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Confirmation of Inorganic Arsenic and Dimethylarsinic Acid in Urine and Plasma of Dog by Ion-exchange and TLC

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The nature of the toxicity of arsenic in man and animals has been well documented (FOWLER 1977). Several methylated forms of arsenic were found to be widespread in the environment (BRAMAN and FOREBACK 1973). They were also found in urine following the ingestion of inorganic arsenic by dog, cow (LAKSO and PEOPLES 1975) and man (CRECELIUS 1977). These arsenic compounds were measured after selective reduction and volatilization. Based on the different forms of arsines detected after reduction, the arsenic compounds were assumed to be arsenite, monomethylarsonic acid and dimethylarsinic acid. Recently, different forms of arsenic metabolites were also detected in plasma and urine of Beagle dogs following intravenous administration of inorganic ^{74}As (TAM et al. 1978). These arsenic metabolites had the same chromatographic behaviour on a cation-exchange column as inorganic, monomethylated and dimethylated arsenic compounds. The exact nature of these arsenic metabolites was however not determined.

The present study describes an ion-exchange--TLC method to confirm that inorganic arsenic (arsenite and arsenate) and dimethylarsinic acid are the major arsenic metabolites in urine and plasma of the Beagle.

METHODS AND MATERIALS

Instrumentation

^{74}As radioactivity was measured using a Beckman gamma 300 scintillation counter. The photopeaks at 0.593 and 0.633 Mev. were measured together.

Arsenic determination was conducted using a Philips Universal Vacuum X-ray Spectrometer PW1410 with a W target X-ray tube (50 kV, 50 mA), a LiF crystal (2d=4.028 Å) and a NaI scintillation detector. The count rate at 33.92° (2θ) was measured and corrected for background at 35° (2θ).

Reagents

^{74}As (>1 mCi/μg As) was obtained from Amersham Corporation in the form of arsenic acid in 0.04M HCl.

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necessary because ^{74}As would stay at origin without the carrier. The sheet was cut into strips and transferred to counting vials containing 10 ml water. The vials were shaken for 1 min. The ^{74}As radioactivities were then measured in the Beckman 300 system.

RESULTS AND DISCUSSION

Various chromatographic plates (silica gel, alumina and cellulose) and solvent systems were initially tried to separate the arsenic compounds. Best results were obtained with cellulose thin layer sheet with methanol-ammonia as the developing solvent. After spraying with 1N $AgNO_3$ in 5% (v/v) NH_4OH solution, arsenic compounds showed up as different color spots on the sheet as described in Table 1. Confirmation of the presence of arsenic in the color spots was made by measuring the arsenic content of the developed TLC strips using the X-ray fluorescence method. Table 1 shows the R_f values of these compounds. It can be seen that the compounds could be separated from each other except for methylarsenate and methylarsonate, which have the same R_f value of 0.36.

TABLE 1

TLC of Arsenic Compounds[a]

Compounds	R_f	Region[b]	Color of spot[c]
sodium arsenate	0.15	1.0--4.3	brown
sodium methylarsenate	0.36	4.3--6.7	pale yellow
sodium methylarsonate	0.36	4.3--6.7	pale yellow
sodium arsenite	0.54	6.7--9.3	yellow
dimethylarsinic acid	0.68	9.3--12.0	brownish yellow

a) Chromatographed ascendingly on cellulose thin layer sheet
solvent system MeOH:1N NH_4OH=80:20 (v/v)
solvent front 14.5 cm
b) Region in cm along the direction of development
c) Spraying reagent: 1N $AgNO_3$/5% (v/v) NH_4OH solution

In another experiment, the recovery of each compound in the region (Table 1) corresponding to the color spot was found to be >90% as determined by X-ray fluorescence method. The chromatograms and recoveries were similar whether the arsenic compounds were present individually, in an aqueous mixture or in dog urine. Recovery in dog plasma was not obtained due to lack of sample.

Earlier studies from this laboratory have shown that, following an intravenous administration of ^{74}As to dog, ^{74}As in plasma or urine was present predomi-

Individual arsenic standard solutions were prepared by dissolving analytical reagent grade sodium arsenite, sodium arsenate, sodium methylarsenate, sodium methylarsonate and dimethylarsinic acid in water. Each solution contained 20 mg compound/ml.

A mixture of arsenic standard solution was prepared by mixing 10.0 ml each of the above arsenic standard solutions. The final solution contained 4 mg of each arsenic compound/ml.

Cation exchange resin AG50W-X8, 100-200 mesh was obtained from Bio-Rad Laboratories.

Thin layer cellulose sheets were obtained from Eastman Kodak Company.

Sample Preparation

^{74}As (0.4 mCi) was neutralized with equal volume of 0.04M Na_2CO_3 solution and administered intravenously to an adult male Beagle. Blood samples were obtained at 10 min and 2 h after dosing. Plasma was separated out immediately. 0-8 h urine samples were also collected. Plasma, and urine samples were then submitted to ion-exchange and TLC analyses.

Procedure

Ion-exchange chromatographic separation of arsenic compounds: One ml of arsenic standard solution, plasma or urine was applied to the top of the column (0.85x16.5 cm). The column was eluted successively with 10.5 ml 0.5N HCl, 15 ml H_2O, 15 ml 5% (v/v) NH_4OH and 15 ml 20% (v/v) NH_4OH solution. The eluates were collected in 2.0 ml fractions and submitted to γ-ray counting. The method was described in detail by TAM et al. (1978). The two fractions containing the highest radioactivity (>90%) in each of the eluting solutions were then concentrated to 0.2 ml in a hot water bath and used in TLC studies.

TLC confirmation of arsenic metabolites: Thin layer cellulose sheets with fluorescence indicator were used. The solvent system was MeOH:1N NH_4OH (80:20) and the solvent was allowed to travel 14.5 cm from origin (~80 min). Arsenic standard solutions (individual standard or a mixture of standards) were made basic with NaOH solution, 5 µl of each solution was then chromatographed on the cellulose sheet. Arsenic compounds were detected by spraying with 1N $AgNO_3$ in 5% (v/v) NH_4OH solution (MIKETUKOVA et al. 1968). They were also detected by measuring the arsenic content using X-ray fluorescence analysis after extracting with methanol. For the radioactive urine and plasma samples from in vivo studies, the concentrated samples after ion-exchange chromatography were fortified with 10 µl of a mixture of arsenic standard solutions, made basic with NaOH solution and chromatographed. This was

nantly in two fractions (>98%) after chromatographing on the cation-exchange column. One was collected in the inorganic arsenic fraction; the other in the dimethylarsinic compound fraction (TAM et al. 1978). In the present study, TLC of these ^{74}As metabolites showed that the compounds in the first fraction were a mixture of arsenite and arsenate, whereas the compound in the other fraction was dimethylarsinic acid.

Furthermore, ion-exchange--TLC studies of control plasma and urine samples fortified with ^{74}As (as arsenate) and a mixture of arsenic standard solutions *in vitro* showed no exchange of ^{74}As with the arsenic in methylarsenate, methylarsonate or dimethylarsinic acid; however some exchange of ^{74}As with the arsenic in arsenite was observed at room temperature. This indicates that methylation of ^{74}As actually occurred in the body instead of *in vitro*. However, the actual arsenite/arsenate ratio in plasma or urine could not be determined by this method.

The methods used by BRAMAN and FOREBACK (1973), LAKSO and PEOPLES (1975) and TALMI and BOSTICK (1975) to confirm the presence of arsenic metabolites were only indirectly deductive ones. The method described in this paper allows the arsenic metabolites to be separated from each other and then confirmed by TLC method. Little or no exchange of the metabolites would occur except for ionic equilibrium. The metabolites were identified directly in their original form present in plasma and urine. Thus arsenite, arsenate and dimethylarsinic acid are confirmed to be the major arsenic metabolites in dog plasma and urine.

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