fractions obtained were further purified by two-fold washing in Tris-Cl buffer followed by centrifugation. Microsomal subfractions were prepared by successive centrifugations on discontinous sucrose gradients according to Tata (1972).

One part of the purified subcellular fractions was ultrasonicated (Ultrasonic Disintegrator 150W, MSE) for 10 min at a frequency of 20 kHz/s and an amplitude of 8 μm and centrifuged for 90 min at 105,000 *g*. The chemical form of arsenic was determined after ultrafiltration as for the other tissues.

Another part of the purified fractions, suspended in 1 ml 0.01 M Tris-Cl, pH 7.8, were dialyzed over night against 200 ml of Tris-Cl containing 1 mM reduced glutathione, 10 mM cacodylate or 0.1% suspension of BAL (2,3-dimercaptopropanol). The ^{74}As content of the fractions was determined before and after the dialysis.

Radioactivity Measurements

A gamma scintillation counter [Searle Nuclear, Chicago, Ill., USA, Model 1195, with a 3-in diameter NaI(T1) crystal] was used for measurements of the ^{74}As-radioactivity. The amount of ^{74}As-arsenic in animal tissues and fractions was determined by comparison with ^{74}As standard solutions of known specific activity.

Results

Elimination and Tissue Distribution

Table 1 shows the elimination of arsenic in urine and feces during the first 4 days after administration. About 22% of the dose was recovered in the urine and about 4% in the feces. Another 8% of the dose was recovered in the climbing tree and in the water used for washing the cage. This arsenic originated probably from urine. Methylated forms of arsenic were not detected in any of the urine samples.

Table 1. Elimination of ^{74}As in urine and feces and chemical form of arsenic in urine of Marmoset monkey i.p. administered ^{74}As-arsenite (0.4 mg As/kg body weight). Contamination of the cage, recovered at washing after termination of the experiment, was considered mainly of urinary origin

Hours after exposure	% of dose		% inorganic arsenic in urine
	Urine	Feces	
24	8.4	0.9	> 99
48	5.5	1.3	> 99
72	4.3	1.3	> 99
96	3.6	0.6	> 99
Contamination of cage	8.0		
Total	29.8	4.1	

As shown by the autoradiogram, the ^{74}As-distribution 24 h after administration was characterized by a high concentration in liver, squamous epithelium of the upper gastrointestinal tract (oral cavity and esophagus), intestinal walls, kidneys and skin (Fig. 1). These tissues still showed the highest concentrations in the monkey sacrificed 4 days after administration (Fig. 2), indicating a long term retention of arsenic in these tissues. A relatively high concentration of ^{74}As was observed also in the testes, where the radioactivity was localized in the seminiferous tubules (Fig. 3). High ^{74}As-activity was also observed in some segments of the epididymis, apparently in the lumen.

There was an increase in the concentration of ^{74}As in the central nervous system of the monkey with 4 days survival time compared to that at 24 h, indicating a slow passage of arsenic through the blood-brain barrier. The radioactivity was localized mainly to the cortex.

The concentration of ^{74}As-arsenic in the different organs 4 days after the administration is shown in Table 2. By far the highest concentration was found in the liver, where about 20% of the administered arsenic was retained. As was seen in the autoradiograms, the tongue and the esophagus had high concentrations of ^{74}As. As for the other organs, the highest arsenic concentrations, in descending order, were found in the penis, kidney cortex, skin, semen ducts, gall bladder and intestinal walls. About 77% of the ^{74}As in the blood was found in the red blood cells.

Subcellular Distribution and Chemical Form of Arsenic

Table 3 shows the total and the ultrafiltrable arsenic in the soluble cytoplasmic fraction of tissues of the monkey 4 days after administration. In general, tissues with high concentration of ^{74}As also showed a high association of ^{74}As with cell organelles. Liver, esophagus and kidney cortex also showed a high degree of binding to macromolecules in the cytosol (low ultrafiltrable fraction of arsenic), while in skin, seminal ducts and gall bladder the major part of the cytosolic ^{74}As

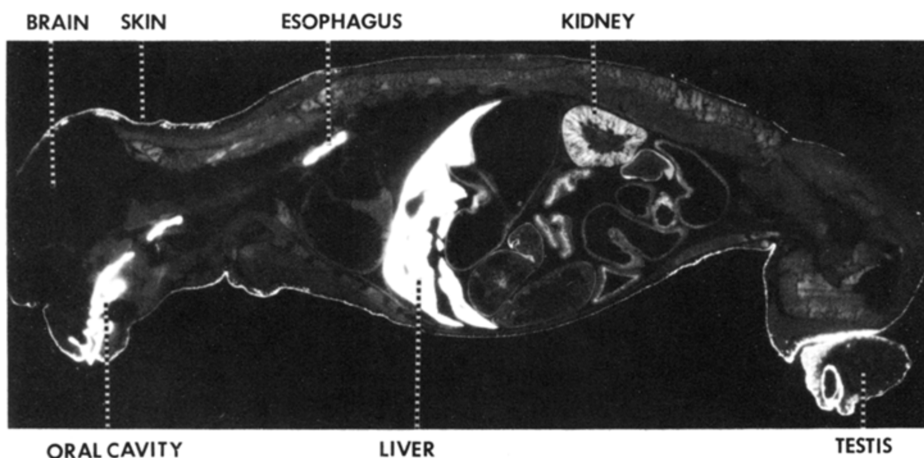


Fig. 1. Autoradiogram showing the distribution of radioactivity 24 h after a single i.p. dose of ^{74}As-arsenite to a male Marmoset monkey. Note the high concentration (white areas) in the liver, the epithelium of oral cavity and esophagus, the skin and the hair. (× 0.65)

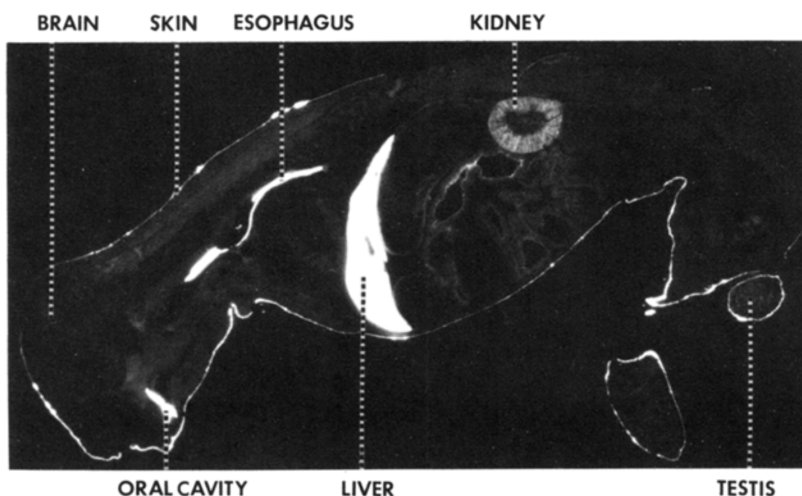
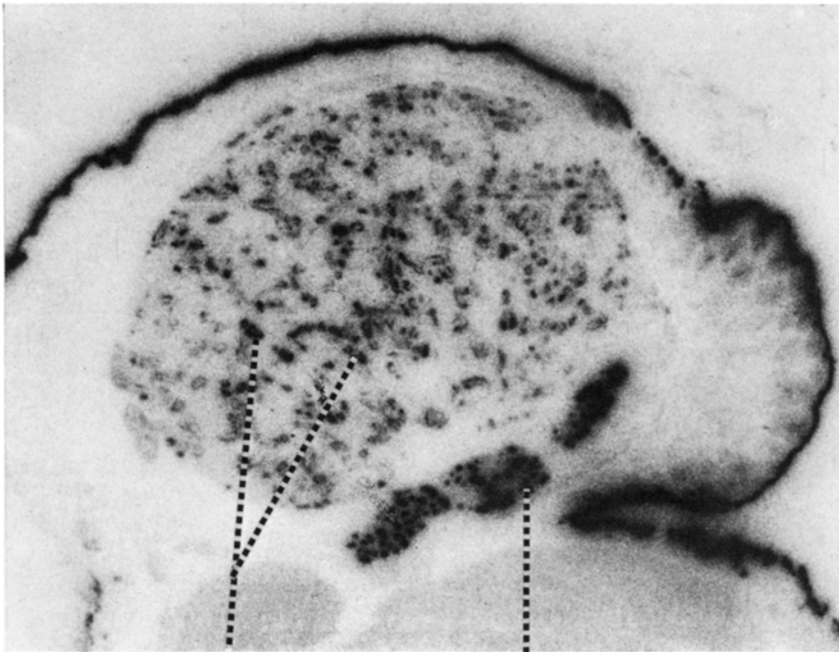


Fig. 2. Autoradiogram showing the distribution of radioactivity 4 days after a single i.p. dose of ^{74}As-arsenite to a male Marmoset monkey. The distribution pattern is essentially the same as after 24 h. There is a relative increase in the concentration in the brain and in the testes. (× 0.65)

was ultrafiltrable. At least for the seminal ducts and the gall bladder this may be related to an excretory function of these tissues.

Also in the small intestine, testes, muscle, heart and brain, showing fairly low concentrations, most of the ^{74}As was associated with cellular organelles. In the testes and the heart a substantial part of the cytosolic arsenic was ultrafiltrable, while in the other tissues the ultrafiltrable fraction was relatively low, indicating a high degree of binding to macromolecules. In the large intestine and the red blood cells most of the ^{74}As was present in the cytosol, but only



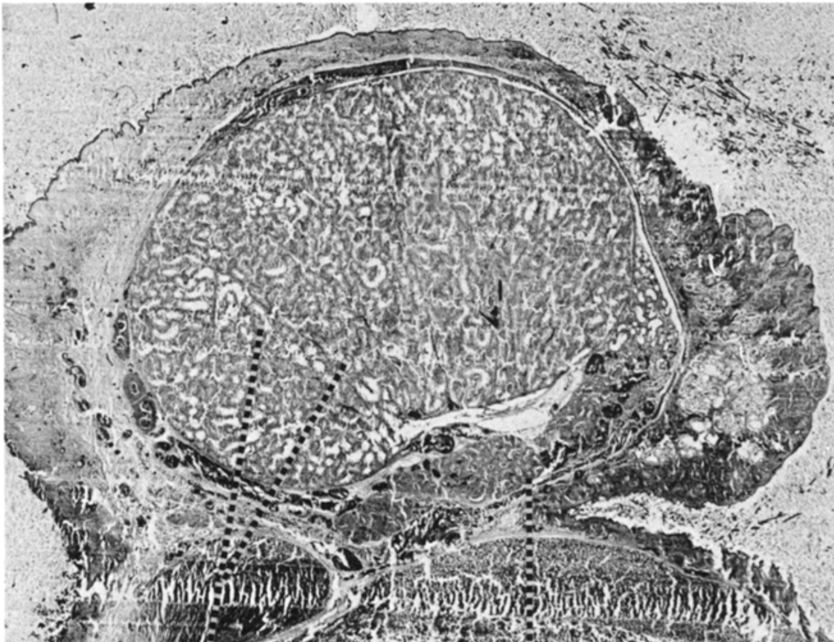


Fig. 3. Detail of autoradiogram *(upper)* and corresponding tissue section *(lower)* showing the testis and epididymis from the same monkey as in Fig. 2. Dark areas in the autoradiogram correspond to radioactivity. There is an accumulation in many (but no all) tubuli seminiferi, which may be related to differences in the stage of spermatogenesis at the time of injection. (× 6)

Table 2. Concentrations of ^{74}As-arsenic in tissues of Marmoset monkey 4 days after i.p. injection of ^{74}As-arsenite (0.4 mg As/kg body weight)

Tissue/fluid	^{74}As-arsenic (μg/g)	% of dose/organ
Liver	1.33	18.9
Esophagus	0.94	0.25
Tongue	0.76	0.41
Penis	0.49	0.05
Kidney cortex	0.36	0.56
Skin	0.36	–
Seminal ducts	0.35	0.06
Gall bladder	0.30	0.05
Large intestine	0.22	0.61
Duodenum	0.21	0.06
Teeth and jaw bones	0.18	–
Kidney medulla	0.18	0.02
Small intestine	0.17	0.65
Stomach	0.16	0.22
Epididymis	0.15	0.01
Spleen	0.13	0.02
Testes	0.13	0.11
Muscle	0.12	–
Salivary glands	0.12	0.07
Heart	0.10	0.18
Pancreas	0.10	0.05
Lungs	0.09	0.09
Trachea and larynx	0.08	0.04
Cerebrum	0.06	0.21
Cerebellum	0.06	0.03
Medulla oblongata	0.06	0.02
Skeleton (femur)	0.05	–
Fat	0.03	–
Hair	0.03	–
Eyes	0.02	0.02
Blood	0.01	–

10–15% of it was ultrafiltrable. All of the ^{74}As in the diffusible fraction of the tissue cytosols was in the form of inorganic arsenic, independent of the degree of binding to intracellular components.

Table 4 shows the distribution of ^{74}As between subcellular fractions of the liver. About 50% of the arsenic was found in the microsomal fraction and 25% in the nuclear fraction. A substantial part of the ^{74}As in the nuclear, mitochondrial and lysosomal fractions was released at the washing, indicating a fairly weak binding. The binding of ^{74}As to microsomes seemed to be considerably stronger since almost no ^{74}As was released by the washing.

Exhaustive dialysis with Tris-Cl buffer, glutathione (GSH) and cacodylate did not release any significant amount of ^{74}As from the nuclear, mitochondrial or microsomal fractions (Table 5). Dialysis with BAL released about 50% of the ^{74}As bound to the nuclei and mitochondria, but only about 16% of the arsenic bound to microsomes. This, together with the observation that only a very small amount of arsenic was released by ultrasonication (Table 4), gives further

Table 3. ^{74}As-arsenic in the soluble cytoplasmic fraction of tissues of Marmoset monkey 4 days after i.p. administration of ^{74}As-arsenite (0.4 mg As/kg body weight) and the percentage of ultrafiltrable arsenic. Organs are listed according to their total concentration of ^{74}As as in Table 2

Tissue	^{74}As in cytosol, % of total homogenate	% ultrafiltrable ^{74}As in the cytosol
Liver	14.2	9.4
Esophagus	14.9	11.4
Kidney cortex	17.1	18.5
Skin	9.4	63.4
Seminal ducts	21.8	80.0
Gall bladder	21.1	70.6
Large intestine	87.3	11.5
Kidney medulla	31.8	39.4
Small intestine	9.9	10.3
Epididymis	39.9	66.7
Testes	17.8	50.1
Muscle	7.4	20.0
Heart	7.7	43.5
Lungs	27.5	52.6
Brain	8.6	20.7
Eyes	50.2	40.6
RBC	92.4	14.6
Plasma	–	27.1

Table 4. Distribution of ^{74}As-arsenic in the subcellular fractions of liver of Marmoset monkey 4 days after i.p. administration of ^{74}As-arsenite (0.4 mg As/kg body weight), and the percentage of ultrafiltrable arsenic

Fraction	^{74}As in liver fractions, % of total ^{74}As in the homogenate	% ^{74}As released by ultrasonication	% ultrafiltrable ^{74}As
Nuclear	25.1		
Purified	8.5	26.1	33.4
1st washing	14.9	–	10.2
2nd washing	1.7	–	3.6
Mitochondrial	11.2		
Purified	1.8	31.0	51.7
1st washing	8.6	–	5.8
2nd washing	0.8	–	3.4
Lysosomal	3.8		
Purified	2.8	11.2	27.4
1st washing	0.7	–	13.1
2nd washing	0.3	–	6.3
Microsomal	47.7		
Purified	45.8	0.5	4.0
1st washing	1.9	–	< 1
Cytosol	14.2	–	9.4

Table 5. Dialysis of purified subcellular liver fractions (1 ml of fraction suspended in 0.01 M Tris-Cl buffer, pH 7.8, dialyzed over night against 200 ml of Tris-Cl with or without different complexing agents). Figures represent dialyzed ^{74}As in % of total ^{74}As in the fractions

Fraction	Tris-Cl (10 mM)	GSH (1 mM)	Cacodylate (10 mM)	BAL (0.1% susp.)
Nuclei	6.2	5.9	4.9	55.9
Mitochondria	2.1	1.8	3.1	45.4
Microsomes	0.1	6.3	2.4	16.3

Table 6. Distribution of ^{74}As-arsenic in submicrosomal fractions of liver of Marmoset monkey 4 days after i.p. administration of ^{74}As-arsenite (0.4 mg As/kg body weight)

Fractions	% ^{74}As
Fractionation of microsomes	
Cell sap	1.4
Smooth microsomes	4.6
Rough microsomes	94.6
Fractionation of rough microsomes	
Light rough microsomes	58
Heavy rough microsomes	41
Precipitate (free or non-membrane bound ribosomes, polysomes and glycogen)	< 1

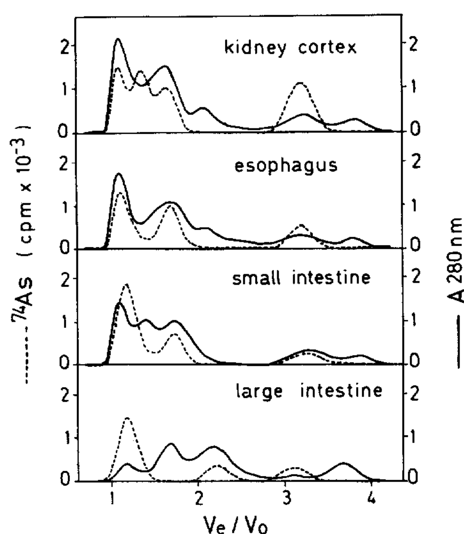


Fig. 4. Elution profiles from Sephacryl S 200 columns of ^{74}As in the soluble fraction of kidney cortex, esophagus, small and large intestines of Marmoset monkey at 4 days after a single i.p. dose of 0.4 mg As/kg body weight as ^{74}As-arsenite

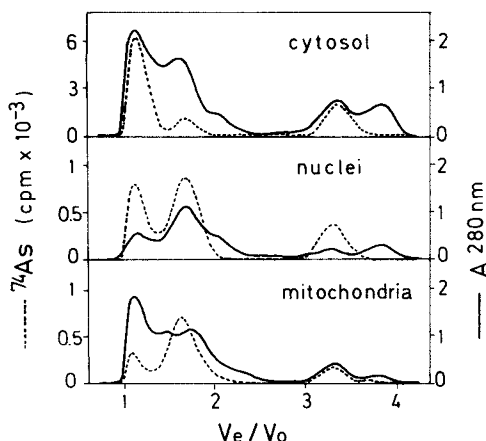


Fig. 5. Elution profiles from Sephacryl S 200 columns of ^{74}As in the soluble cytoplasmic fraction and the soluble extract of nuclear and mitochondrial fractions of liver of Marmoset monkey at 4 days after a single i.p. dose of 0.4 mg As/kg body weight as ^{74}As-arsenite

evidence for the strong binding of ^{74}As to the microsomes compared to that of the other subcellular fractions.

Further fractionation of liver microsomes showed that almost all of the ^{74}As-arsenic was associated with rough microsomes (Table 6). No differences in binding of ^{74}As was found between light and heavy rough microsomes. Practically no arsenic was present in free or non-membrane bound ribosomes, polysomes or glycogen.

Of the ^{74}As present in the liver cytosol and the soluble extract of ultrasonicated microsomes, only 9.4 and 4.0%, respectively, was ultrafiltrable, indicating that the major part of the arsenic was associated with macromolecular components. Elution profiles obtained on gel filtration of the cytosols of kidney cortex, esophagus and small intestine are given in Fig. 4. All cytosols showed a high degree of binding of ^{74}As to high molecular weight compounds, eluted at Ve/Vo 1–2. However, a minor part of the ^{74}As was recovered in fractions of low molecular weight components (Ve/Vo > 3), corresponding to the ultrafiltrable arsenic. Similar distribution of ^{74}As between components of high and low molecular weight was obtained on gel filtration of liver cytosol and soluble extracts of the nuclear and mitochondrial fractions of the liver (Fig. 5).

Discussion

The present study shows that the metabolism of arsenic in Marmoset monkeys is quite different from that reported for other animal species. Only about 30% of the dose was eliminated during the 4 days studied. The long term whole-body retention was mainly due to accumulation of arsenic in the liver. Such a prominent liver accumulation in other species, including man, has not been reported (Hunter et al. 1942; Du Pont et al. 1941; Liebscher and Smith 1968; Vahter and Norin 1980; Marafante et al. 1981; Wester et al. 1981). In rats, also showing a low elimination rate of arsenic, the high retention is mainly due to accumulation in the red blood cells, probably binding to the hemoglobin (Hunter et al. 1942; Lanz et al. 1950; Klaassen 1974; Marafante et al. 1982).

The Marmoset monkey is the only animal species, studied so far, which seems unable to methylate arsenic. All of the diffusible arsenic of the tissues, as well as the arsenic excreted in urine, was found to be in inorganic form. Dimethylarsinic acid is considered to be the main detoxification product of inorganic arsenic in both experimental animals and man. The former has a higher rate of elimination (Vahter 1981) and a lower affinity for tissue constituents (Marafante and Vahter 1982), than the latter.

The fact that no biotransformation of arsenic occurred in the Marmoset monkey may partly explain the interaction with tissue constituents and thus the long whole-body retention. The chromatographic studies showed a strong binding of arsenic to macromolecular constituents, probably high molecular weight proteins in the soluble extracts of the tissues and liver organelles. In the liver, the binding strength of arsenic varied considerably among the different subcellular components. In the purified fractions of the nuclei, mitochondria and lysosomes, a substantial fraction of the bound arsenic was released by ultrasonication or dialysis with BAL, which is known to form strong complexes with arsenic (Peters 1955; Webb 1966). Arsenic bound to the microsomes, however, was not influenced to the same extent by this treatment, indicating a very strong association. The difference in binding of arsenic between smooth and rough microsomes may possibly be related to the differences in surface charge between the two types of microsomes (Dallner and Azzi 1972).

A similarly strong binding of arsenic to microsomes has not been observed in other animal species. Studies on intracellular distribution in rabbits showed that only a few percent of the ^{74}As-activity was found to be associated with the microsomes of liver, kidney and lung homogenates, 1–2 days after a single dose of ^{74}As-arsenite (Sabbioni et al. 1979; Marafante et al. 1981). In addition, the intracellular binding of arsenic in rabbit tissues was fairly weak and there was a continuous release of arsenic from the intracellular components between 1 and 16 h after exposure, mainly in the form of dimethylarsinic acid.

The whole-body distribution pattern of arsenic in the Marmoset had several characteristics in common with that seen in mice or hamsters (Lindgren et al. 1982), e.g., the accumulation in skin, hair, squamous epithelium of the oral cavity and esophagus. However, the retention time was longer in most tissues of the Marmoset. On the other hand, some organs with high accumulation in the mice, namely the thyroid gland and the ocular lens, almost completely lacked ^{74}As-activity in the Marmoset. As in the rodents high ^{74}As-activity was observed in the lumen of some segments of the epididymis. In contrast to the rodents, however, the Marmoset showed testicular accumulation as well. The arsenic appeared to be localized in the spermatogenic epithelium. The possible influence of arsenite on reproduction in families of workers at a Swedish smelter has been discussed (Beckman 1978).

Since the Marmoset monkey did not methylate inorganic arsenic, the distribution of ^{74}As among the tissues will reflect that of inorganic arsenic, while in other animal species, tissue arsenic will be a mixture of inorganic arsenic and methylated metabolites. There is, however, a need for further studies on the interaction of different arsenic metabolites with tissue constituents of various animal species.

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