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ABSTRACT

The *in vitro* conversion of ^{131}I-labeled sodium iodide to volatile iodine was investigated in the artificial rumen and in simulated abomasal and intestinal fluids. In addition, the association of ^{131}I-labeled iodide with rumen juice sediment, which includes microflora and feed debris, was also studied. The results show that under the conditions reported here, ^{131}I is not volatile. As much as three percent of the ^{131}I was shown to be associated with rumen juice sediment in the artificial rumen. This value was reduced to 0.52 percent and 0.038 percent in the simulated abomasal and intestinal fluids, respectively.

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INTRODUCTION

Gases of the rumen may be expelled by eructation and subsequently absorbed by the lungs. At this laboratory it was observed that ^{131}I appears in the blood and milk of cattle very soon after an oral administration of ^{131}I iodide. It was therefore postulated that ^{131}I could have been converted to volatile iodine in the rumen and transported into the respiratory system by eructation where it could be absorbed. Experiments using the artificial rumen were designed to test this hypothesis.

Dougherty et al. (1965), discussing work of others concerning eructated rumen gas, stated the following: A repetition by Dougherty et al. (1962) of an experiment performed many years ago by Dougherty (1940) very effectively demonstrated that components of rumen gas which reach the lungs are absorbed. Dougherty et al. (1962) and Shipe et al. (1962) demonstrated that certain odors carried in eructated gases from the rumen to the lungs were absorbed, thereby contaminating the mammary blood supply and transmitting off-flavors to the milk. The same substances tested in these experiments were also absorbed by the portal blood; however, contamination of milk was much slower by this route than it was when eructated gases were allowed to enter the lungs.

This study was designed to assess the possibility that ^{131}I iodide might be converted to volatile iodine or methyl iodide in rumen juice which could then be eructated into the respiratory tract. If iodide were indeed

converted to iodine in the gastrointestinal tract, it would then be necessary to account for ^{131}I losses by eructation as well as recycling of ^{131}I via the respiratory system. Volatilization of ^{131}I iodide from simulated abomasal and intestinal juice was included in this study. In addition, the binding of ^{131}I to rumen microflora and other sediment was also studied.

Barth and Bruckner (1969 a,b) effectively employed an artificial rumen followed by simulated abomasal and intestinal fluids to study the uptake by binding agents of ^{134}Cs, strontium, and other essential cations in order to reduce radionuclide transport to milk. This procedure also provides a means for investigating the solubility or availability for absorption of fallout radionuclides in a natural medium, taken directly from a rumen-fistulated steer, in the presence of viable rumen microflora.

METHODS AND MATERIALS

The artificial rumen and simulated abomasal and intestinal fluid procedure used is similar to that employed by Barth and Bruckner (1969 a,b) and is briefly described here with necessary modifications. Each digestion flask was prepared by addition of 250 ml of rumen juice to 250 ml of basal medium in a one-liter Ehrlenmeyer flask containing 3.75 g of powdered cellulose. The CO_2 outlet from each Ehrlenmeyer flask was connected to an ^{131}I scrubber consisting of a 1.5- by 20-cm column of activated charcoal. The content of each flask was adjusted to pH 6.5 with saturated sodium carbonate solution and dosed with 0.7 to 1.2 μCi of carrier-free $Na^{131}I$. Digestion flasks were incubated for about 21 hours in a water bath at 39°C, with a continuous stream of CO_2 passing through the contents of each flask.

The artificial rumen fluid in each flask was then converted to simulated abomasal fluid by addition of 125 ml of simulated abomasal juice containing 0.25 g pepsin and by adjusting the pH to three with 5.0 N HCl. The resulting mixture was incubated for three hours. The CO_2 flow was allowed to continue slowly in order to sweep gaseous ^{131}I that might be produced to the charcoal scrubber.

Following the abomasal digestion phase, the simulated abomasal fluid was converted to a simulated intestinal fluid. One-hundred milliliters of 0.1N $NaHCO_3$ was added followed by 40 ml of bile. Fifty milliliters of an enzyme preparation containing 6.0 g pancreatin, 0.3 g trypsin, and 0.3 g erepsin was added and the pH was adjusted to six with 5.0 N NaOH. Incubation continued for an additional three hours with a slow rate of CO_2 flow.

At the beginning and end of each digestive phase, a 5-ml sample of rumen juice was removed and pipetted directly into 50 ml of 0.1N NaOH for radioanalysis of ^{131}I. Upon completion of each digestive phase, an additional 10-ml sample from each flask was removed and centrifuged for 20 minutes at the maximum speed of a Servall Table Model Centrifuge, Type M (over 5000 rpm). The supernatant was decanted into 0.1N NaOH for radioanalysis while the sediment was washed twice by resuspension in 5 ml of water and recentrifuged. All supernatants were combined. The washed sediment was analyzed for radioiodine. Fresh charcoal scrubbers were used during each digestive phase for collection of gaseous ^{131}I. Gamma spectrometry was carried out using opposed 5- by 9-inch sodium iodide crystals associated with a TMC 400-Channel Analyzer. Digestion flasks were run in duplicate for three separate trials.

A preliminary experiment was designed so that gas chromatography could be used to determine the chemical form of any volatile iodine. This operation was similar to the procedure described above except that the CO_2 outlet was connected to a small filter flask which served as a trap. This was followed by passing the gas through a 1.5- by 20-cm column of a mixture of calcium chloride pellets and anhydrous calcium sulfate which served as a drying agent. Plastic tubing was used to connect this column to the first scrubber which consisted of a "U" tube containing 4 ml of toluene. The toluene scrubber was placed in a Dewar flask containing a slurry of ground dry ice and isopropyl alcohol. This was subsequently attached to a column of activated charcoal which served as a backup scrubber. Toluene was used only during the artificial rumen phase, while the charcoal scrubber was used during all digestion phases.

During the preliminary trial only, one digestion flask was used. Rumen juice was sampled at the beginning of the phase and at one, six, and 16-1/2 hours after dosing. Samples were collected at the beginning and end of the abomasal and intestinal phases. The collection of ^{131}I by the toluene liquid scrubber permitted later separation of organic and inorganic gaseous ^{131}I by gas-liquid chromatography and possible identification of organic and other forms.

RESULTS AND DISCUSSION

Results of the preliminary trial (data not shown) indicate practically no loss of ^{131}I from the digestive juices during the incubation periods. Virtually no ^{131}I was recovered in the toluene and charcoal scrubbers or

in the plastic tubing used. Chromatographic analysis, originally scheduled on the toluene to determine the form of ^{131}I present, was not necessary since the toluene contained no radioactivity.

The averages of the results of the three in vitro trials are shown in Table I. An analysis of variance indicates that there was no significant difference ($p >> 0.05$) between the activity levels in any of the nine fluids sampled. This indicates little, if any, loss due to volatility of ^{131}I or binding of ^{131}I to sediment.

Radioactivity associated with the sediment was not significantly above background. Therefore, although some activity is associated with the sediment, it is within the limits that might be expected from experimental error. There was a sharp drop in sediment-associated ^{131}I following abomasal digestion and almost no ^{131}I was associated with sediment during the intestinal phase. This may be due to enzymatic digestion of the rumen microflora and other sediment present.

Absence of ^{131}I deposited on the charcoal scrubbers also demonstrates little or no ^{131}I volatility in these digestive fluids. Though not statistically significant ($p >> 0.05$), there appears to be a slight drop in ^{131}I remaining in whole juice and supernatant between the beginning and the end of the abomasal phase. This cannot be accounted for by ^{131}I association with sediment or deposition on the charcoal scrubbers and is possibly due to binding of ^{131}I to the container during incubation or centrifugation.

TABLE I

^{131}I DISTRIBUTION DURING *IN VITRO* DIGESTION

	Artificial Rumen %	Abomasal Phase %	Intestinal Phase %
Whole juice start	100.00	100.00	100.00
Percent of ^{131}I remaining at end of phase	102.36*	96.52	98.59
Percent of ^{131}I in supernatant at end of phase	98.16	94.53	99.82
Percent of ^{131}I in sediment at end of phase	3.13	0.521	0.038
Percent of ^{131}I deposited on charcoal during phase	0.00062	0.001038	0.0000

*Value greater than 100% is due to counting error.

These results suggest that ^{131}I iodide present in rumen contents is not volatilized and it is not likely that it would be eructated as gaseous iodine into the bovine respiratory system where it might be absorbed through the tissues of the respiratory tract.

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