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In Vitro Methylation of ^{74}As in Urine, Plasma and Red Blood Cells of Human and Dog

G. K. H. Tam, S. M. Charbonneau, G. Lacroix, and F. Bryce

Food and Toxicology Research Division, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ont., Canada, K1A 0L2

BRAMAN and FOREBACK (1973) found that dimethylarsinic acid and methylarsonic acid were widespread in the environment. Methylated forms of arsenic were also found in urine following ingestion of inorganic arsenic by dog, cow (LAKSO and PEOPLES 1975) and man (CRECELIUS 1977). Dimethylarsinic acid was detected and confirmed to be the major arsenic metabolite in urine and plasma of the Beagle dog following intraveneous administration of inorganic ^{74}As (TAM et al. 1978a,b). A study was conducted to determine whether in vitro methylation of inorganic ^{74}As can also occur in the urine, red blood cells and plasma of the dog and human.

METHODS AND MATERIALS

^{74}As (>1 mCi/μg As) was obtained from Amersham Corporation in the form of arsenic acid. ^{74}As radioactivity was measured using a Beckman gamma 300 scintillation counter.

Fresh blood samples were obtained from an adult male subject and a male Beagle dog. Freshly voided urine was obtained from the same male subject, whereas fresh dog urine was obtained via a urinary catheter. A 24 h urine sample was also collected from the same dog using a metabolism cage. Plasma, red blood cells or urine samples were spiked with 0.05 μCi ^{74}As/g sample. Urine samples were kept for 0, 24, 48 or 72 h at 4°C and 37°C. Plasma and red blood cells were kept for 0, 24, 48 h at 4°C and 24 h at 37°C. Duplicate samples were analysed by an ion-exchange chromatographic method which resolves inorganic arsenic, monomethylated arsenic and dimethylarsinic acid (TAM et al. 1978a). Percentages of the various forms of arsenic compounds were calculated after radioactivity measurements.

RESULTS and DISCUSSION

No methylation of ^{74}As was found in the plasma samples of either human or dog at 0, 24, 48 h at 4°C and 24 h at 37°C, whereas a small amount (~0.2%) of

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dimethylarsinic acid was detected in both human and dog red blood cells after 24 and 48 h storage at 4°C and 24 h at 37°C.

Fresh human and dog urines showed no methylation of ^{74}As at 4°C or 37°C after 24, 48 or 72 h storage. ^{74}As was recovered as inorganic arsenic which was present originally in the tracer solution. No methylation of ^{74}As was observed in the 24 h dog urine sample for the same storage times at 4°C, but methylated forms of ^{74}As were definitely detected at 37°C as shown in Table 1.

TABLE 1

In Vitro Methylation of ^{74}As in the 24 h Dog Urine at 37°C

Time of storage	Inorganic arsenic	Monomethylated arsenic	Unidentified arsenic	Dimethyl-arsinic acid
0 h	96.8*	2.9*	0.2*	0*
24	91.5	2.8	2.6	3.1
48	88.0	2.9	3.5	5.6
72	78.1	4.4	5.9	11.6

* % of ^{74}As, average of duplicate analyses

It has been shown that microorganisms can methylate inorganic arsenic to organic forms (McBRIDE and WOLFE 1971, COX and ALEXANDER 1973). Contamination of urine by microorganisms would be much greater in urine sample collected over a 24 h period using a metabolism cage than urine obtained using a urinary catheter. Subsequent storage of the 24 h sample at 37°C would result in a further increase in the quantity of microorganisms present. Therefore the presence of increased amounts of methylated arsenic derivatives in 24 h dog urine stored at 37°C is likely due to the action of microorganisms present in the urine.

The results of the present study showed no difference between the plasma, red blood cells and fresh urine of human and dog in methylation of inorganic ^{74}As. No methylated forms of arsenic were observed under the *in vitro* condition except for red blood cells.

Recent studies in our laboratories showed that more than 10% of ^{74}As in urine, plasma and red blood cells was present as dimethylarsinic acid 1 h after intraveneous administration of ^{74}As to the Beagle dog (CHARBONNEAU and TAM, unpublished). Dimethylarsinic acid was also found in urine of man (CRECELIUS 1977), cow and dog (LAKSO and PEOPLES 1975) at 24 h following ingestion of inorganic arsenic. The slow process of *in vitro* methylation which occurred in red blood cells and 24 h dog urine indicated different mechanisms were involved under *in vivo* and *in vitro* conditions.

Methylation which occurred *in vivo*, possibly in the liver, would likely proceed through enzymatic reaction, whereas *in vitro* methylation in the urine might result from microbial contamination.

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