Physiological Studies of Environmental
Pollutants

MASTER

FINAL REPORT

September 1, 1975 - March 31, 1978

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Prepared for the U. S. Energy Research
and Development Administration
under Contract # E(11-1)-2784

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PHYSIOLOGICAL STUDIES OF
ENVIRONMENTAL POLLUTANTS

Final Report
for period September 1, 1975 to March 31, 1978

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Administration under contract number E(11-1)-2784

U. S. E. R. D. A. :

Expenditure of Effort for September 1, 1976 to March 31, 1978

F. W. Lengemann	0.2 man years
R. A. Wentworth	0.3 man years

In early September, 1976, Dr. Clifton Blincoe came to Cornell on sabbatic leave from the University of Nevada. Dr. Blincoe chose to work along lines that would supplement the effort of this contract. Thus an additional 0.3 man years should be added to the above efforts.

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ABSTRACT

Physiological studies of environmental pollutants are reported in which a major emphasis is on factors involved in the secretion of these materials into milk. Elements of concern relate to the energy production field, both nuclear and non-nuclear. The distribution of ^{207}Bi , ^{203}Pb , ^{210}Po and ^{201}Tl between milk, urine and feces of lactating goats was determined after oral and intravenous administration. Data is presented showing that these elements are poorly absorbed and in consequence appear in milk in only small amounts. However levels in goats appear to be greater than in cows. Experiments relating to mammary transfer of non-actinide series elements are summarized. Included are observations of significant temperature effects of radioiodine transfer, sites and rates of resorption of iodine, zinc and calcium from the mammary gland; and the use of radio indicators in determining in vivo milk volume. Experiments with zinc have provided blood level, secretion and balance data necessary for modeling zinc metabolism in lactating goats. A method is presented for compartmental analysis of models involving a pool of cycling volume such as the mammary gland.

The Metabolism of ^{207}Bi , ^{203}Pb , ^{210}Po and ^{201}Tl by Lactating Goats

Abstract

The distribution of ^{207}Bi , ^{203}Pb , ^{210}Po and ^{201}Tl between milk, urine and feces of lactating goats was determined after oral and I.V. administration of the radioactivity. These elements were poorly absorbed and in consequence only small amounts appeared in the milk. For at least ^{210}Po and ^{203}Pb more of these radionuclides appeared in the milk of these goats than has been reported for cows.

A major effort of this research was to look at some member of the actinium decay series to determine the amounts that might get into the milk of lactating goats. The use of nuclear reactors for the production of electrical power and concern over the possible harmful effects of the heavy elements if a reactor accident should occur is the impetus for such a study. Milk represents a significant path of contamination for the human population since the cow and the goat consume vegetation that represents tremendous areas of leaf surface upon which these elements could deposit. Very few studies have been carried out on the transfer of these elements into milk and most have been done with cattle. It is recognized that the goat is sometimes more efficient in its transfer of a contaminant to milk than is the cow, and since little information was available it seemed worthwhile to begin with this species. We looked at the transfer to milk after oral administration and after intravenous administration to be able to estimate if the digestive tract or the mammary gland would be the major block to transfer into milk.

Methods

For these studies we used classical animal metabolism methods. The goats were put into galvanized steel metabolism stalls that were equipped with screens and pans to separate and collect urine and feces. The metabolism stalls were contained in an environmental chamber that was set at 20°C and had a diurnal cycle of 12 hours of light and 12 hours of darkness. The animals were conditioned to the metabolism stalls for about one week before dosing and sample collection began. There was always one sample of milk, urine, feces and blood taken before the radionuclide was administered.

Feces, milk and urine were collected and weighed twice daily; the morning samples were taken at 0800 and the evening sample at 1500; blood was collected at about 1030 hrs on a preset schedule. The amount of hay and grain consumed during the day was also recorded. The length of the experimental period was determined by the amount of radioactivity appearing in the milk.

Since it was expected that little radioactivity would actually transfer into milk a special point was made to reduce the exterior contamination of the udder prior to milking. To accomplish this before milking, the udder of the goat was washed and dried and the milker donned a clean pair of gloves. This probably reduced the extraneous contamination of the milk but may not have eliminated it completely.

Dosing of the animals at the start of the experiment occurred at 1030 hours. For the oral dose the desired amount of the dosing solution was pipetted into a small capsule containing tissue paper; this small capsule was placed in a much larger capsule which also contained tissue paper. The capsules were capped and then given by balling gun. For an intraveous injection the dose was given by syringe directly into the jugular vein. In most cases the standard was made up immediately using the same pipette or syringe.

The method of preparation of the samples and the counting of the samples depended upon the type of emission and its energy. Polonium-210 is an alpha emitter and therefore the samples had to undergo preparation before counting. For feces this meant a $\text{HNO}_3\text{-HClO}_4$ digestion and for whole blood a $\text{H}_2\text{O}_2\text{-HClO}_4$ digestion was used. Aliquots of the digests were put into Aquasol and counted in a liquid scintillation counter. Milk, plasma and urine were pipetted directly into Aquasol and counted.

For the other radionuclides the samples were in quart screwcapped jars and were counted in a small animal (Tobor) gamma counting system. All of the sample was counted and so no partitioning was necessary. Comparison was made to a dose standard made up in 1 liter of water. Where the sample being counted was less than 1 liter in volume water was added as needed so that self absorption differences would be minimized.

The radionuclides administered to the animals were examined by use of a multi-channel analyzer which had a Ge-Li detector. Only in the case of ^{201}Tl was it possible to demonstrate that ^{202}Tl was present as a contaminant.

Results and Discussion

The results of the studies to date are summed up in Table 1. Each value listed in this table is the average of two goats and represents experimental periods ranging upward from 20 days. Most often the length of the experimental period was determined by the presence of detectable amounts of the radioisotope in the milk. As was expected the transfer of these heavy elements into milk was quite low. The value for the percent dose in milk represents the sum of the activity in milk over the entire experimental period. To obtain the percent dose/liter the total amount of radioactivity secreted into the milk was divided by the average amount of milk secreted daily as determined by the milk production records of the first 18 days or during the period in which detectable radioactivity was present in the milk.

Of the radioelements studied ^{207}Bi showed the least transfer into milk, less than 0.1% after an oral dose. As judged by the excretion values it is very poorly absorbed; that which is absorbed or reaches the body proper is preferentially disposed of in the urine. By comparing the urinary excretions of the oral and I.V. doses and assuming that I.V. dose to represent 100% of the radionuclide in the body it can be calculated that only 1% of the oral dose could have been absorbed. If milk is used for comparison a similar calculation suggests that 17.5% of the dose was absorbed. Since the recovery in the feces was 100% after an oral dose it is not likely that 17.5% was absorbed. Possible explanations for this overestimate of absorption using milk ratios could be that some of the radioactivity in the milk after oral dosing was from external contamination or that after absorption from the digestive tract bismuth is in some form that is more readily transferred into the milk than the $\text{Bi}(\text{NO}_3)_3$ that was injected intravenously.

More ^{203}Pb was transferred to milk than ^{207}Bi . After an I.V. dose about 3.4%/L was found in the milk and 0.2%/L after an oral dose. Lead given I.V. was readily retained in the body; about 50% was still in the body when the experiment was terminated. Of that lead excreted after an I.V. dose two-thirds was in the feces and one-third in the urine. Using the milk values for I.V. and oral routes of administration and that the I.V. dose represents 100% of the ^{203}Pb in the body the absorption of lead nitrate via oral administration is estimated as 5.2%. When the urine is used in similar calculations absorption is estimated as 5.7%. These estimates are in close agreement with the 4.5% from the balance study and with one another. In contrast to the bismuth data this suggests that the lead which is given I.V. and that given orally are in the same form and equally available for milk secretion. The goat seems to put 10 times as much dietary lead into the milk per liter as the cow [Stanley et al Health Physics 21: 211 (1971)].

The transfer of ^{210}Po to milk seemed to resemble that for lead. Of the polonium that got into the body only about 50% was excreted and 80% of that went via the feces. From comparison of milk values after the two modes of administration (3.1 and 0.5%/L I.V. and oral, respectively) it was estimated that 15% of an oral dose of polonium was absorbed; a similar calculation using the urine gave a value of 0.1% absorption. Since the fecal polonium collections approximated 100% these calculations suggest that polonium absorbed from the digestive tract may be in a form that is more readily secreted into milk than that given intravenously. The secretion of ^{210}Po into the milk of the cow is about 0.18%/liter as determined from the data of Schrecklise and Walters [J. Dairy Sci. 52: 1867 (1969)] and Walters and McInroy [Health Physics 16: 22 (1969)]; the goats used in our studies put $2\frac{1}{2}$ times as much into their milk as did the cows.

At this point it should be noted that our recovery of ^{210}Po after an oral dose was greater than 100%. The reason resides solely with the fecal analysis. In some samples we have obtained spurious counts which are, in all probability, related to large amounts of HNO_3 and/or HClO_4 remaining in the samples after the liquid ashing process. This was true only for the oral dosing trial; our technique had improved by the time we analyzed

the feces for the I.V. study so that these spurious results were not encountered. In addition, it is possible that we encountered absorptive losses for our standard in the oral dosing trial. Calculations showed that the DPM recovered in the standard was but 88% of theoretical for the oral dose study but was 98.1% for the I.V. study. If the data are corrected for this the underestimate of the standard the total recovery for ^{210}Po for the oral dose experiment for milk, urine and feces was 102.5%.

Thallium-201 exhibited a greater mobility in the body of the goat after an I.V. dose than either lead or polonium; the intestine was the preferred pathway for excretion of thallium once it entered the body. About 78% of the I.V. dose was recovered with three-quarters appearing in the feces; about 2% of the administered dose was recovered in the milk.

After an oral dose more radiothallium was recovered in the urine than for either polonium, lead or bismuth. Based on the percent of the dose of thallium in the urine the absorption from an oral dose was estimated as 64% of the dose and based on the milk the absorption was 42%. Both of these estimates are very much higher than the zero absorption indicated by the fecal collections. Since the urine values were high the fecal data is obviously in error.

Investigation of the ^{201}Tl dosing solution using a GELi detector system indicated the presence of ^{202}Tl with possible other unidentifiable radioimpurities. The half life determined for the counting standard was 100 hours which is significantly different from the 74 hour $t_{1/2}$ listed for ^{201}Tl .

The data, overall, indicate ready absorption of ^{201}Tl after an oral dose with about 0.8%/L of the dose appearing in the milk. Little data is available in the literature for comparative purposes.

The data for these members of the actinide series show that transfer to milk can occur even though the intestine serves to bar major proportions of most of these elements from entering the body. Both bismuth and polonium showed greater than expected transfer to milk after an oral dose suggesting that during absorption a change in chemical state may occur. All of the elements used show at least two possible valence states and were administered in the lower oxidation state. If further oxidation occurred in the intestine or in the intestinal cells this could produce a form which had different transfer characteristics than the original. Another possibility is that bismuth and polonium have an affinity to combine with particular anions in the digestive tract or cells and remain in close association in their distribution through the body. Further work is indicated in this area.

Where comparisons are possible it has been noted that the proportion of the oral dose of the radioelement transferred to milk seems to be greater for the goat than for the cow.

The curves depicting the course overtime for these elements in urine, milk, feces, blood and plasma have been reported in our first progress report (COO-2784-1) and will not be repeated here.

Table 1: Partitioning of selected members of the actinium decay series into milk, urine, feces of lactating goats after oral and intravenous administration.

<u>Element</u>	<u>Route of Administration</u>	<u>Milk</u>		<u>Urine</u>	<u>Feces</u>
		<u>Percent of Dose</u>	<u>Percent Dose/L</u>	<u>Percent of Dose</u>	<u>Percent of Dose</u>
^{207}Bi	I.V.	0.4	0.74	66.1	21.2
	Oral	0.07	0.09	0.6	100.3
^{203}Pb	I.V.	3.5	3.4	15.5	31.5
	Oral	0.2	0.2	0.8	94.5
^{210}Po	I.V.	3.3	3.1	9.6	48.1
	Oral	0.5	0.47	0.01	113.8
^{201}Tl	I.V.	1.9	2.0	17.7	59.1
	Oral	0.8	0.84	11.5	111.3

Summary of Experiments Relating to Mammary Transfer
of Non-Actinide Elements

Abstract

For the experiments performed under this heading it was found a) that milking goats maintained at 33° C secreted 6.6 times as much radioiodine into their milk as at 5° C; b) ¹⁴C-labelled lactose and ¹⁴¹Ce gave essentially equal results when infused into the udder to serve as a means of determining milk volume; c) radioiodine was readily absorbed from the mammary gland with the rate of removal being greater for glands containing large amounts of milk.

a. The effect of temperature on the transfer of radioiodine into milk.

The transfer of radioiodine to milk has been intensively investigated because of the large amounts likely to be produced as a result of atomic fission and because it is readily transferred into milk. A large body of data has accumulated on radioiodine; however, there are few definitive reports on the effect temperature might have on the transfer of iodine into milk. Since it is known that the thyroid and mammary gland compete for circulating iodine and that the thyroidal production of thyroxine responds to temperature it is reasonable to expect that the environmental temperature could affect transfer of radioiodine into milk. The purpose of our studies was to look at the possible relation between temperature and the amount of iodine secreted into milk of goats.

The details of the procedures have been reported (See our first progress report (COO-2784-1) and will not be repeated here. Two milking goats were adapted to 33° C temperatures and then given an oral dose of ¹²⁵I and NaI and collections of milk, blood, urine and feces were made over a 50 day period. The animals were then adapted to 5° C temperatures and the experiment repeated. The important result of this study was that 17.1% of the radioiodine appeared in milk when the goats were at 33° C while only 2.6% was in the milk at 5° C. To put it another way the milk iodine at 5° C was only 15% of that at 33° C. At both 33° and 5° C the urine was the main pathway for the excretion of radioiodine and constituted about 80 percent of the total iodine lost in the urine and feces. The urine radioiodine was 36% greater at 5° C than at 33° C while the fecal ¹²⁵I was 85% greater.

Counts of the thyroids at the end of the experiment showed only a trace of ¹²⁵I in the thyroids (0.5%) of the goats when at the high temperature and an average of 13% when the goats were at 5° C. This represents a 26 fold increase in thyroidal radioiodine at the lower temperature.

It is worth noting that the milk production of the two goats averaged 821 ml at the start of the 33° C experiment and 745 at the end. For the 5° C period the average production was 689 ml at the start and 460 ml at the end. Though the rigors of the low temperature environment reduced milk yield by one third the lower production alone cannot explain the 6.6 fold decrease in total milk radioiodine. From a plot of the data of these experiments it was seen that the plasma ^{125}I curves essentially paralleled the milk curves though at a much lower level; the peak radioiodine concentrations occurred within 24 hours for plasma as well as for the milk. The milk iodine was about 4 times greater than the plasma at 33° C and 2 times greater at 5° C during the entire experiment.

The thyroid counts of the goats at the two temperatures showed that thyroid iodine peaked at about 5 days at 29% of the dose when the temperature was 33° C and decreased with a half-time of 31 days thereafter. For the low temperature (5° C) study the thyroid peaked by the first day and declined very rapidly until about 5 days when a slower decline took over. The rate of loss in the last phase had a half time of 14 days.

It can be deduced that part of the additional iodine that appears in milk at 33° C is derived from the thyroid. At 24 hours after dosing the ^{125}I content of the thyroids of the goats at 5° and 33° C was essentially equal. However, by 48 hours, there was a large decrease in the ^{125}I of the thyroid gland when the goats were kept at 33° C. An explanation for this is that at 33° C the thyroid is operating at a very reduced level and relatively little thyroxine is being produced. The thyroid iodide concentrating mechanism, however, is operative and so initially the thyroid ^{125}I content is high. Because of a reduced thyroxine formation little of the radioiodide is bound and soon returns to the blood to be available for distribution to other body compartments - particularly the mammary gland.

The iodide-concentrating mechanism of the mammary gland is qualitatively similar to that of the thyroid but is not as efficient; this concentrating mechanism is sensitive to the level of circulating thyroxine. High temperatures can lead to a decreased thyroxine output and this decreased thyroxine may be responsible for the increase in the efficiency of the mammary iodide concentrating mechanism seen in our experiment. The high thyroxine output induced by low temperature may have led to the reduced efficiency of the mammary gland iodide concentrating mechanism.

Overall, high temperature has made more iodide available for secretion into milk and made the mammary gland more efficient in clearing the blood of this iodide. The net result is for greater amounts of radioiodine to be secreted into milk than was seen for low temperatures.

The practical significance of these findings is to suggest that the use of a single grass-milk iodide transfer function in equations used for predicting the ^{131}I intake of the human population may be misleading. The data of this paper suggest that a series of transfer functions may be needed to cover seasonal and geographical temperature ranges.

b. Comparison of ^{14}C -lactose to ^{141}Ce as a milk volume indicator.

An objective of this research project was to provide information on the transfer rates of actinide elements across mammary epithelium from blood into milk and from milk into blood. Important to this study is the ability to determine the expansion of the milk compartment so that the concentration of the radioelement in the mammary gland can be appropriately corrected. Several markers have been used in this laboratory but we have settled on ^{141}Ce since it is not absorbed and its β and γ emissions make it easy to detect.

Another marker that can be used to determine milk volume is ^{14}C -lactose, the sugar normally present in milk. Radiocerium combines readily with milk proteins and it can be postulated that this large dimension molecule might impede diffusion of the ^{141}Ce into the smaller spaces of the gland. Lactose may be better able to penetrate because of its smaller dimension and lesser tendency to interact with milk components. For this reason we sought to compare ^{14}C -lactose to ^{141}Ce .

For this study we used 2 goats in 3 separate trials and into each half of the glands we infused known quantities of ^{141}Ce and ^{14}C -lactose. We waited $\frac{1}{2}$ hours or 4 hours after the infusion of $2\mu\text{Ci}$ of the markers before beginning the sampling. One-half of the gland was unmixed before serial sampling (25 to 50 ml volumes) while the other half of the gland was mixed just prior to sampling. The first milk samples approximating 20% of the total yield of the gland were used to calculate the volume of milk in the udder. This estimate was made by dividing 100 by the percent of the radionuclide dose per ml; this was considered the Milk Volume Estimate (MVE). The data of activity recovered in the milk samples was plotted as percent accumulated dose versus accumulated milk volume. From these computations and plots the accumulated percent error of the M.V.E. was calculated using each milk sample successively in the following equation:

$$\text{Accumulated Percent Error for MVE} = \frac{(\% \text{ of MVE} - \% \text{ accumulated dose})}{\% \text{ accumulated dose}} \times 100 \quad (1)$$

A line was fitted to the plot of accumulated % error of the MVE versus percent accumulated dose by linear regression analysis; extrapolation beyond the last sample to 100% volume gave the total percent error of the MVE. The samples from the unmixed glands showed that in all three tests the ^{141}Ce and ^{14}C failed to distribute uniformly through the gland. This nonuniform distribution was very apparent for two of the goats but required close inspection to detect in the third goat. The ^{14}C lactose seemed to be distributed into a greater volume than the ^{141}Ce in two of these three trials as judged by the lower percent dose/ml. The total recovery of the ^{14}C averaged $92 \pm 19\%$ with one goat showing a recovery of the dose of ^{14}C lactose of only 71%; recovery of ^{141}Ce from the glands of all three goats (unmixed) was $97 \pm 11\%$.

When the gland was kneaded before the milk was removed by serially sampling the ^{141}Ce accumulation fell on a straight line for goats 166 (4 hr) and 185 (4 hr); however for 185 ($\frac{1}{2}$ hr) a slight deviation was apparent at about 1250 ml. For ^{14}C the deviation away from a straight line began at about 850 ml for 185 ($\frac{1}{2}$ hr). For 166 (4 hr) the ^{14}C values tended to form a curvilinear function; this is not the case for the ^{141}Ce

Table 1 The calculation of milk volume estimates based upon ^{14}C labelled lactose and ^{141}Ce as non-absorbable dilution markers. These data are for glands where mixing preceded sampling.

<u>Goat</u>	<u>Period</u>	<u>Initial Milk Volume Estimate (ML) *</u>	<u>Error of Total Milk Volume Estimate (%) **</u>	<u>Revised Milk Volume Estimate (ml)</u>
185	$\frac{1}{2}$ hr ^{14}C	1609	5.4	1696
	^{141}Ce	1453	3.2	1500
185	4 hr ^{14}C	571	5.5	602
	^{141}Ce	536	-1.1	530
166	4 hr ^{14}C	125	-1.6	123
	^{141}Ce	137	0.6	138

* Based upon either ^{14}C or ^{141}Ce concentration in the first 20% of the milk collected.

** From Figure 3.

values for this same goat. For all of the goats recovery of the ^{14}C averaged $91 \pm 5.4\%$ compared to $94 \pm 12\%$ of the ^{141}Ce when mixing preceded milk sampling.

From the equation (1) it is possible to calculate the total error of the M.V.E. and use these to correct the Milk Volume Estimate based upon the concentration of the radiotracer in the first 20% of the milk. Table 1 presents such calculations for the glands where mixing took place before serial sampling began. The average Accumulated Percent Error was $3.1 \pm 4.1\%$ for ^{14}C and $0.9 \pm 2.1\%$ for ^{141}Ce .

Overall, the results suggest that ^{14}C -lactose and ^{141}Ce are both useful for mammary gland milk volume estimates. The ^{141}Ce does seem to provide superior results showing a smaller error and a more uniform distribution through the milk. The ^{14}C -lactose curves tended to exhibit a curvilinear characteristic that implies either poorer distribution or some absorption of the ^{14}C -lactose from the gland.

c. Resorption of radioiodine from the mammary gland

Most often the secretion of milk is considered a one way transfer without recognition that milk components may indeed be traveling in both directions across the mammary epithelium. Infusion of the radiotracer into the mammary gland would make it possible to study directly the back-flow of materials into the body of the lactating animal and the factors which may control this back flow.

In these studies we used ^{131}I as the tracer since it is known that iodine moves readily across the mammary gland in both directions. Radiocerium

was also present to serve as a milk volume indicator at the time of sampling. We have compared the rate of absorption of iodine when glands are relatively empty and when approaching their physical capacity; moreover, both studies were carried out in the same goats at the same time. (For further details on methods see our report COO-2784-1).

One additional factor looked at in this research was to determine if distention of the gland with milk could cause milk accumulation to cease. For this purpose one half of the gland was milked out at the start of the experiment and the other half allowed to retain its 16 hour accumulation of milk.

For the "empty" glands the estimated milk content was 89 ml at the start and 506 ml at the end of the 24 hour period. The total milk produced during the trial averaged 417 ml which is a rate of 17.4 ml/hour. The increase in milk volume was linear over the 24 hr period for all of the goats but one indicating that a shut off mechanism did not come into play.

The glands that were full at dosing contained an average of 470 ml at the start of the experiment and 900 ml at the end. The average milk production was 17.9 ml/hour, remarkably close to that for the empty glands. Examination of the individual milk volume plots showed that 3 of the goats exhibited a linear increase over this time. Three goats, however, demonstrated a definite tendency to reduce their secretion rate over time; the reduction was seen when the contained milk volumes approached 700 ml. Most interesting was that the other half of the gland showed a milk secretion rate which was linear over the entire time period. This suggests that the control mechanism resides within the particular half and is not mediated by some external mechanism. The linear increase in milk volume for periods up to 48 hours for some of the goats suggests that the control is not related to the length of time that milk stays in the mammary gland.

The curves for the ^{131}I content of the mammary glands showed a greater removal of radioiodine from glands that were "full" at the time of the dose infusion as compared to glands that were "empty".

At the end of the 24 hour period there was an average of 12.5% of the dose in the "empty" halves and 9.2% in the "full" halves. There was a large variation between animals with a range of 1.7 to 32.2% of the infused dose remaining in the half at the end of 24 hours. These values represent inorganic iodine - the bound iodine contribution was subtracted from the data.

The equations for the ^{131}I disappearance are:

Full Half

$$\text{Fraction of Dose} = 0.27 e^{-1.88t} + 0.48 e^{-0.412t} + 0.248 e^{-0.03t}$$

$$\text{Fraction of Dose/liter} = 1.28 e^{-0.342t} + 1.12 e^{-0.072t}$$

Empty Half

$$\text{Fraction of Dose} = 0.33 e^{-0.92t} + 0.4 e^{-0.29t} + 0.27 e^{-0.022t}$$

$$\text{Fraction of Dose/liter} = 1.89 e^{-1.4t} + 5.0 e^{-0.35t} + 1.89 e^{-0.077t}$$

From the data it is apparent that iodine is readily removed from the mammary gland. This iodine is then moved into the blood and is available for transfer to all other sites in the body. In fact, some of the radioiodine can be returned to the mammary gland. In experiments where radioiodine was infused into only one half of the gland it was found that radioiodine did accumulate in the other half. The peak amount varied between goats from 0.5 to 4.0% of the infused dose and the time the peak was reached was from 4 to 24 hours after dosing.

An attempt was made to fit the data to a simple model of the milk producing system. Using the procedure described by Shipley and Clark (Tracer Methods for In Vivo Kinetics, Academic Press, N. Y., 1972) the following k values were found.

From studies on iodide transfer to milk of goats we have shown that the milk/plasma iodide ratio can be as high as factor of 10. In order for this to occur the transfer coefficients must be such that the pathway to milk predominates over the return from milk. The transfer coefficients of both conditions of state of fill of the gland actually suggest a flow of iodine from milk into blood; this needs further investigation.

d. Resorption of zinc from the mammary gland

In addition to metabolic studies experiments were run to determine the rate of removal of ^{65}Zn from the mammary gland of the goat and cow. For this approximately $3\mu\text{Ci}$ of ^{65}Zn and $2\mu\text{Ci}$ of ^{141}Ce was infused into the mammary glands of two goats and a cow about 2 hours after milking. The glands were massaged for 3 minutes and 20 ml milk samples were collected at $\frac{1}{2}$, 1, 2, 4, 8, 16 and 24 hours. The ^{141}Ce was used as a nonabsorbable volume indicator and this data was used to correct the zinc concentration for the expansion of the milk compartment the volume of the infused dose, and the amount of ^{65}Zn removed in the samples.

The striking between the goat and the cow is that little of the zinc moves from milk to blood for the goats, however, a relatively rapid loss was seen for the dairy cow. The equation for the milk concentration of ^{65}Zn for one goat was determined to be: Fraction of Dose = $0.12 e^{-0.202t} + 0.83 e^{-0.0024t}$. The equation for the cow was: Fraction of dose = $0.305 e^{-0.356t} + 0.695 e^{-0.0863t}$.

Since the data obtained from both of these animals indicate the possibility of at least 2 compartments the model of the system is postulated to be

The values calculated for the various k's by compartmental analysis is

	<u>Cow</u>	<u>Goat</u>
k_{ba}	0.169	0.0262
k_{ab}	2.441	7.783
k_{ob}	0.0185	0.1822

If compartment "a" is milk and "b" is mammary secretory tissue these data show that both the cow and the goat show a major movement of zinc into the milk and a very small movement out. The k_{ab}/k_{ba} ratio is 297 for this goat and 14.5 for the cow. This indicates a greater propensity of the goat to transfer zinc into milk and to retain it there than for the cow.

Resorption of ^{45}Ca in the Mammary Gland

W. T. Beilman

Abstract

Autoradiography was used to determine the site of reabsorption of ^{45}Ca injected into the mammary gland of the goat and the guinea pig. Tissue slices of lactating guinea pig mammary gland were also incubated in vitro with radiocalcium to compare cellular ^{45}Ca uptake to that observed in vivo. All tissues were frozen, sectioned and exposed in the frozen condition.

In general, the alveolar and intralobular duct epitheliums showed a similar pattern of labelling; only about 70% of the cells showed radio-activity. The labelling of the cells of the interlobular ducts was less frequent. The conclusion was drawn that resorption of calcium can occur at the alveolus and in the intralobular ducts; however, the transfer was markedly less for the interlobular ducts and no conclusion can be drawn.

While some of the reactions that take place in the mammary gland that result in the formation of organic fractions of milk have been elucidated, very little information is available on the mechanism of secretion of the electrolyte components of milk (1). J. Linzell, one of the most active investigators of electrolyte transfer across the mammary epithelium, contends that once milk is formed in the alveoli its electrolyte composition undergoes little modification in the gland (2). This conclusion is counter to the hypothesis of Zaks et al (3) and Mackenzie and Lascelles (4) who suggest that the mammary gland produces its secretion in a manner like the salivary, pancreatic and sweat glands where a precursor secretion is produced that then undergoes modification during passage through the ducts. (5)

Fractionation of milk during withdrawal from the mammary gland resembles the classical "stop flow" experiments used with other secretory tissues. The last drawn milk, particularly when oxytocin is administered, is presumed to represent alveolar milk and to have the composition of the primary secretion. The different composition of this primary secretion from the bulk of the milk led Zaks et al and Mackenzie and Lascelles to conclude that the ducts and the cisterns of the gland modified the electrolyte composition of milk (3,4).

Linzell explains the results of Zaks et al and Mackenzie and Lascelles as being artifacts produced by injecting oxytocin to obtain the milk held by the alveoli. Linzell has obtained evidence that the tight junctions between alveolar cells become leaky under the influence of oxytocin allowing ions and small molecules to pass down their concentration gradients from blood to milk thus producing a secretion that differs from the bulk of the milk (6,7,8).

Using a teat preparation Linzell (2,6) determined that radioactive Na, Cl, K and Rb were unable to traverse from milk to blood or from blood to milk while $^3\text{H}_2\text{O}$ and ^{14}C urea were able to pass across teat epithelium. Further, milk removed from the mammary gland 25 minutes after the I.V. injection of $^3\text{H}_2\text{O}$, ^{14}C urea, ^{86}Rb , ^{42}K , ^{24}Na and ^{36}Cl showed very low concentrations of the ions in teat milk with increasing concentrations in the order of bulk milk, milk obtained by stripping and milk obtained by injection of oxytocin.

Since mixing of gland content is possible, 7 and 14% of a dose of ^{14}C lactose introduced into the teat was recovered in oxytocin milk at 10 and 20 minutes, Linzell concluded that the small amounts of labelled ions in duct, cistern and teat milk arrived there by downward diffusion and that the epithelium lining these areas is actually impermeable to ions. Resorption of radioactivity introduced into the udder (9-14) then reflects the permeability characteristics of the secretory portions of the mammary gland.

No information is available on the site of resorption of calcium from milk and since this is a major ion of milk we were interested in determining if absorption is also limited to the alveolar epithelium. The work presented here demonstrates that calcium is resorbed equally well in the alveoli and in the intralobular ducts. There is no definite indication of calcium resorption in the interlobular ducts.

Methods

The goat used in this study had been locally obtained, milking for more than two years and was non-pregnant. To carry out the experiment a 30 ml volume of milk was removed from each gland. Each 30 ml sample was mixed with 40 μCi ^{45}Ca , and then drawn through PE90 tubing into a 30 ml syringe. The PE90 tubing, coated with sterile lubricant, was inserted through the teat canal of each gland and the doses injected. The udder was massaged for 5 minute periods at 15 minute intervals to distribute the ^{45}Ca . The goat was killed after one hour by I.V. injection of 15 ml of T-61 Euthenasia Solution.

Prior to sacrifice the glands were completely milked out after I.V. injection of 0.1 ml of oxytocin. The glands of the goat were removed rapidly after the animal died and the tissue quickly sectioned into small sections and frozen by immersion into liquid N_2 . The tissue was handled as described below for the guinea pigs.

The guinea pigs used for this study came from Buckberg Lab Animals, Tomkins Cove, New York and were bred while being maintained in the laboratory animal facilities of the N. Y. State College of Veterinary Medicine. The animals were used for experiments within one week after parturition.

For dosing the lactating guinea pigs, 40 μCi of the stock ^{45}Ca was pipetted from its container into a 1.5 ml testtube. Using a 1 ml syringe and PE 10 tubing, the dose was drawn into the PE 10 tubing. The tubing was then coated with sterile lubricant and inserted into the teat canal and the dose ejected into the gland of the anesthetized guinea pig. The gland was then massaged for 3 minutes every 15 minutes for 2 hours to insure distribution of the dose throughout the gland. The guinea pigs were killed at the end of the 2 hour period by stunning. The entire mammary gland was

then removed by dissection without prior milking and submerged in liquid N₂. After freezing the glands and pieces of tissue were then wrapped in a double layer of saran wrap and stored at temperatures ranging from -25° C to -80° C until sectioning.

For an in vitro study two lactating guinea pigs were killed by stunning and the glands immediately dissected out and placed in a petri plate containing oxygenated medium [Medium 199 (GIBCO) containing 20% fetal calf serum]. The glands were rapidly cut into 1 mm thick sections and the sections transferred, in duplicate, to petri dishes containing 20 ml of the oxygenated Medium 199 plus 20% FCS and 93μCi of ⁴⁵Ca and incubated for ½, 1, 2 and 4 hours. At the termination of incubation, the tissue slices were removed from the media, placed in small vials, frozen in liquid nitrogen and then stored at -80° C until sectioning.

Sectioning of the tissue was performed on a Lipshaw cryostat at chamber temperatures of -30° to -34° C. The tissues were mounted by imbedding the tissue in Tissue Tek (imbedding fluid) and freezing immediately with Cryokwik (frozen spray). The tissue was allowed to equilibrate with the cryostat temperature for 1 hour before being cut.

The tissues were sectioned into 2μ thick slices, transferred with a fine brush to a brass plate mounted inside the cryostat and arranged for easy pick up.

At this point the lights of the dark room were turned off and the sections were picked off the brass plate, one by one, by pressing firmly against them with precooled slides previously coated with Kodak NTB-2 emulsion. The autoradiographs were then placed in slide boxes containing drierite and the boxes were then wrapped in aluminum foil. The exposure of the emulsion took place in a freezer or in the cryostat chamber at temperatures of -20° to 34° C. Exposure periods ranged from a minimum of 14 days to a maximum of 57 days.

For the development and staining process the slides were allowed to warm at room temperature for 5 minutes; they were then fixed in 5 percent acetic ethanol for 5 minutes and then rinsed for 5 minutes in distilled water. The autoradiograms were developed in Kodak developer for 5 minutes, rinsed for 5 minutes in distilled water, fixed for 8 minutes in Kodak fixer and rinsed for 10 minutes in distilled water.

The slides were stained with Delafield's Hematoxylin, washed, counter-stained with aqueous eosin Y, and dried using 95 and 100% ethanol.

The autoradiographs were examined through a Nikon Inverted Model M light microscope equipped with a Zeiss Neofuar oil objective (N.A. 1.30) and a Zeiss Achromatic-aplanatic phase contrast condenser VZ (N.A. 1.40) not utilized in the phase mode. Only cells with visible nuclei were examined to eliminate the possibility of counting radioactivity contained in an underlying lumen.

Since the labelling of the cells depended upon the amount of isotope present in the lumen of the ducts or alveoli the cells containing grains were

arbitrarily separated into three classifications according to the degree of labelling present. These classifications are: Class 1, 1 to 5 grains/cell; Class 2, 6 to 15 grains/cell; Class 3 >16 grains/cell. The luminal radioactivity was categorized as follows: Category A, 1 to 4; Category B, 4.1 to 6.0; Category C, 6.1 to 8; Category D, 8.1 to 10; and Category E, >10 grains/square mm. At least twenty-six random microscopic fields were examined for each experimental group. The total number of cells for each epithelial type (alveolar, intralobular, and interlobular) in each microscopic field was recorded as well as the number of grain-containing cells in Class 1, 2 or 3 for each epithelial type. The number of labelled cells within a particular class and cell type were then expressed as a percentage of the total number of cells in that cell type.

Grain counts were made of the alveolar and duct lumens in each field using an ocular grid of 1 mm squares; each square was equivalent to 0.0001mm^2 using a 1000 power magnification of the tissue. These lumens were classified as Category A, B, C, D or E as described previously, so that the labelling in the cells of the different anatomical units could be compared taking into account the amount of radioactivity that actually was available for cell uptake.

The data were expressed as the mean of the cells labelled, the range and the numbers of observations (n) falling within a particular class and category. Where only one observation was made for a particular Class and Category the percentage determined is designated with an asterisk.

Results

The labelling percentages for the autoradiographs of the goat mammary epithelium are presented in Table 1. All the lumens examined fell in grain count Category A. Low luminal grain counts were expected since the mammary glands were milked out prior to collection of tissue, thereby removing ^{45}Ca . Labelling percentages in both Class 1 and 2 were similar for the cells of the alveoli and intralobular ducts. Interlobular labelling was considerably lower than alveolar and intralobular labelling and was restricted to Class 1.

Background grain counts averaged from 1.5 to 2.0 grains/sq of the ocular grid on the autoradiographs examined. Therefore, some of the labelling of alveoli and intralobular ducts classified as Class 1 is due to the random distribution of grains arising from the background. However, because of the low percentage of labelling for the interlobular ducts and that 70% of the cells of the alveoli and intralobular ducts showed no labelling at all, a large part of the grain counts over the alveolar and intralobular cells can be inferred to be from ^{45}Ca actually taken into the cells.

The data of the goat strongly suggest that movement of calcium from milk to epithelial cells occurs readily in the alveolus and intralobular duct and occurs to a lesser extent, if at all, in the interlobular ducts.

The data for the in vivo guinea pig study is presented in Table 2. The differences between this experiment and the goat experiment include the use

of a relatively larger dose of ^{45}Ca , a two hour interval between dosing and sacrifice and the sectioning of the mammary tissue without removing the labelled milk.

The labelling exhibited by the cells can be seen to be roughly related to the amount of activity contained in the lumens. For Category A only 4% of the alveolar cells were in Class 3 whereas for Category E labelling of the lumen 29% was in Class 3. A similar trend can be seen for the intralobular labelling. Few data are available for the interlobular labelling because the tissue preparations seldom contained these structures. From the data it can be seen that resorption of calcium from milk into the cells took place in the alveoli and the intralobular ducts. It is difficult to be certain about the cellular uptake of ^{45}Ca for the interlobular ducts. The data suggest little difference exists between the capabilities of the alveolar and intralobular epithelium but probably there is a reduced ability of the interlobular epithelium to resorb calcium.

Autoradiographs of guinea pig mammary tissue maintained in vitro up to 4 hours in a medium containing ^{45}Ca were made for purposes of comparison with in vivo studies. The labelling percentages for each maintenance period ($\frac{1}{2}$, 1, 2 and 4 hours) are presented in Table 3.

The labelling percentages in Class 2 generally increased as the luminal labelling Category increased for both the alveolar and intralobular epitheliums. Class 3 labelling was observed only in the $\frac{1}{2}$ hour maintenance period and suggests that with time the viability of the tissue slices declined.

The interlobular epithelium demonstrated some Class 2 labelling, the percentages, however, were lower than those for the alveolar and intralobular epitheliums.

A feature that stands out in this study as well as in the in vivo study is that there was little discernable difference between the labelling of the alveolar epithelium and that of the intralobular epithelium. The interlobular epithelium, however, had a decidedly lesser ability to transfer calcium as indicated by the number of cells labelled and the degree of labelling.

Discussion

It should be noted that the preparation of the autoradiographs resulted in tissue specimens that represented essentially only those portions of the mammary gland that have been recognized to have secretory capability i.e., the alveoli and the intralobular ducts. Only some of the time were the interlobular ducts included in the sections. The larger ducts, the cisterna and the teat epithelium were not included; therefore, no conclusions can be made concerning the absolute or relative permeability of the entire mammary gland epithelium to calcium contained in milk. The suggestion of Linzell and Peaker (2,6) that reabsorption can occur only in the upper reaches of the gland has not been tested.

Unfortunately, the amount of radioactivity injected into the mammary gland of the goat was relatively small and so it became difficult, in many cases, to distinguish true labelling from background counts. The data suggest, however, that reabsorption of calcium occurs in the alveolar and intralobular areas/and only possibly of the gland in the interlobular ducts.

The in vitro study using slices of lactating guinea pig mammary gland tissue are not directly comparable to in vivo guinea pig studies since the labelling patterns seen in vitro could be a summation of calcium movement into the cell from both the apical and basal ends of the cells. It is of interest, however, to note that the total percent of cells showing radioactivity was approximately the same for both kinds of experiments. For the in vivo experiment (Table 2) for Category E the average percent of labelled cells in the alveolus was 67%. It is also of interest that the proportion of alveolar cells (Category E) for the in vitro experiment - Class 2 was approximately twice that for the in vivo experiment. A similar trend can be noted for the intralobular cells though direct comparisons are more difficult to make because of a relative lack of data. The labelling of only about 70% of the cells suggests that either some cells have different functioning characteristics than the majority of the cells or that they are at a different secretory phase than the other cells.

The higher proportion of cells showing Class 2 labelling for the in vitro experiment as opposed to the in vivo experiments could imply that a large number of the cells that resorb calcium are also important in the secretion of calcium. This is not to suggest that the other cells are not involved in the secretion of calcium; again, it may be that these cells are in a different secretory phase as compared to the other cells of that structure.

The interlobular epithelium showed a markedly lesser labelling percentage (>25%) than for the other two epithelial types; moreover the amount of label tended to be Class 1 with only the in vitro study showing some Class 2 labelling. Before a definite conclusion can be made about calcium resorption from this part of the gland structure studies will have to be made to determine if the grains over the cells could result from the activity actually present in the lumen. Since there was no attempt to determine the type of cell showing labelling no judgement can be made for any of the tissues as to whether or not the labelling was function of cell type. It would be important to carry this out in future studies since Linzell and Peaker (2) have shown that the proportion of cells showing secretory activity increases towards the upper reaches of the gland.

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Table 1. Goat mammary epithelial cell labelling as a function of anatomical structure. The values in the table are the mean percent of cells in a structure showing labelling with ^{45}Ca .

<u>Structure</u>	<u>Alveolus</u>		<u>Intralobular duct</u>		<u>Interlobular duct</u>	
Labelling Class	<u>1⁺</u>	<u>2⁺⁺</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Mean	30	3	30	3	8	0
Range	9-42	0-20	12-44	0-18	0-30	0
n	15	15	14	14	11	11

⁺ Class 1 labelling represents 1-5 grains per cell

⁺⁺ Class 2 labelling represents 6-15 grains per cell

Table 2. Guinea pig mammary epithelium-in vivo labelling with ^{45}Ca as a function of location

<u>Luminal Category</u>	<u>Alveolus Labelling Class</u>			<u>Intralobular Duct Labelling Class</u>			<u>Interlobular Duct Labelling Class</u>		
	<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>
A	28 (22-35) n = 4	18 (11-23) 4	4 (0-10) 4	31 (22-38) n = 4	8 (0-33) 4	0 (0-10) 4	-	-	-
B	41 (18-54) n = 5	20 (8-36) 5	0 (0-29) 5	20 (10-29) n = 4	37 (20-71) 4	3 (10-10) 4	-	-	-
C	-	-	-	-	-	-	18 (0-32) m = 6	0 (0) 6	0 (0) 6
D	7 (0-15) n = 4	27 (21-31) 4	18 (0-29) 4	3* n = 1	28* 1	14* 1	-	-	-
E	17 (13-25) n = 3	21 (14-31) 3	29 (19-50) 3	0* n = 1	60* 1	0* 1	-	-	-

The group of values for each Category-Class represents the mean percentage of cells that fell within a particular labelling class, the range of the percentages, and the number of animal observed. A dash (-) implies that no observations were made in this category and an asterisk (*) that this value is for a single microscopic field.

Table 3. Labelling of epithelium of guinea pig mammary gland after tissue sections were immersed in a labelling solution containing ^{45}Ca .

	<u>Luminal Category</u>	<u>Alveolus Labelling Class</u>			<u>Intralobular Labelling Class</u>			<u>Interlobular Labelling Class</u>		
		<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>
$\frac{1}{2}$ hour incubation	1		-			-			-	
	2		-			-			-	
	3	18 (15-24) n = 3	22 (15-27) 3	15 (0-27) 3	13 (13-14) n = 2	54 (50-57) 2	0 (0-31) 2	15 (0-29) n = 10	4 (0-31) 10	0 (0) 10
	4	4 (0-9) n = 2	49 (46-52) 2	11 (0-23) 2	0 (0) n = 2	53 (40-67) 2	5 (0) 2	25 (17-33) n = 2	0 (0) 2	0 (0) 2
	5	5 (0-28) n = 6	50 (21-71) 6	11 (0-42) 6	2 (0-12) n = 6	70 (59-83) 6	0 (0) 6		-	
1 hour incubation	1		-			-			-	
	2		-			-			-	
	3		-		17 (0-40) n = 5	41 (31-56) 5	0 (0) 5		-	
	4	21 (14-27) n = 2	52 (47-57) 2	0 (0) 2	18 (12-25) n = 2	46 (41-50) 2	0 (0) 2	33 (25-38) n = 3	9 (6-12) 3	0 (0) 3
	5	8 (0-19) n = 7	58 (37-75) 7	0 (0) 7	16 (14-18) n = 2	52 (47-57) 2	0 (0) 2	12 (8-19) n = 4	14 (6-23) 4	0 (0) 4

Table 3 Continued

	Luminal Category	<u>Alveolus</u> <u>Labelling Class</u>			<u>Intralobular</u> <u>Labelling Class</u>			<u>Interlobular</u> <u>Labelling Class</u>		
		<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>
2 Hour incubation	1		-			-			-	
	2							23 (0-67) n = 4	0 (0) 4	0 (0) 4
	3		-			-		23 (20-25) n = 2	0 (0) 2	0 (0) 2
	4		-			-			-	
	5	7 (0-33) n = 9	69 (47-89) 9	0 (0) 9	8 (0-40) n = 7	68 (50-100) 7	0 (0) 7	20 (15-29) n = 3	7 (0-21) 3	0 (0) 3
4 Hour incubation	1		-			-			-	
	2		-			-		9 (0-23) n = 9	0 (0) 9	0 (0) 9
	3	21 (11-31) n = 3	44 (31-50) 3	0 (0) 3	21 (0-40) n = 6	48 (40-63) 6	0 (0) 6		-	
	4	8 (0-15) n = 2	46 - 2	0 (0) 2	24 (0-40) n = 3	38 (20-50) 3	0 (0) 3		-	
	5	6 (0-14) n = 4	40 (13-55) 4	0 (0) 4		-			-	

COMPARTMENT MODELS INVOLVING A COMPARTMENT OF CYCLING VOLUME

Clifton Blincoe

1. INTRODUCTION

In the study of the transfer of materials between compartments the assumption is made that all compartments have constant volume (Atkins, 1969). Although this assumption is generally valid for many physiological spaces, notable exceptions are the mammary gland contents of lactating mammals and the urinary bladder contents. Mammary excretion of iodide and exchange between the cycling milk compartment and other iodide spaces were included in a computer simulation of iodide metabolism (Blincoe, 1975).

The objective of this study was to develop equations representing the weight of solute and its concentration in a compartment of variable volume under a variety of assumptions concerning the mechanism of transfer of solute into and out of such a compartment. All symbols conform to the recommendations of the International Commission on Radiation Units (Brownell, Berman and Robertson, 1968) in so far as practical.

2. GENERAL DEVELOPMENT

The volume cycle of compartment i is assumed to be a sawtooth function, increasing gradually during a time interval Δt from a minimum volume V_{oi} to a maximum volume followed by an instantaneous reduction to the original volume. The difference between the maximum and minimum volume V_f is assumed to be removed from the system. This results in equations that are continuous but not single-valued at the times of volume reduction. Various modes of introduction of solute into the variable volume compartment are considered. The equations were developed as a finite series of terms, one for each of n volume cycles. This approach is quite applicable to simulation using a digital computer. The exact form of the volume cycle need not be specified as is represented functionally as $V(t)$. Two reasonable functions that would find general physiological application are:

- (a) Volume growth as a linear function of time:

$$V(t) = V_{oi} + R_i \Delta t$$

- (b) Exponential approach to a maximum:

$$V(t) = V_{oi} + V_f (1 - e^{-R_i \Delta t})$$

where R_i is the rate of change of the volume of compartment i . In these cases the ratio F of the volume remaining after volume reduction and removal to the volume immediately prior to reduction are

$$F = \frac{V_{oi}}{V_{oi} + R_i \Delta t_m}$$

$$F = \frac{V_{oi}}{V_{oi} + V_f (1 - e^{-R_i \Delta t_m})}$$

where Δt_m is the time interval between successive volume cycles ($t_n - t_{n-1}$) or the maximum value of Δt .

The assumption of instantaneous reduction of volume is probably physiologically valid since such processes are nursing, milking, etc. take place in a much shorter time than the preceeding accumulation of fluid.

In all cases the equations behave as expected in the limiting cases of time approaching zero and infinity. As the various process rates approach zero the equations approach the appropriate simpler case.

3. SPECIFIC CASES

a. Removal of solute only by the volume cycle.

For a fixed initial weight of solute g_0 the weight of solute remaining at the start of the n -th volume cycle is

$$g_{n-1} = g_0 F^{n-1}$$

An example of this case would be the introduction of a non-absorbed drug into the mammary gland immediately after milking. If a fixed weight of non-absorbed solute G_0 is introduced at the start of each volume cycle, the amount remaining at the start of the n -th cycle is

$$g_{n-1} = G_0 (F + F^2 + \dots + F^{n-1})$$

Since the series is convergent, being a power series with argument less than unity, the amount remaining at the start of each cycle before the addition of new solute approaches the constant value

$$g_{\infty} = \frac{1}{1-F} - 1$$

The concentration during the n -th cycle would be

$$C = G_0 (1 + F + F^2 + \dots + F^{n-1}) \frac{1}{V(t)}$$

A time invariant addition of solute at rate B could result from a one-way diffusion from a compartment of constant concentration. In this case

$$g_{n-1} = F^{n-1} g_0 + B \Delta t_m (F + F^2 + \dots + F^{n-1})$$

and the concentration during the n -th cycle will be

$$C = [g_{n-1} + B (t - t_{n-1})] \frac{1}{V(t)}$$

t_{n-1} is the time at the start of the n -th volume cycle and $t - t_{n-1}$ is the time elapsed since the last reduction of volume. If we have a constant rate of loss ($B < 0$) from the cycling compartment the concentration of solute will be extinguished and remain zero after the first cycle when

$$-F^n g_0 \geq B \Delta t_m (F + F^2 + \dots + F^n)$$

This can be easily approximated on a digital computer. If B is small, then n for the first occurrence of $g_n \leq 0$ will be large and approximately

$$n = \log_F \left[-\frac{B}{g_0} \Delta t_m \left(\frac{1}{1-F} - 1 \right) \right]$$

Note that the base of the logarithm is the number F .

Now consider the addition of solute by a first-order process from compartment 1 of constant volume V_1 to compartment 2 of variable volume. Solute may leave compartment 1 by n first-order processes k_{i1} only one of which, k_{21} , is the transfer to the compartment of cycling volume. For this case, we have

$$g_{n-1,2} = \frac{R_{,1}}{K} V_1 C_{01} [F^{n-1} g_{02} + F^{n-1} (e^{-Kt_0} - e^{-Kt_1}) + F^{n-2} (e^{-Kt_1} - e^{-Kt_2}) + \dots + F (e^{-Kt_{n-1}} - e^{-Kt_n})]$$

where for notational simplicity

$$K = \sum_{i=1}^n k_{i1}$$

and C_{01} is the initial concentration in compartment 1.

The concentration in the n -th cycle will be described by

$$c = [g_{n-1,2} + V_1 C_{01} (e^{-Kt_n} - e^{-Kt_{n-1}})] \frac{1}{V(t)}$$

Again as n approaches infinity the series converges since it is pointwise less than a convergent power series.

b. Removal of solute by the volume cycle and by a first-order process

First assume that all removals from the compartment with cycling volume are irreversible. Addition of solute to this compartment is by the same processes as previously considered but removal is both by a concentration-dependent first-order process of rate constant k_2 and by the volume cycle.

First consider a fixed weight of solute added initially and removed by both processes. In this case it follows directly that

$$g_{n-1} = F^{n-1} g_0 e^{-(n-1)k_2 \Delta t_m}$$

and

$$c = \frac{g_{n-1}}{V(t)} e^{-k_2 (t - t_{n-1})}$$

If a fixed weight of solute is added at the start of each volume cycle then

$$g_{n-1} = G_0 (F e^{-k_2 \Delta t_m} + F^2 e^{-2k_2 \Delta t_m} + \dots + F^{n-1} e^{-(n-1)k_2 \Delta t_m})$$

and the series is convergent as n increases without limit since it is termwise less than a convergent power series. During the n -th cycle

$$c = \frac{1}{V(t)} (g_{n-1} + G_0) e^{-k(t - t_{n-1})}$$

An example of such cases would be the introduction of an absorbable drug into the mammary gland.

If the solute is added to the cycling compartment by a time invariant process of rate B

$$g_{n-1} = F^{n-1} g_0 e^{-(n-1)k_2 \Delta t_m} + B \Delta t_m (F e^{-k_2 \Delta t_m} + F^2 e^{-2k_2 \Delta t_m} + \dots + F^{n-1} e^{-(n-1)k_2 \Delta t_m})$$

and

$$c = \frac{1}{V(t)} [g_{n-1} + B (t - t_{n-1})] e^{-k_2 (t - t_{n-1})}$$

The series are convergent.

If the solute is introduced by a first-order process of rate constant k_{21} from compartment 1 of constant volume to compartment 2 of variable volume but the reverse transfer is not permitted.

$$g_{n-1,2} = F^{n-1} g_{02} e^{-(n-1)k_2 \Delta t_m} - g_{01} [F^{n-1} e^{-(n-1)k_2 \Delta t_m} (e^{-k_{21} t_0} - e^{-k_{21} t_1}) + \dots + F e^{-k_2 \Delta t_m} (e^{-k_{21} t_{n-2}} - e^{-k_{21} t_{n-1}})]$$

and

$$c = \frac{1}{V(t)} [g_{n-1,2} + g_{01} (e^{-k_{21} t_{n-1}} - e^{-k_{21} t})] e^{-k_2 (t - t_{n-1})}$$

Consider the case of reversible transfer between compartment 1 of constant volume and compartment 2 of variable volume. Other concentration dependent losses are permitted from both compartments, as is transfer from compartment 2 to compartment 1 with rate constant k_{12} . In this case

$$g_{n-1,2} = \frac{1}{(1 - \frac{k_{12}}{K} e^{-K \Delta t_m})^{n-1}} \left\{ F^{n-1} g_{02} e^{-(n-1)K \Delta t_m} - g_{01} [F^{n-1} e^{-(n-1)K \Delta t_m} (e^{-k_{21} t_0} - e^{-k_{21} t_1}) + \dots + F e^{-K \Delta t_m} (e^{-k_{21} t_{n-2}} - e^{-k_{21} t_{n-1}})] \right\}$$

and

$$c = \frac{1}{V(t)} [g_{n-1,2} + g_{01} (e^{-k_{21} t_{n-1}} - e^{-k_{21} t})] \frac{e^{-K(t - t_{n-1})}}{1 - \frac{k_{21}}{K} e^{-K(t - t_{n-1})}}$$

An example of such a case would be that of excretion of a solute into milk with the solute in the formed milk being available for exchange with blood. This case has been simulated (Blincoe, 1975) for iodide excretion by the mammary gland and may be rather generally applicable.

4. SUMMARY

Equations have been presented to describe the weight and concentration of a solute in a compartment of cyclically variable volume. The cases considered included several of physiological and pharmacological interest.

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TABLE I. Symbols

c = Concentration of solute in the compartment of variable volume at time t .

c_{ni} = Concentration of solute in compartment i at the end of cycle n .

F = Ratio of the volume remaining after each volume cycle to the maximum volume during that cycle.

G_o = Fixed weight of solute added at the start of each volume cycle.

g_i = Weight of solute in compartment i at time t .

g_{ni} = Weight of solute in compartment i after cycle n is completed.

k_{ij} = Transport rate constant from compartment j to compartment i .

k_i = Transport rate constant from compartment i to outside the system.

$\sum k$ = Sum of all transport rate constants from compartment j .

n = Volume cycle number.

R_i = Rate of change of volume in compartment i .

t = Time.

t_{n-1} = Time at the start of the n -th volume cycle.

$t = t - t_{n-1}$

$\Delta t_{\max} = t_n - t_{n-1}$ (Maximum value of Δt).

V_{oi} = Volume of compartment i at time zero and at the start of each volume cycle.

$V^R(t)$ = Volume as a function of time.

$V_R = F$

THE METABOLISM OF ^{65}Zn BY LACTATING GOATS

Abstract

Studies of zinc as an environmental pollutant and trace element have been undertaken. Balance studies of radiozinc and stable zinc have been carried out with lactating goats following single oral and I.V. administration of ^{65}Zn . Findings regarding the fixing of zinc to red blood cells in vivo, the appearance of significant amounts of zinc in the hair of goats, and the ready transfer of zinc into milk are reported. Kinetic analysis of blood data suggest zinc pools within the body with large differences in turnover times.

A study of zinc metabolism by lactating goats was undertaken both because it is a significant element in nutrition and health, and because such a study would fall within the scope of the ERDA contract in the area of mineral metabolism and at the same time provide training in radioisotope techniques of a visiting fellow from the Sudan who worked on the initial portion of these studies. Zinc deficiencies do occur in domestic livestock [Kirchgessner and Schwarz, Zbl. Vet. Med. A. 22: 572 (1975)]. Stable zinc is an environmental contaminant [Dorn et al, Environmental Res. 9: 159 (1975); Pfeilstucker and Markard, Z. Lebensm. Unters. - Forsch. 158: 129 (1975)] and ^{65}Zn has been found in people, plants and animals as a result of bomb tests and reactor operations [Agr. Natl. Lab. Rept. No. ANL-5755, p. 53-60, 1957; Perkins and Nielsen, Sci. 129: 94 (1959)]. Since our point of interest is milk secretion we used lactating goats for these studies. Oral and I.V. doses of ^{65}Zn were administered. We decided to make complete metabolic collections in hopes

that the data produced would allow for the development of a model useful in describing zinc metabolism in particular, or many other mineral elements in general.

Experiments

In the initial phase of this study three goats received ^{65}Zn as ZnCl both orally (50 μCi) and after a minimum of $2\frac{1}{2}$ months another dose intravenously (30 μCi). Two goats also received ^{65}Zn intramuscularly. These goats were housed in metabolism stalls during each study to allow for complete collections. Each goat was placed in the stall a few days before dosing to allow for accommodation. Feed included hay and grain from similar batches. Water was provided by automatic watering devices.

Dosing with ^{65}Zn was done during late morning followed by sampling of blood several times during that first day. Subsequently, samples were drawn daily during the first week and at longer intervals thereafter. Aliquots of whole blood and of plasma were assayed for ^{65}Zn in a well-type scintillation counter (3" $\text{NaI}(\text{tl})$). Total zinc in these blood fractions was determined by atomic absorption analysis. Whole blood was ashed prior to this latter analysis. Radiozinc and total zinc of red blood cells were calculated from the difference between plasma and whole blood concentrations.

Milking of the goats occurred at about 7 AM and 4 PM. Assay for ^{65}Zn was done in a large sample counter with two opposing 5" $\text{NaI}(\text{th})$ crystals as detectors (Nuclear Chicago - "Tobar"). Aliquots of milk were ashed for atomic absorption analysis for Zn.

Like milk, urine and feces were collected in total twice daily, and assayed in a similar manner. Feces were ashed; urine only diluted for

A.A. analyses. Rations were weighed before feeding; uneaten material was weighed back prior to the next feeding. These feeds were sampled for stable Zn analysis.

In order to examine another route of Zn loss from the body hair was also sampled periodically. Unwashed, full length samples were cut from the shoulder area of the goats. This area was chosen to minimize contamination from licking, as this was an area the goats could not reach.

Additional studies involving uptake of ^{65}Zn by red blood cells in vitro and the effects of dietary supplementation with Zn SO_4 on blood levels of radiozinc were undertaken and will be reported later.

Detailed data for these studies is presented in the appendix tables of this report. Plasma and cell uptake and disappearance of zinc were calculated both as percent of ^{65}Zn administered per ml and as specific activity, i.e., the fraction of ^{65}Zn administered associated with a given mass of total Zn in a given tissue. In Figures 1, 2 and 3 are shown average plasma and cell S.A. of goats administered ^{65}Zn I.V. and orally. The plasma curves of goats receiving I.V. injections show three distinct rates of removal with half times of removal averaging 39 min., 46 hr. and 39 days. Except during a short period after dosing the ^{65}Zn associated with the cells is very much higher than that of the plasma. However, when the specific activities are calculated the cell S.A. exceed that of plasma only modestly and does not exceed that of plasma until several days after dosing. This would indicate a slow exchange of Zn between plasma and cells; this will be examined later in the discussion of kinetics. The early data of Zn uptake by cells is interesting in that it appears to be biphasic; a rapid rise and fall, followed by a more gradual rise and a very slow decent over longer periods (tables 1, 2 and 3).

Since the cellular ^{65}Zn was obtained by difference between plasma and blood this biphasic curve may imply that ^{65}Zn was loosely associated with the exterior of the cell at early times and later was incorporated into cells. At about one and half hours after dosing the cellular ^{65}Zn reached a temporary low point and then began to increase. By 10 days the cell ^{65}Zn essentially had reached a plateau. All goats showed the same pattern of events after intravenous injection suggesting this is a real phenomenon.

Average blood curves following oral administration showed a rise over the first 2 days followed by a slow decent (Fig. 3). This decent was at approximately the rate of the I.V. dose, however levels were at least an order of magnitude lower, which would seem to indicate an absorption of Zn somewhat under 10%. The erratic appearance of the plasma zinc curves is due to the extremely low counts encountered 20 days or so after dosing, especially after oral dosing.

Milk ^{65}Zn concentrations and S.A. are shown in figures 4 through 7. In the case of I.V. administration, values reached a peak at the first or second milking, whereas following oral administration peak concentrations were reached during the second day. Thereafter levels fell with essentially a two component exponential slopes with half times similar to plasma. Again levels following oral dose were about 1/10 those following I.V. doses. The counting statistics of milk samples were better than that of plasma due to larger samples and higher counts.

Average urine concentrations and S.A. are plotted in figures 8 and 9. The patterns seen in milk samples were also seen in urine. However much larger variations were seen in counts and specific activity.

The following table shows the distribution of zinc in excretions and

secretions. Total zinc excretions ranged from 50.5 to 79.8 mg/day.

	<u>Feces</u>	<u>Urine</u>	<u>Milk</u>
Total Zn (stable)			
Lactating goats	90.0-94.4%	1.9-4.2%	2.7-8.1%
Non-lactating goat	94.3-95.8	4.2-5.7	-
⁶⁵ Zn - oral dose			
Lactating goats	94.8-97.9	0.4	1.7-4.8
Non-lactating goat	99.6	0.4	-
⁶⁵ Zn-I.V. dose			
Lactating goats	59.3-76.8	0.7-1.7	21.8-40.1
Non-lactating goat	98.9	1.1	

As can be seen by these data, excretion is mainly via the feces with very little via urine, even when the ⁶⁵Zn was given intravenously. Milk is a significant route by which Zn leaves the body in goats.

Zinc does become incorporated into hair as shown in figures 10 and 11. It appears to take somewhat over 10 days before activity of significant levels is detectable, however this is undoubtedly due to the inability to clip hair close enough to the growth site at times earlier than 10-15 days. There were considerable differences between goats in the activity measured. This may in part be due to length of hair in each goat, but perhaps also indicates real differences among goats in rate of hair growth and zinc incorporation. It should be noted that goat 183 had been used in a previous study with ⁶⁵Zn; thus explaining the high level in hair at the start of this work.

Blood data of goats injected with ⁶⁵Zn intravenously were subjected to a compartmental analysis procedure. The specific activities of plasma over time were fit to exponential equations of three terms each.

$$\begin{aligned} \text{Goat 181} \quad SA &= 50e^{-44.23t} + 3.15e^{-.292t} + .82e^{-.023t} \\ 183 \quad SA &= 50e^{-25.7t} + 2.8e^{-.399t} + .50e^{-.015t} \\ 185 \quad SA &= 90e^{-18.0t} + 6.45e^{-.411t} + .60e^{-.017t} \end{aligned}$$

where $SA = \text{Dose } ^{65}\text{Zn} \times 10^{-5} / \mu\text{g Zn}$

$t = \text{Time in days}$

These equations were utilized to solve for the parameters of a three compartment model as follows:

Pool a is the plasma space pool into which the radioisotope was injected. Pool b is a slowly exchanging pool whereas pool c is a more rapidly exchanging pool.

The following rate constants (k) with units of fraction per day were computed:

	<u>Goat 181</u>	<u>183</u>	<u>189</u>	<u>Average + S.D.</u>
k_{ba}	1.96	3.54	2.48	$2.66 \pm .81$
k_{ab}	.075	.063	.044	$.061 \pm .016$
k_{ca}	37.9	19.3	12.5	23.2 ± 13.2
k_{ac}	3.47	1.92	1.66	$2.35 \pm .98$
k_{oa}	1.14	1.26	1.73	$1.38 \pm .31$

Pool sizes relative to pool "a" ($a = 1$) were pool "b", 46 ± 17 ; pool "c" 9.5 ± 1.8 . The flow of zinc between pools and out of pool "a" expressed as a % of the zinc in pool "a" flowing per hour work out to be:

between "a" and "b", 11.1%; between "a" and "c" 96.7%; and out of "a", 5.7%.

In the analysis used, pool sizes and flow rates expressed in mass units of zinc necessitate knowledge of the size of pool "a". This has been estimated by the zero time intercept of the equations fit to the specific activity curves. Since the specific activity of plasma changes very rapidly the precision of estimating these zero intercepts are limited by sampling frequency, radiochemical assay accuracy and on the number of exponential terms used to describe this early portion of S.A. curves. With these limitations in mind, the following approximations are presented.

Pool size (mgZn)

a	1.85	1.88	1.03	$1.59 \pm .48$
b	48.4	104.4	58.7	70.5 ± 29.8
c	20.2	18.9	7.74	15.6 ± 6.8

Flow (mg/hr)

$f_{ba} = f_{ab}$	.15	.28	.11	$.19 \pm .09$
$f_{ca} = f_{ac}$	2.9	1.5	5.4	1.7 ± 1.2
f_{oa}	.09	.10	.07	$.09 \pm .01$

The specific activity of zinc in pools "b" and "c" over time were predicted by the following equations, also derived by these kinetic analysis procedures.

Pool "b"

$$\begin{aligned} \text{goat 181} \quad \text{SA} &= -.08e^{-44.23t} - 1.09e^{-.292t} + 1.17e^{-.023t} \\ 183 \quad \text{SA} &= -.12e^{-25.7t} - .53e^{-.399t} + .65e^{-.015t} \\ 189 \quad \text{SA} &= -.22e^{-18.0t} - .76e^{-.411t} + .98e^{-.017t} \end{aligned}$$

Pool "c"

$$SA = 4.26e^{-44.23t} + 3.44e^{-.292t} + .82e^{-.023t}$$

$$SA = 4.04e^{-25.7t} + 3.54e^{-.399t} + .50e^{-.015t}$$

$$SA = 9.17e^{-18.0t} + 8.56e^{-.411t} + .61e^{-.017t}$$

where $SA = \text{Dose } ^{65}\text{Zn} \times 10^{-5} / \mu\text{g Zn}$

t = Time in days

Composites of these curves are shown in figures 1 and 2.

In this analysis a three compartment model was employed with two compartments exchanging with plasma Zn. This undoubtedly represents a summing of pools with similar characteristics, however the plasma curve does show three distinct slopes. Further investigations with frequent sampling during the first hours after I.V. dosing may enable dividing the rapidly changing portion of the curves into more components.

A complete modeling of zinc metabolism in the goat remains to be carried out, data for which this work has furnished. The analysis of blood curves presented here is useful in that it points to areas to be considered and provides data as to the magnitude of rate constants, flow rates and comparative sizes of pool groups. Pool "B", for example, is the largest pool and has a rather slow turnover time. It can be speculated that this pool represents the red blood cells and indeed the specific activity of these cells appears to follow that predicted by the equation for pool B. However, pool B must also represent tissues other than the red blood cells as reflected by the size of this pool compared to the total zinc content of red blood cells. During the early portion of the curves the RBC specific activity is higher than that predicted for pool B. This would suggest a loose binding of zinc to the outside of the cells which is not part of pool B. Pool C represents tissues which come to rapid equilibrium with plasma zinc.

In this model the simplifying assumption was made that there was no significant back flow of zinc from the mammary gland into plasma. The following experiments tended to support this assumption in the goat.

Experiments were run to determine the rate of removal of ^{65}Zn from the mammary gland of the goat and cow. For this approximately 3 μCi of ^{65}Zn and 2 μCi of ^{141}Ce was infused into the mammary glands of two goats and a cow about 2 hours after milking. The glands were massaged for 3 minutes to mix the radioactivity into the residual milk. Samples were collected at $\frac{1}{2}$, 1, 2, 4, 8, 16 and 24 hours. The sampling procedure consisted of injection of 1 I.U. of oxytocin. I.V. for the goats and 10 I.U. for the cow. The udder was then massaged carefully to mix the milk well and then a 20 ml or greater volume of milk was removed. The milk was counted by gamma counting for ^{65}Zn and ^{141}Ce . Some of the milk was mixed with Dowex 50 cation exchange resin to determine the amount of bound ^{65}Zn present in the milk. Urine and blood samples were collected but neither showed detectable amounts of radioactivity.

The ^{141}Ce was used as a nonabsorbable volume indicator and determined the expansion of the milk compartment over time. The ^{65}Zn concentration was corrected for the volume of the infused dose and the amount of ^{65}Zn removed in the samples.

Figure 12 presents the results of the three experiments; the data are expressed as the percent of the ^{65}Zn dose left in the gland at each sampling time interval. The striking difference shown is that little of the zinc moves from milk to blood for the goats; however, a relatively rapid loss was seen for the dairy cow. Figure 13 is the data expressed as percent of dose per ml of milk. From this it can be seen that the removal function can easily be described by a single exponential function in the case of the goats but is a more complex function for the cow.

Using the data of figure 12, percent dose remaining in the gland, a compartmental analysis was attempted. The equation for the data of Goat 180 was determined to be: Fraction of Dose = $0.12e^{-0.202t} + 0.83e^{-0.0024t}$. The equation for the cow was: Fraction of Dose = $0.305e^{-0.356t} + 0.695e^{-0.0863t}$.

Since the data obtained from both of these animals indicate the possibility of at least 2 compartments the model of the system is postulated to be:

The values calculated for the various k's by compartmental analysis* is:

	<u>Cow</u>	<u>Goat</u>
k_{yx}^{**}	0.169	0.0262
k_{xy}	2.441	7.783
k_{oy}	0.0185	0.1822

If compartment "x" is milk and "y" is mammary secretory tissue these data show that both the cow and the goat show a major movement of zinc into the milk and a very small movement out. The k_{xy}/k_{ya} ratio is 297 for this goat and 14.5 for the cow. This indicates a greater propensity of the goat to transfer zinc into milk and to retain it there than for the cow.

* Tracer methods for in vivo kinetics, R. A. Shipley and R. E. Clark, Academic Press, N.Y., 1972.

** k has the dimension of fraction per hour.

Figure 1. Compartmental analysis of plasma curve of goats administered ^{65}Zn intravenously. "A" represents pool or observed plasma specific activity. "B" and "C" represent calculated S.A. of pools B and C, respectively. Point plotted represent S.A. of red blood cells as determined experimentally.

Figure 2. Compartmental analysis of plasma curve of goats administered ^{65}Zn intravenously. "A" represents pool or observed plasma specific activity. "B" and "C" represent calculated S.A. of pools B and C, respectively.

Figure 3. Average specific activity of plasma and red blood cells following oral dosing of goats with ^{65}Zn .

Figure 4. Concentration of ^{65}Zn in milk of Goat 183.

Figure 5. Specific activity of ⁶⁵Zn in milk of Goat 183.

Figure 6. Concentration of ^{65}Zn in milk of Goat 189.

Figure 7. Specific activity of ^{65}Zn in milk of Goat 189.

Figure 8. Average concentration of ^{65}Zn in urine of goats.

Figure 9. Average specific activity of ^{65}Zn in urine of goats.

Figure 10. Concentration of ^{65}Zn in hair of goats. Day 0 indicates time of oral administration. Arrows indicate time of I.V. administration. Goat 189 was previously administered ^{65}Zn .

Figure 11. Specific activity of ^{66}Zn in hair of goats. Day 0 indicates time of oral administration. Arrows indicate time of I.V. administration. Goat 189 was previously administered ^{66}Zn .

PERCENT DOSE

HOURS

FIGURE 12: The percent of a ^{65}Zn dose in milk at various time intervals after dosing. □—□ Goat 180; x—x Goat 185; +—+ a Cow.

FIGURE 13: The percent dose per ml of milk of ^{65}Zn infused into the udder. $\square$ — $\square$ Goat 180; $\times$ — $\times$ Goat 185; $+$ — $+$ a Cow.

Table 1. ^{65}Zn in Blood of Goat 181*

ORAL DOSE

Days	<u>Whole Blood</u>		<u>Plasma</u>		<u>Cells</u>	
	Concentration	S.A.	Concentration	S.A.	Concentration	S.A.
.008	4.595	6.046	6.713	47.624	.831	.446
.035	2.938	4.738	4.115	32.415	.751	.489
.049	2.020	1.924	2.845	16.740	.488	.182
.092	1.305	1.611	1.725	11.819	.558	.280
1.092	.630	.724	.483	3.175	.881	.421
2.092	.540	.857	.332	2.595	.944	.588
5.092	.405	.579	.177	1.312	.880	.469
8.092	.405	.460	.137	.896	.903	.405
12.092	.365	.392	.051	.320	.901	.401
16.092	.281	.702	.069	.657	.657	.711
19.092	.314	.850	.052	.505	.825	.926
23.092	.350	.625	.075	.620	.861	.626
26.092	.280	.667	.055	.514	.737	.698
30.092	.255	.375	.055	.414	.626	.369
33.092	.245	.395	.045	.354	.633	.402
42.092	.185	.303	.060	.476	.439	.275
49.092	.125	.321	.025	.241	.319	.338
56.092	.100	.208	.032	.279	.233	.195
63.092	.105	.188	.016	.128	.270	.197

INTRAVENOUS DOSE

.172	.019	.040	.003	.029	.044	.042
1.172	.081	.198	.112	.983	.033	.038
2.172	.081	.166	.097	.792	.058	.056
3.172	.097	.176	.062	.483	.151	.125
6.172	.089	.185	.031	.255	.184	.172
7.172	.054	.123	.043	.363	.072	.077
8.172	.070	.155	.027	.229	.142	.140
9.172	.070	.162	.035	.299	.129	.134
10.172	.062	.147	.004	.033	.165	.172
13.172	.059	.062	.024	.144	.118	.052
16.172	.041	.052	.009	.060	.111	.051
20.172	.032	.040	.005	.030	.091	.041
24.172	.032	.031	.010	.055	.079	.028
27.172	.014	.011			.032	.013
30.172	.005	.005			.013	.006
37.172	.009	.009			.025	.010
46.172	.015	.009			.023	.010

* Concentration = % dose/ml $\times 10^{-3}$
 S.A. = % dose/mgZn

Table 2. ^{65}Zn in Blood of Goat 183*

DOSE		Whole Blood		Plasma		Cells	
Days	Concentration	S.A.	Concentration	S.A.	Concentration	S.A.	
.010	6.280	6.542	8.635	48.071	1.276	.487	
.023	3.385	4.285	4.565	28.070	.758	.347	
.038	2.365	2.920	3.175	19.285	.562	.250	
.080	1.560	2.261	2.035	13.333	.503	.266	
1.080	.645	.430	.540	2.311	.879	.203	
2.080	.545	.540	.340	1.842	.981	.355	
5.080	.445	.371	.170	.835	1.029	.310	
8.080	.385	.306	.106	.506	1.103	.278	
12.080	.335	.447	.083	.523	.952	.433	
16.080	.320	.451	.051	.327	.980	.473	
19.080	.335	.453	.042	.266	.987	.485	
23.080	.300	.455	.065	.434	.875	.458	
26.080	.300	.441	.025	.165	.942	.492	
30.080	.295	.360	.065	.392	.784	.355	
33.080	.215	.279	.080	.498	.516	.242	
42.080	.170	.205	.045	.270	.476	.194	
49.080	.170	.246	.045	.295	.476	.237	
56.080	.140	.311	.040	.311	.385	.311	
63.080	.115	.202	.011	.078	.370	.228	

INTRAVENOUS DOSE

.170	.035	.026	.054	.214	.003	.001
1.170	.066	.058	.081	.349	.031	.010
2.170	.058	.045	.062	.249	.050	.014
3.170	.058	.042	.054	.210	.067	.017
6.170	.050	.040	.031	.126	.098	.026
7.170	.050	.058	.027	.132	.107	.044
8.170	.044	.078	.023	.131	.096	.062
9.170	.043	.058	.019	.100	.097	.048
10.170	.054	.061	.008	.037	.127	.065
13.170	.047	.030	.016	.057	.127	.026
16.170	.037	.022	.005	.016	.118	.023
20.170	.028	.019	.005	.017	.089	.019
24.170	.019	.016	.005	.019	.051	.015
27.170	.023	.014	.005	.017	.079	.014
29.170	.014	.010			.035	.011
34.170	.009	.007			.028	.008
41.170	.019	.022			.048	.026
50.170	.015	.012			.029	.014
59.170	.010	.008			.030	.010

* Concentration = $\% \text{ dose/ml} \times 10^{-3}$
 S.A. = $\% \text{ dose/mgZn}$

Table 3. ^{65}Zn in Blood of Goat 189*

<u>L DOSE</u>		<u>Whole Blood</u>		<u>Plasma</u>		<u>Cells</u>	
Days	Concentration	S.A.	Concentration	S.A.	Concentration	S.A.	
.010	15.502	13.250	25.530	120.456	1.072	.421	
.021	9.095	8.194	12.555	60.963	3.905	1.583	
.042	4.915	5.461	7.325	39.606	1.300	.659	
.083	3.165	3.403	4.290	22.826	1.478	.723	
.208	2.045	1.598	2.595	11.640	1.185	.404	
1.000	1.090	1.172	.915	4.868	1.390	.631	
4.000	.875	.803	.295	1.446	1.821	.718	
7.000	.725	.763	.165	.869	1.678	.748	
11.000	.896	1.066	.249	1.389	1.907	1.018	
15.000	.739	1.055	.079	.476	1.862	1.156	
18.000	.713	.938	.079	.459	1.704	1.014	
21.000	.673	.748	.111	.600	1.516	.769	
24.000	.543	.603	.053	.284	1.377	.650	
29.000	.556	.539	.007	.033	1.414	.607	
35.000	.567	.630	.070	.378	1.379	.667	
42.000	.476	.529	.021	.114	1.359	.594	
49.000	.420	.467	.056	.303	1.127	.492	
53.000	.427	.474	.077	.416	1.077	.483	

INTRAVENOUS DOSE

.083	.014	.010	.004	.014	.031	.009
.175	.067	.047	.084	.264	.035	.010
1.175	.146	.052	.155	.341	.129	.018
2.175	.142	.084	.080	.232	.267	.061
5.175	.111	.049	.031	.077	.265	.045
8.175	.098	.067	.036	.111	.229	.059
12.175	.092	.044	.014	.036	.244	.045
16.175	.088	.044	.009	.025	.233	.047
19.175	.085	.042	.033	.088	.178	.036
23.175	.135	.076	.005	.014	.356	.085
26.175	.090	.045	.020	.054	.214	.044
30.175	.085	.041	.035	.092	.174	.035
33.175	.090	.054	.030	.088	.201	.049
42.175	.060	.040	.025	.077	.122	.034
49.175	.050	.043	.010	.035	.128	.045
56.175	.065	.055	.016	.053	.153	.055
63.175	.065	.051	.011	.036	.165	.053

* Concentration = $\% \text{ dose/ml} \times 10^{-3}$
 S.A. = $\% \text{ dose/mgZn}$

Table 4. Secretion of ^{65}Zn into Milk of Goat 183 Following Intravenous Dose.

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
.87	.419	2.715	6.474	2109
1.20	.222	1.063	4.781	1370
1.87	.445	1.488	3.346	1036
2.20	.196	.550	2.804	794
2.87	.447	1.050	2.351	719
3.20	.200	.401	2.005	548
3.87	.431	.746	1.731	539
4.20	.188	.300	1.595	433
4.87	.408	.546	1.340	456
5.20	.169	.209	1.236	368
5.87	.391	.462	1.180	311
6.20	.206	.232	1.126	312
6.87	.418	.422	1.008	280
7.20	.178	.162	.912	263
7.87	.367	.309	.843	203
8.20	.202	.172	.851	259
8.87	.343	.245	.715	177
9.20	.195	.133	.681	209
9.87	.362	.254	.700	167
10.20	.179	.110	.618	159
10.87	.385	.230	.597	144
11.20	.173	.097	.563	163
11.87	.376	.207	.552	135
12.20	.146	.075	.513	145
12.87	.332	.176	.530	123
13.20	.163	.079	.483	126
14.20	.141	.061	.436	121
14.87	.376	.161	.430	100
15.20	.149	.070	.473	473
15.87	.349	.137	.392	96
16.20	.150	.061	.406	100
16.87	.313	.101	.324	94
17.20	.152	.040	.262	94
17.87	.342	.099	.290	101
18.20	.141	.040	.285	96
18.87	.353	.129	.364	80
19.20	.155	.061	.390	85
19.87	.316	.141	.448	133
20.20	.125	.052	.416	84
20.87	.355	.130	.367	100
21.20	.142	.046	.323	82
21.87	.353	.109	.309	80
22.20	.169	.047	.276	75
22.87	.386	.097	.252	74

Table 4 (Continued).

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
23.20	.162	.034		
23.87	.387	.083	.212	67
24.20	.173	.033	.213	67
24.87	.387	.075	.191	65
			.192	63
25.20	.180	.031		
25.87	.388	.073	.173	61
26.20	.182	.033	.188	59
26.87	.392	.073	.180	57
			.186	56
27.20	.176	.032		
27.87	.417	.071	.181	48
28.20	.193	.032	.170	53
28.87	.431	.076	.164	53
			.175	51
29.20	.162	.027		
29.87	.396	.067	.168	46
30.20	.183	.030	.170	46
30.87	.423	.067	.161	45
			.157	38
31.20	.172	.024		
31.87	.417	.059	.138	39
32.20	.185	.025	.142	42
32.87	.405	.057	.134	34
			.141	42
33.20	.128	.017		
33.87	.419	.054	.130	42
34.20	.187	.034	.129	39
34.87	.388	.052	.184	58
			.134	41
35.20	.170	.022		
35.87	.416	.053	.127	34
36.20	.179	.021	.128	39
41.87	.376	.043	.115	37
			.115	19
42.20	.153	.017		
48.87	.293	.037	.109	33
49.20	.125	.014	.127	34
55.87	.396	.036	.114	34
			.091	26
56.20	.173	.015		
62.87	.388	.029	.084	25
63.20	.155	.011	.075	16
			.073	16

* SPECIFIC ACTIVITY IN PERCENT DOSE/G. ZN

Table 5. Secretion of ^{65}Zn into Milk of Goat 183 Following Oral Dose.

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
.20	.243	.0013	.0054	1.70
.87	.527	.0066	.1643	53.51
1.20	.253	.0575	.2269	77.97
1.87	.547	.1471	.2691	83.33
2.20	.250	.0584	.2342	72.28
2.87	.576	.1204	.2091	32.12
3.20	.236	.0437	.1851	56.10
3.87	.579	.0926	.1600	47.48
4.20	.251	.0347	.1381	44.13
4.87	.534	.0656	.1229	36.26
5.20	.270	.0286	.1061	29.55
5.87	.550	.0556	.1011	29.99
6.20	.268	.0231	.0862	25.66
6.87	.525	.0447	.0852	24.40
7.20	.260	.0191	.0732	23.10
8.20	.266	.0164	.0617	18.75
8.87	.567	.0344	.0607	12.70
9.20	.239	.0137	.0575	17.06
9.87	.549	.0297	.0541	14.77
10.20	.268	.0131	.0491	12.30
10.87	.558	.0267	.0478	13.64
11.20	.246	.0112	.0456	12.70
11.87	.579	.0244	.0421	11.39
12.20	.236	.0095	.0401	10.71
12.87	.547	.0206	.0376	10.66
13.20	.249	.0082	.0332	9.04
13.87	.517	.0179	.0346	9.52
14.20	.238	.0077	.0325	8.43
14.87	.530	.0172	.0325	7.81
15.20	.235	.0072	.0304	3.26
15.87	.509	.0146	.0287	7.37
16.20	.255	.0070	.0273	4.60
16.87	.533	.0142	.0267	5.86
17.20	.291	.0079	.0272	7.43
17.87	.528	.0140	.0265	6.84
18.20	.207	.0055	.0266	6.67
18.87	.543	.0135	.0249	7.02
19.20	.222	.0049	.0219	5.92
19.87	.517	.0111	.0216	5.89
20.20	.213	.0044	.0206	5.13
20.87	.484	.0103	.0213	5.78
21.20	.239	.0047	.0196	5.46
21.87	.567	.0106	.0187	5.62
22.20	.208	.0037	.0180	5.12

Table 5. (Continued).

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
22.87	.447	.0080	.0180	4.68
23.20	.219	.0035	.0161	3.80
23.87	.598	.0091	.0152	4.26
24.20	.230	.0035	.0153	3.86
24.87	.548	.0078	.0142	3.83
25.20	.221	.0028	.0127	2.94
25.87	.522	.0065	.0125	3.77
26.20	.229	.0033	.0144	4.25
26.87	.510	.0070	.0138	3.59
27.20	.238	.0032	.0137	3.63
27.87	.504	.0071	.0140	2.22
28.20	.243	.0029	.0121	3.45
28.87	.513	.0066	.0129	3.42
29.20	.218	.0025	.0114	3.15
29.87	.522	.0058	.0110	3.31
30.20	.250	.0023	.0092	2.67
30.87	.451	.0052	.0116	3.36
31.20	.217	.0026	.0120	3.66
31.87	.387	.0056	.0145	4.16
32.20	.200	.0021	.0104	3.24
32.87	.522	.0053	.0101	2.99
33.20	.212	.0024	.0114	3.54
33.87	.531	.0050	.0095	2.93
34.20	.237	.0018	.0074	2.47
34.87	.526	.0050	.0096	2.95
35.20	.238	.0018	.0075	2.21
35.87	.519	.0046	.0089	2.59
36.20	.213	.0017	.0082	2.39
36.87	.512	.0047	.0093	2.83
37.20	.220	.0028	.0125	3.22
42.87	.428	.0040	.0092	2.67
43.20	.198	.0015	.0073	2.54
49.87	.453	.0032	.0070	1.86
50.20	.202	.0012	.0058	1.28
56.87	.497	.0027	.0055	1.60
57.20	.191	.0013	.0067	1.94
70.87	.485	.0019	.0039	2.09
71.20	.194	.0017	.0090	2.65
77.87	.466	.0031	.0066	1.94

* SPECIFIC ACTIVITY IN PERCENT DOSE/G. ZN

Table 6. Secretion of ^{65}Zn into Milk of Goat 189 Following Intravenous Dose.

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
.20	.173	1.491	8.627	1646
.87	.450	6.670	14.805	2742
1.20	.234	2.992	12.787	2351
1.87	.514	5.440	10.593	1625
2.20	.221	2.110	9.533	1390
2.87	.494	3.386	6.851	1052
3.20	.225	1.376	6.107	990
3.87	.499	2.357	4.723	833
4.20	.225	.977	4.338	761
4.87	.490	1.839	3.751	632
5.20	.175	.639	3.658	603
5.87	.462	1.505	3.257	494
6.20	.171	.481	2.816	453
6.87	.589	1.434	2.433	453
7.20	.266	.576	2.164	410
7.87	.588	1.235	2.100	365
8.20	.331	.650	1.964	348
8.87	.520	.877	1.686	334
9.20	.217	.425	1.954	618
9.87	.324	.555	1.711	290
10.20	.230	.397	1.727	267
10.87	.282	.644	2.289	264
11.20	.127	.272	2.136	259
11.87	.293	.665	2.267	268
12.20	.127	.240	1.889	240
12.87	.358	.662	1.847	265
13.20	.143	.250	1.749	261
13.87	.279	.458	1.645	223
14.20	.172	.265	1.543	234
14.87	.238	.327	1.374	201
15.20	.155	.188	1.208	194
15.87	.267	.258	.968	209
16.20	.170	.171	1.005	194
16.87	.241	.271	1.126	185
17.20	.137	.150	1.094	177
17.87	.314	.297	.946	168
18.20	.134	.124	.927	173
18.87	.209	.231	1.107	167
19.20	.113	.128	1.137	175
19.87	.283	.258	.912	166
20.20	.123	.089	.721	160
20.87	.249	.195	.784	162
21.20	.134	.124	.924	152
21.87	.267	.275	1.030	134

Table 6. (Continued).

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
22.20	.126	.134	1.059	160
22.87	.251	.226	.900	145
23.20	.146	.115	.790	125
23.87	.332	.216	.650	132
24.20	.151	.093	.612	134
24.87	.330	.183	.555	100
25.20	.188	.110	.586	99
25.87	.373	.236	.634	111
26.20	.163	.094	.577	104
26.87	.414	.207	.500	106
27.20	.150	.071	.475	99
27.87	.399	.190	.475	109
28.20	.167	.079	.472	101
28.87	.336	.163	.486	87
29.20	.190	.090	.472	93
29.87	.353	.168	.475	98
30.20	.179	.084	.467	86
30.87	.340	.162	.476	84
31.20	.183	.088	.482	96
31.87	.340	.171	.504	88
32.20	.187	.100	.535	83
32.87	.317	.194	.613	95
33.20	.124	.075	.605	84
33.87	.250	.183	.732	97
34.20	.111	.074	.670	101
34.87	.125	.098	.782	105
35.20	.126	.095	.751	96
35.87	.212	.162	.767	102
36.20	.106	.080	.756	103
36.87	.297	.173	.581	103
43.87	.413	.117	.284	73
44.20	.156	.040	.256	74

* SPECIFIC ACTIVITY IN PERCENT DOSE/G. ZN

Table 7. Secretion of ^{65}Zn into Milk of Goat 189 Following Oral Dose.

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
.20	.545	.0140	.0258	3.15
.87	.956	.3666	.3833	351.68
1.20	.488	.2653	.5432	127.81
1.87	.944	.5205	.5516	127.39
2.20	.553	.2829	.5113	116.73
2.87	1.132	.4770	.4214	93.85
3.20	.524	.1990	.3796	86.67
3.87	.964	.3133	.3250	69.30
4.20	.639	.1910	.2990	28.31
4.87	.884	.2013	.2276	48.95
5.20	.563	.1158	.2057	44.71
5.87	1.126	.1874	.1664	34.02
6.20	.570	.0896	.1572	29.55
6.87	1.090	.1557	.1428	32.01
7.20	.572	.0743	.1299	29.86
7.87	1.077	.1284	.1192	26.38
8.20	.647	.0739	.1143	26.10
8.87	.998	.1031	.1033	24.48
9.87	1.097	.1075	.0980	21.40
10.20	.525	.0465	.0885	18.66
10.87	1.101	.0821	.0746	19.78
11.20	.568	.0439	.0772	18.83
11.87	1.114	.0764	.0686	16.52
12.20	.466	.0327	.0702	15.71
12.87	1.088	.0690	.0634	15.49
13.20	.559	.0369	.0659	16.03
13.87	1.012	.0624	.0617	14.97
14.20	.427	.0286	.0670	15.45
14.87	.948	.0570	.0601	13.99
15.20	.485	.0284	.0585	12.72
15.87	.925	.0491	.0531	12.31
16.20	.522	.0265	.0507	11.62
16.87	.878	.0469	.0534	12.50
17.20	.541	.0290	.0535	11.85
17.87	.975	.0453	.0464	11.36
18.20	.488	.0234	.0478	10.11
18.87	.999	.0431	.0432	10.56
19.20	.501	.0215	.0429	10.20
19.87	1.011	.0416	.0412	7.66
20.20	.490	.0200	.0409	3.22
20.87	1.021	.0391	.0383	9.32
21.20	.440	.0164	.0373	8.94
21.87	1.019	.0371	.0364	8.09
22.20	.496	.0194	.0390	8.47

Table 7. (Continued).

DAYS	LITERS	PERCENT	PERCENT/L.	S.A.*
23.20	.457	.0182	.0399	9.04
23.87	.923	.0350	.0379	8.71
24.20	.495	.0195	.0393	8.54
24.87	.956	.0353	.0369	8.84
25.20	.457	.0170	.0371	6.49
25.87	.912	.0321	.0352	8.07
26.20	.419	.0148	.0352	8.08
26.87	.825	.0293	.0343	7.85
27.20	.476	.0163	.0342	7.94
29.20	.319	.0107	.0335	7.55
29.87	.970	.0300	.0309	6.55
30.20	.468	.0137	.0292	6.34
30.87	.970	.0301	.0310	6.60
31.20	.400	.0130	.0325	6.94
31.87	.906	.0280	.0309	6.70
32.20	.398	.0125	.0313	6.24
32.87	.917	.0287	.0313	6.61
33.20	.310	.0099	.0319	7.09
33.87	.786	.0255	.0324	6.79
34.20	.572	.0176	.0308	6.90
34.87	.767	.0219	.0286	11.80
35.20	.418	.0119	.0284	6.25
35.87	.848	.0259	.0305	6.21
36.20	.477	.0139	.0291	6.37
41.87	.776	.0212	.0273	6.22
42.20	.363	.0098	.0270	6.20
48.87	.750	.0195	.0260	5.67
49.20	.302	.0079	.0262	5.58
55.87	.739	.0178	.0241	5.01
56.20	.380	.0090	.0237	5.21
62.87	.748	.0166	.0222	4.58
63.20	.359	.0075	.0208	4.77

* SPECIFIC ACTIVITY IN PERCENT DOSE/G. ZN