meat samples is comparable with the mercury content in meat observed in Sweden and Denmark (Westoo, 1969). The analysis of meat samples reveals that the mercury content is considerably higher in bacon and pork sausage. The determination was based on the wet sample weight. In 1963, Smart and Lloyd (1963) reported a high content of mercury ($\simeq$10 ppm) in eggs from hens fed with seed containing about 6–14 ppm of methylmercury dicyanodiamide. This compound has been used extensively in the United States for the treatment of seed. The only egg white sample analyzed shows the mercury content to be greater than 0.1 ppm, much higher than the average Swedish value of 0.029 ppm.

In order to check the applicability of this method to most all types of biological samples, several hair samples were digested and analyzed for their mercury content. The hair samples dissolved easily and presented no problems. Values ranged from 0.255 to 1.32 ppm of Hg for the 18 samples analyzed.

LITERATURE CITED

Bache, C. A., Lisk, D. J., *Anal. Chem.* **43,** 950 (1971).
Basely, T. M., *Environ. Sci. Technol.* **5,** 634 (1971).
Bucknell, M., *Br. J. Ind. Med.* **8,** 181 (1951).
Cranston, R. E., Buckley, D. E., *Environ. Sci. Technol.* **6,** 274 (1972).
Fagerstrom, T., Jernelov, A., *Water Res. Program Press* **5,** 121 (1971).
Fishman, M. J., *Anal. Chem.* **42,** 1462 (1970).
Gage, J. G., Watten, J. M., *Ann. Occup. Hyg.* **13,** 115 (1970).
Hatch, W. R., Ott, W. L., *Anal. Chem.* **40,** 2085 (1968).
Johnasson, B., Ryhage, R., Westoo, G., *Acta Chem. Scand.* **24,** 2349 (1970).
Kalb, G. W., *At. Absorpt. Newsl.* **9,** 84 (1970).
Kimura, Y., Miller, V. L., *Anal. Chim. Acta* **27,** 325 (1962).
Lindstedt, G., *Analyst* **95,** 264 (1970).
Lindstedt, G., Skare, I., *Analyst* **96,** 223 (1971).
Malaiyandi, M., Barrette, J. P., *Anal. Lett.* **3,** 579 (1970).
Mayer, J., *Bull. Environ. Sci. Toxicol.* **5,** 383 (1970).
Moffitt, A. E., Jr., Kupel, R. E., *At. Absorpt. Newsl.* **9,** 113 (1970).
Newsome, W. H., *J. Agric. Food Chem.* **19,** 567 (1971).
Nobel, S., *Stand. Methods Clin. Chem.* **3** (1961).
Noren, K., Westoo, G., *Acta Chem. Scand.* **22,** 2289 (1968).
Pillay, K. K. S., Thomas, C. C., Jr., Sondel, J. A., Hyche, C. M., *Anal. Chem.* **43,** 1419 (1971).
Poluektov, N. S., Vitkun, R. A., Zelyukova, Ya. V., *Zh. Anal. Khim.* **19,** 937 (1964).
Rathje, A. O., *J., Am. Ind. Hyg. Assoc.* **30,** 126 (1969).
Rottschafer, J. M., Jones, J. D., Mark, H. B., Jr., *Environ. Sci. Technol.* **5,** 336 (1971).
Sandell, E. B., "Colorimetric Determination of Traces of Metals", Vol. 3, Interscience, New York, N.Y., 1959.
Sjostrand, B., *Anal. Chem.* **36,** 814 (1964).
Smart, N. A., Lloyd, M. K., *J. Sci. Food Agric.* **14,** 734 (1963).
Sumino, K., *Kobe J. Med. Sci.* **14,** 115 (1968a).
Sumino, K., *Kobe J. Med. Sci.* **14,** 131 (1968b).
Suzuki, T., Miyama, T., Katsunuma, H., *Ind. Health* **8,** 39 (1970).
Suzuki, T., Takemoto, T., Shimano, H., Miyama, T., Katsunuma, H., Kagawa, Y., *Ind. Health* **9,** 1 (1971).
Takizawa, Y., *Acta Med. Biol. (Niigata)* **17,** 293 (1970).
Westoo, G., *Acta Chem. Scand.* **20,** 2131 (1966).
Westoo, G., *Acta Chem. Scand.* **21,** 1790 (1967).
Westoo, G., *Acta Chem. Scand.* **22,** 2277 (1968).
Westoo G., *Chem. Fallout, 1969,* 75–93 (1969).

Received for review May 3, 1974. Accepted April 22, 1975. Presented before the Southeastern Regional Meeting of the American Chemical Society, Birmingham, Ala., Nov 1972. The authors are indebted to the Murray State University Committee on Institutional Studies and Research for financial support of this research.

Methylation of Inorganic Arsenic by Mammals

Jolean U. Lakso*[1] and Stuart A. Peoples

Four cows and four dogs were fed sodium arsenate and potassium arsenite daily for 5 days. Their urine was analyzed for methylated arsenic (MA) and inorganic arsenic (IA) during the control, feeding, and feed-off periods. The control values of MA and IA in cow urine were 0.12–0.26 and 0.05–0.17 ppm, respectively. These levels peaked at 3.53 ppm of MA and 1.33 ppm of IA during arsenate feeding and at 4.78 ppm of MA and 1.57 ppm of IA during arsenite feeding. For the dog urine, the MA and IA control values were 0.20–0.31 and 0.11–0.16 ppm, respectively. Feeding arsenate caused a peak in MA and IA values of 6.23 and 10.48 ppm, respectively. Feeding arsenite caused a rise to 5.03 ppm of MA and 5.16 ppm of IA. Values for both arsenic forms reached control levels 5 days after feeding stopped in both mammalian species. It was concluded that both species produced methylated arsenic when given either trivalent or pentavalent inorganic arsenic.

McBride and Wolfe (1971) have given evidence, using laboratory cultures, that methanogenic bacteria (*Methanobacterium* strain M.o.H.) can methylate arsenic. Braman and Foreback (1973) pointed out that because suitable analytical procedures were lacking, it has been difficult to establish if such methylation or its by-products existed in nature. Braman et al. (1972) described a spectral emission system for arsenic. Braman and Foreback further refined the procedure to analyze for inorganic trivalent and pentavalent arsenic as well as several methylated forms of arsenic. They subsequently analyzed samples taken from "natural water, bird eggshells, seashells, and human urine" (Braman and Foreback, 1973). They found dimethylarsenic acid to be the "major and ubiquitous form of arsenic in the environment". Methanearsonic acid was also present, but in lower concentrations.

In two previous reports the authors (Peoples et al., 1971; Lakso et al., 1973) have shown that the principal form of arsenic in mammalian urine and plants was a methylated form of arsenic (MA). The presence of MA in the normal background of plants suggested the possibility that the MA in the mammalian urine could have its origin in the vegetative portion of the diet and not from the metabolism of the animal. However, the findings of McBride and Wolfe (1971) that bacterial methylation occurred under anaerobic conditions suggested arsenic methylation could occur in the rumen of the cow, a site of intense anaerobic bacterial action (Hungate, 1966).

The experiments reported here were done to ascertain if

Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, California 95616.
[1] Present address: School of Pharmacy, University of the Pacific, Stockton, California 95207.

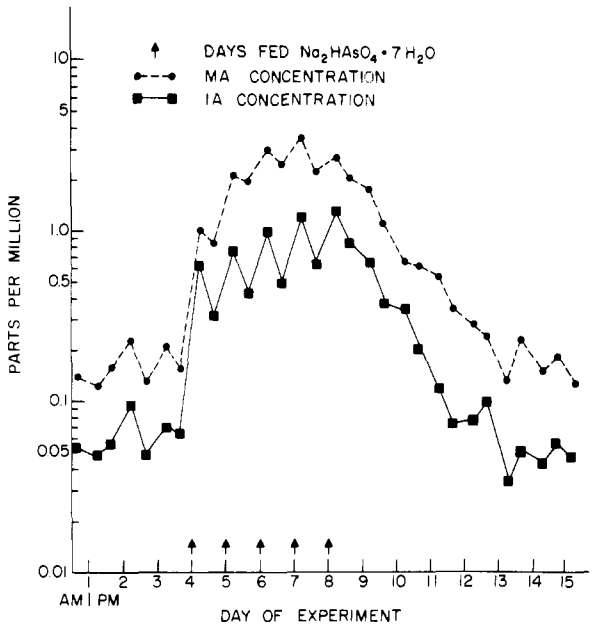

Figure 1. Average concentration of methylated arsenic (MA) and inorganic arsenic (IA) in urine from four cows fed 2.75 mg/kg sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$).

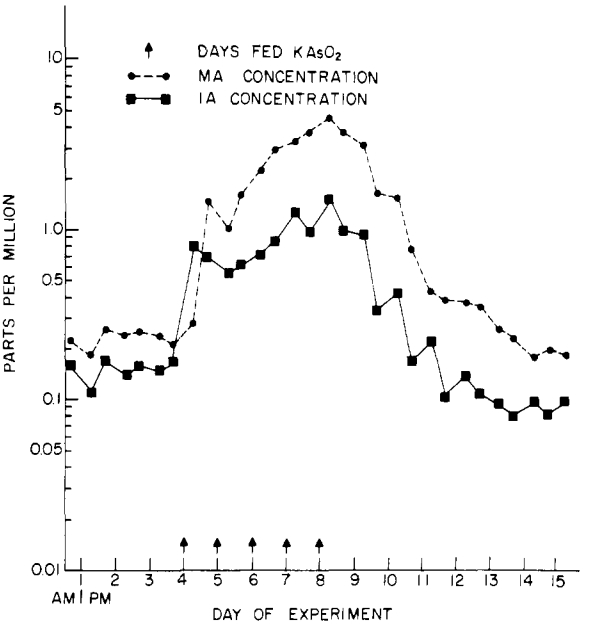

Figure 2. Average concentration of methylated arsenic (MA) and inorganic arsenic (IA) in urine from four cows fed 1.75 mg/kg potassium arsenite ($KAsO_2$).

mammals can methylate arsenic or were only excreting the background MA that could be in their diet. Two types of mammals, carnivore and ruminant, were used to determine if the rumen is essential for inorganic arsenic (IA) methylation.

EXPERIMENTAL SECTION

Four cows (two Jerseys, one Hereford, one Holstein) were fed five consecutive daily doses (2.75 mg/kg) of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) with their morning dairy ration. Urine samples were collected approximately 12 hr apart, in the morning and evening, 3 days before, the 5 days during and for 7 days after feeding. One week after the 7-day feed-off period, the same cows were fed 1.57 mg/kg of potassium arsenite ($KAsO_2$) and the urine samples were collected as described for the sodium arsenate experiment.

Four male dogs (two beagles, two pound-run) were placed in metabolism cages and 24-hr urine samples were collected 3 days before, the 5 days during, and for 7 days after arsenic feeding. They were fed 3.40 mg/kg of sodium arsenate in canned dog food daily for 5 days, followed by a 7-day feed-off period. Seven days later, the same experiment was repeated using 1.94 mg/kg of potassium arsenite. All the doses used in these experiments are less than the recommended therapeutic doses of arsenic as listed by Milks (1949).

The samples were analyzed using the method reported by Peoples et al. (1971) and Lakso et al. (1973) which simultaneously determines MA and IA in urine and plant extracts, with a limiting sensitivity of 0.02 ppm, with 85–100% recovery from arsenic-spiked samples of urine and plant extracts. In this method dimethyl and monomethyl arsenate and monomethyl arsenite are reduced to their corresponding arsines. All of these arsines react with a solution of silver diethyldithiocarbamate to give a colored complex which has a maximum absorption at 450 mμ. The IA reacts with this reagent to give a complex which has a maximum absorption at 550 mμ. The concentrations of MA and IA are then determined using a two-color calculation method.

All urine samples were preserved by the addition of formaldehyde and refrigerated at 4° until analyzed. Each urine sample was analyzed in triplicate and the results varied from the mean not more than 2.1%. There were no signs of toxicity in any of the animals. None of the animals gave off any garlic-like odors. No differences in methylating ability were noted among the various breeds of cows or breeds of dogs.

RESULTS

Figures 1 and 2 show the results of feeding inorganic arsenic to cows. It can be seen that in the sodium arsenate trials the range of normal urine levels of MA (average of four cows) was 0.12–0.23 ppm and for IA 0.05–0.08 ppm. Twelve hours after the first dose both the MA and IA rose rapidly and, following the third dose, plateaued with the MA varying between 2.02 and 3.53 ppm and IA between 0.43 and 1.33 ppm. There was a peak each day; this was probably related to the arsenic dose given 12 hr earlier. Five days after feeding stopped, the levels of MA and IA both dropped to control levels.

Cow urine taken prior to the potassium arsenite feeding had arsenic levels of 0.18–0.26 ppm for MA and 0.11–0.17 ppm for IA. The increase in MA lagged behind the increase in IA by 12 hr after the first day of arsenic feeding before reaching a peak level of 4.78 ppm of MA and 1.57 ppm of IA. There was a gradual increase in both MA and IA levels. MA and IA levels both dropped to control levels, 5 days after feeding stopped.

The results of feeding dogs inorganic arsenic are shown in Figures 3 and 4. In the sodium arsenate experiment the control values for MA were 0.20–0.28 ppm and for IA, 0.11–0.15 ppm (average of four dogs). The level of MA rose quickly to a rounded peak height of 6.23 ppm. The IA level formed a sharp peak at 10.48 ppm. Both levels dropped off rapidly to control values 5 days after arsenic feeding stopped. In the potassium arsenite experiment both the MA and IA levels rose slowly to essentially the same peak values (5.03 and 5.16 ppm, respectively) after the fifth feeding from control values of 0.25–0.31 ppm for MA and 0.13–0.16 ppm for IA. Control values were again reached 5 days after the last feeding.

DISCUSSION AND CONCLUSIONS

Both mammals readily produced methylated arsenic when given either trivalent or pentavalent IA, with approximately equal amounts of MA and IA being excreted rapidly into the urine. The short delay in increased MA ex-

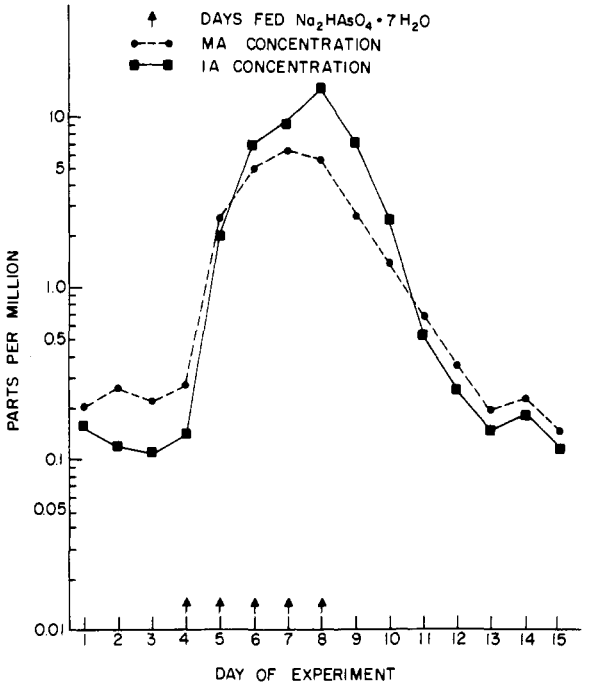

Figure 3. Average concentration of methylated arsenic (MA) and inorganic arsenic (IA) in urine from four dogs fed 3.40 mg/kg sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$).

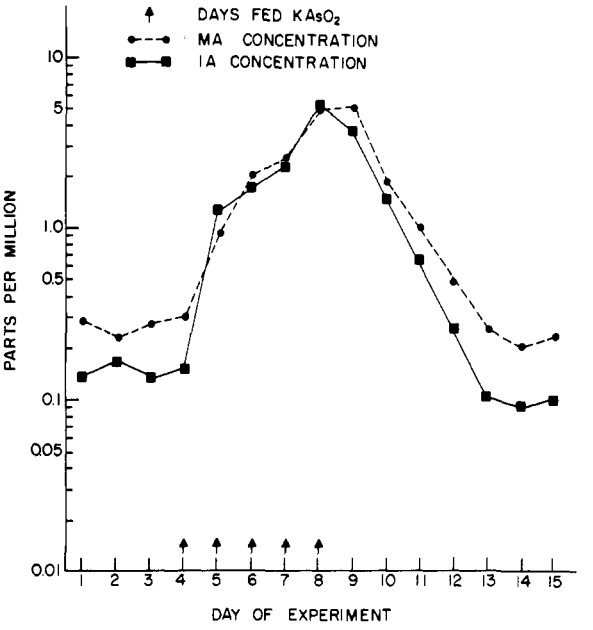

Figure 4. Average concentration of methylated arsenic (MA) and inorganic arsenic (IA) in urine from four dogs fed 1.94 mg/kg potassium arsenite ($KAsO_2$).

cretion after arsenite feeding suggests that it may be oxidized to arsenate before being methylated. In both species the gradual rise in both MA and IA urine levels after arsenite feeding suggests that there could be a gradual induction of the necessary metabolic processes required to oxidize arsenite to arsenate. Support for this concept is the work of Winkler (1962). He found that when rats were fed trivalent IA, the major form of arsenic found, in their livers, was pentavalent IA. On the other hand, McBride and Wolfe (1971) found that before methylating IA, *Methanobacterium* reduced the arsenate to arsenite.

Although the cow excretes a larger percentage of her arsenic overload in a methylated form, the results of the dog experiments cast doubt that the site of methylation in the cow is solely in the rumen. The $T_{1/2}$ excretion rate was about 1.5 days in both species after being fed both forms of arsenic. Unlike the methylation of mercury, methylation of arsenic is probably a detoxification mechanism, given that methanearsenate is much less toxic than sodium arsenate (Christensen, 1973).

LITERATURE CITED

Braman, R. S., Foreback, C. C., *Science* **182,** 1247 (1973).
Braman, R. S., Justen, L. L., Foreback, C. C., *Anal. Chem.* **44,** 2195 (1972).
Christensen, H. E., Ed., "The Toxic Substances List", U.S. Department of Health, Education, and Welfare, Rockville, Md., 1973, pp 96–97, 590–591.
Hungate, R. E., "The Rumen and Its Microbes", Academic Press, New York, N.Y., 1966, pp 8–81.
Lakso, J. U., Peoples, S. A., Bayer, D. E., *Weed Sci.* **21,** 166 (1973).
McBride, B. C., Wolfe, R. S., *Biochemistry* **10,** 4312 (1971).
Milks, J. H., "Practical Veterinary Pharmacology, Materia Medica and Therapeutics", Alex Eger, Inc., Chicago, Ill., 1949, pp 490–500.
Peoples, S. A., Lakso, J. U., Lais, T., *Proc. West. Pharmacol. Soc.* **14,** 178 (1971).
Winkler, W. O., *J. Assoc. Off. Agric. Chem.* **45,** 80 (1962).

Received for review July 29, 1974. Accepted February 15, 1975. Presented in part at the 16th Western Pharmacology Society Meeting, Squaw Valley, Calif., Feb 1973. This investigation was supported, in part, by General Research Support Grant, NIH 05457.