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Phase I Radiation and Radioisotope Studies
with Laboratory and Domestic Animals

Subsection "Physiological Studies of
Environmental Pollutants.

Submitted by

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Progress Report

Phase I Radiation and Radioisotope Studies

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Subsection - Physiological Studies of

Environmental Pollutants

Foreward

As a result of a request from the Division of Biomedical and Environmental Research at this renewal date the various groups of research under Phase I of AEC Contract No. AT(11-1)-3167 are being written as if they were separate, new projects. This creates some problems in submission of a progress report for Phase I and so it has been suggested that each investigator submitting a proposal under Phase I would report that work for which he is responsible and which is pertinent to the particular research proposal. Since only the effort studying milk secretion is truly a continuation we are submitting the work in the past year which is pertinent to that project. The report of the current effort is covered in Part I of this report.

Since the proposals of the separate projects previously carried out under Phase I will be sent out to outside reviewers we have included as well a summary of the work reported on the transfer of elements into milk carried out in the three years preceding the 1973-1974 contract year. This summary is from our AEC Progress Report for 1972-1973 and consists of pages 415 to 491 of that report. To avoid any misunderstanding we have not changed the page numbering. This section is intended only for those reviewers who have not had the opportunity of reading the previous report or who are not familiar with our work.

Subsection: Physiological Studies of Environmental
Pollutants.

Progress during the 1973 - 1974 contract year

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G. Distribution of ^{131}I Secreted into Milk

When the stimulation of a radiolabel in the milk of the goat is based upon a single sample of withdrawn milk it is necessary to establish that the distribution of the label is uniform. Such evidence is required before a milk sample may be considered as a representative fraction of the total milk volume.

In the guinea pig the distribution of marker in the milk of the unagitated gland was decidedly nonuniform, with concentration decreasing toward the alveoli. The average error in milk volume estimation under such conditions, therefore, was large and amounted to 12.8% (59). In the goat, on the other hand, the error in milk volume estimation associated with the mixed (agitated) gland was small, amounting to 1 % (82). Moreover, the uniformity extended to alveolar fraction of milk when oxytocin was administered before mixing.

When a radionuclide enters milk at the alveolar end of the gland its concentration would be expected to decrease from alveolar spaces to cisternal spaces in a fashion somewhat the reverse of the distribution of radiomarker which had been transferred to milk through the teat. An important consideration regarding product secretion into milk, therefore, was the evaluation of the uniformity of distribution of this product in milk.

The purpose of this experiment was to determine the reciprocal distributions of radiocerium injected into milk via the teat and radioiodine secreted into the milk from blood. Comparisons were made for $\frac{1}{2}$ and 2 hour equilibration periods, that is, for time intervals between intravenous ^{131}I injection and milk removal. Comparisons also were made of marker distributions between mixed and unmixed milk collected from glands that were purposely agitated for 2 minutes or unagitated prior to milk removal.

Procedure

Four experiments were conducted on two goats in which marker distributions were followed in each gland under mixed and unmixed conditions, two goats were used dosed at once.

On the day preceding the experiment the goats were placed in metabolism stalls and were given oral doses of propylthiouracil at two separate times in order to inhibit the binding of iodine in the thyroid.

The goats were milked at their regular time in the morning. Experiments commenced at 1 P.M., about 6 hours after morning milking, to allow the accumulation of an adequate amount of milk for marker dispersal.

On the day of the experiment a dosing solution of ^{141}Ce was prepared by adding radiocerium to a sample of freshly drawn milk. At the same time ^{131}I dosing solution was prepared by diluting radioiodine with sterile isotonic saline.

Starting at 1 P.M. 5 ml (0.5 uc.) of ^{141}Ce milk dosing was injected into each gland and followed by an intravenous injection of 2 I.U. of oxytocin. The udders were kneaded for 2 minutes to help disperse the marker in the contained milk. Two milliliters (100 μCi) of ^{131}I dosing solution were administered to each animal by way of the jugular vein. After a waiting period of 30 minutes for one goat and 2 hours for the second serial milk sampling was initiated.

Milk samples were first taken from the unmixed gland in volumes of 10-20 ml, using a metal teat cannula. When milk flow ceased 2 I.U. of oxytocin were administered intravenously and the ejected milk was also serially collected. The second gland was then kneaded for two minutes in order to distribute the radionuclides uniformly prior to sampling this mixed gland. Serial sampling of this gland followed. When the free flow of milk stopped another dose of oxytocin was given and the ejected milk also was collected serially.

Counting standards of ^{141}Ce and ^{131}I dosing solutions were prepared after radioiodine dosing. The ^{141}Ce standard was prepared by transferring 5 ml of the ^{141}Ce milk dosing solution to a 100 ml volumetric flask and diluting to volume. The ^{131}I standard was prepared from 0.50 ml of dosing solution and diluted to 100 ml in a volumetric flask.

Milk samples were weighed, diluted to 20 ml in a glass vial then counted in an automatic well counter. The activity of ^{141}Ce and ^{131}I was measured and expressed as percent dose per ml of milk. The $^{141}\text{Ce}/^{131}\text{I}$ ratio was determined for mixed and unmixed milk, and the percent recovery was calculated for ^{141}Ce .

Results and Discussion

For milk collected from the mixed glands, plots of percent dose per ml of milk versus percent accumulated milk volume indicated little variation in concentration of radionuclides (Figures 12 and 13). Radiocerium marker entering the gland from the teat was more uniformly distributed in the first 80-90% of the extracted volume since this label was fixed into the cisternal compartment of contained milk which represents the bulk of the milk volume. In the last 10-20% of the total milk volume variation in the ^{141}Ce concentration was evident but the fractional differences were not dramatically large. In the case of the secreted isotope entering the milk at the alveolus, the radioiodine concentration rose continuously and sharply from the first to last samples. This indicated that the highly concentrated radioiodine found in newly secreted milk is slow to diffuse from the alveoli through the narrow and highly branched duct system in the mammary tissue.

Figures 14 and 15 illustrate the differences in uniformity of distribution of ^{131}I and ^{141}Ce after $\frac{1}{2}$ and 2 hour equilibration periods for radiocerium marker.

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Figure 12. Distribution of ^{131}I and ^{141}Ce marker in mixed glands of goats 18 and 32 after 1/2 hour equilibration time of radiocerium marker.

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Figure 13. Distribution of ^{131}I and ^{141}Ce marker in mixed glands of goats 18 and 32 after a 2-hour equilibration time for radiocerium milk marker.

Figure 14. Percent of ^{131}I dose recovered versus accumulated milk volume (grams) in unmixed glands of goats 18 and 32 after 1/2 and 2-hour equilibration periods.

Figure 15. Percent of ^{131}I dose recovered versus accumulated milk volume (grams) in mixed glands of goats 18 and 32 after 1/2 and 2-hour equilibration periods.

		GLAND	TIME PERIOD
○	○	18 R	0.5 HOURS
x	x	18 L	2 "
●	●	32 R	0.5 "
■	■	32 L	2 "

In Table 14, the Ce/I ratio of the unmixed gland appears small and relatively uniform over the series of samples for goat 18 after a two hour waiting period. These uniform values are probably due to unwanted agitation of the udder when the goat became excited during catheterization, causing the unintended mixing of milk on this side of the udder.

When mixing was carried out, ^{141}Ce and ^{131}I concentrations were relatively constant for all fractions collected up to the point of oxytocin administration. The tabulated values for percent recovery of ^{141}Ce in Table 14 shows that this marker is well distributed in all milk spaces. The ratio of cerium to iodine concentration varied from 0.91 to 1.15 and 0.96 to 1.04 for the $\frac{1}{2}$ hour and 2 hour waiting periods, respectively. The ranges of Ce/I ratios for milk collected after oxytocin injection were 0.54 and 0.61 and 0.80 to 0.86 for the $\frac{1}{2}$ hour and 2 hour periods, respectively. The variation in these ranges are attributed (1) to insufficient time in the $\frac{1}{2}$ hour period for diffusion of the radio-markers to occur uniformly and (2) to more variable concentrations of radio-iodine which is less able to diffuse from the constricted spaces in the alveoli and small ducts. In all cases, the concentration differences of ^{141}Ce in cisternal and oxytocin milk were relatively small. Those differences in cisternal and oxytocin concentrations of ^{141}Ce are most noticeable when comparing concentrations between the $\frac{1}{2}$ hour and 2 hour ratios. The greatest variation occurred among milk samples for shorter intervals. This means that a longer equilibration period allows more effective distribution of marker into the small recesses of the mammary gland.

In Table 15, a comparison of isotopic concentration differences in mixed glands between cisternal and alveolar (oxytocin) milk shows a 5.4% lower ^{141}Ce concentration in cisternal milk than the average alveolar concentration during

Table 14. Ce/I Ratios in Serial Samples of Milk from
Mixed and Unmixed Glands Collected After $\frac{1}{2}$ and
2 Hour Equilibration Periods

Goat Gland Time Periods Milk Samples:	Unmixed				Mixed			
	32	18	18#	32	32	18	18	32
	L	L	R	R	R	R	L	L
	$\frac{1}{2}$	$\frac{1}{2}$	2	2	$\frac{1}{2}$	$\frac{1}{2}$	2	2
1	2.98	4.16	1.21	2.12	1.08	1.15	1.03	1.02
2	2.84	2.94	1.14	1.83	1.07	1.12	1.02	1.04
3	2.70	2.10	1.08	1.76	1.10	1.10	1.03	1.02
4	2.02	1.45	1.06	1.75	1.08	1.11	1.02	1.02
5	1.82	1.20	1.06	1.74	1.10	1.10	1.01	1.03
6	1.81	1.11	1.08	1.79	0.98	1.11	1.01	1.03
7	1.46	0.98	1.08	1.70	0.97	1.08	1.02	1.04
8	0.85*	0.79	1.11	1.22	0.93	1.06	1.01	1.02
9	0.61*	0.24*	1.11	1.12	0.96	0.89	1.00	1.00
10	0.57*	0.18*	1.13	1.11	0.93	0.45*	0.99	1.00
11	0.52*		0.70*	1.04	0.77*	0.47*	0.87*	0.97
12	0.46*		0.50*	0.38*	0.74*		0.85*	0.94
13	0.43*		0.63*	0.21*	0.62*		0.86*	0.94
14	0.34*		0.84*	0.21*				0.86*
15			0.93*	0.22*				0.76*
% Recovery ^{141}Ce	89.3	96.0	95.6	88.8	94.3	93.0	98.6	92.5

* Values for milk samples collected after administration of oxytocin.

Table 15. Comparison of Average Cisternal and Alveolar Concentrations of ^{141}Ce and ^{131}I in Milk of Mixed Glands

Time	Goat	Isotope	Cisternal Ave. pre- oxytocin milk (%/ml milk)	Alveolar	% Difference	Total Milk Vol.	Alveolar Milk Vol.	% Alveolar Milk Vol.
$\frac{1}{2}$ hour	18	^{141}Ce	0.612	0.574	6.2	165.0	12.1	7.3
		^{131}I	0.570	1.254	120.1			
$\frac{1}{2}$ hour	32	^{141}Ce	0.443	0.423	4.5	225.6	14.9	6.6
		^{131}I	0.434	0.601	38.4			
				Ave. Ce	5.4			
				I	79.8			7.0
2 hour	18	^{141}Ce	0.515	0.502	2.5	195.0	18.8	9.6
		^{131}I	0.508	0.583	14.8			
2 hour	32	^{141}Ce	0.412	0.416	1.0	242.6	8.2	3.4
		^{131}I	0.412	0.515	25.6			
				Ave. Ce	1.2			
				I	20.2			6.5

the $\frac{1}{2}$ hour period. In the same interval the concentration of ^{131}I in alveolar milk was 79.8% greater than that in the cisternal milk. For the 2 hour interval, however, these same differences are only 1.2% and 20.2%; furthermore, the volume of oxytocin milk is only 6.5% of the total milk volume at 2 hours while it was 7.0% at $\frac{1}{2}$ hour.

In summary, the distribution of the ^{141}Ce entering the milk by way of the teat and the distribution of the ^{131}I milk from blood is fairly well distributed in approximately the first 95% of the milk collected from glands that have been mixed for 2 minutes prior to milk removal. Concentrations of each radionuclide collected from unmixed glands showed wide variations. However, the changes in the Ce/I ratio in both mixed and unmixed glands in going from cistern to alveolus was primarily due to increasing concentrations of ^{131}I . The error in estimating radionuclide concentrations in the total milk volume are small since the error is associated with a relatively small volume of milk.

H. Distribution of ^{47}Ca Secreted into Milk

This experiment was similar to the previous experiment (Chapter IV, Section G) pertaining to distribution of ^{131}I in milk. Here the radionuclide of primary concern was ^{47}Ca . In addition to verifying the effectiveness of the mixing procedure for uniform distribution of radiocalcium in milk this experiment attempted to determine the constancy or variability of radiocalcium concentration in newly formed milk.

This experiment is especially significant because the kinetic experiments to follow deal with calcium transfer. In order to calculate radiocalcium concentration in milk, blood and tissue from external counts the following sequence of steps were planned for the live goat. An external count of the udder would be taken, then a blood sample removed, oxytocin injected into the jugular vein, hand mixing of milk in the udder, and finally a milk sample would be collected.

Adhering to this procedure, a time lapse of 5 to 10 minutes would intervene between the external count and the milk sampling. This means ^{47}Ca secretion would continue during the interval. The newly secreted radiocalcium would not be included in the external count although it would be mixed to some extent with the older milk and counting in the milk sample and would lead to an over-estimation of the ^{47}Ca in the milk compartment at the time of the external count. Inclusion of the newly secreted ^{47}Ca represented an error, and the question arose as to the significance of this error.

A second objective of this experiment was to compare the concentrations of ^{47}Ca in alveolar milk at various time intervals. This was to be accomplished by administration of oxytocin at several time intervals after the mammary gland was emptied.

Procedure

Dosing solutions of ^{141}Ce and ^{47}Ca were prepared in a manner similar to the previous experiment (Chapter IV, Section 6) except that ^{47}Ca was added to heparinized blood rather than isotonic saline.

The goats were milked at the usual time in the morning of the experiment but not all milk was removed; approximately 200 ml of milk was left in each gland. Later, five milliliters of ^{141}Ce (5 μCi) were injected into each gland by way of a PE-90 cannula. At zero time 10 ml of heparinized blood containing 10 μCi of ^{47}Ca were injected into the jugular vein.

Two hours later the milk was serially collected from the unmixed right gland using a metal teat cannula. When the flow of milk stopped, 2 I. U. of oxytocin were administered and the time of administration recorded. Oxytocin (alveolar) milk was collected serially until milk flow again stopped. The udder was then kneaded for 2 minutes to mix the milk contained in the left

gland, after which the milk from the left gland was sampled serially. A second dose of oxytocin was given and the time recorded. The oxytocin milk was immediately collected from the left gland, then from the right gland. Injection of a third oxytocin dose occurred 35 minutes after the previous injection, and the milk from left and right glands were sampled without any kneading of the udder. Four more oxytocin injections were administered at intervals between injections of 5, 15, 25, and 45 minutes and samples obtained from both glands at these times.

The serial milk samples were weighed and 1 ml aliquots were taken, diluted to 20 ml, then counted in the automatic gamma counter.

The percent dose per ml was calculated for ^{141}Ce and ^{47}Ca and plotted against the percent accumulated milk volume for the serially collected samples. The $^{141}\text{Ce}/^{47}\text{Ca}$ ratio was determined for each of these milk samples. For the oxytocin milk samples cpm/ml/minute were computed and plotted against time.

Figures 16 and 17 and Table 16 illustrate the uniformity in ^{47}Ca and ^{141}Ce concentration found in serially collected milk samples from unmixed and mixed glands. Only in the last 5% of the milk collected does an obvious concentration difference occur in the mixed gland. The distributions shown here resemble those of Knutsson and Sperber (25) which demonstrated marker distributions in milk of goats.

The comparison of ^{47}Ca distribution, as well as the distribution of ^{131}I on pages , illustrate the disparity in concentration in the cisternal and alveolar milk between the mixed and unmixed glands. This comparison points out how erroneous it would be to sample milk from an unmixed gland in determining the total radiocalcium content of milk. Since calcium concentration in milk constitutes one of the more reliable compartmental measurements it is important that gross errors in calcium concentrations, as would occur

in unmixed samples, be avoided. For that reason a two minute mixing procedure is needed to minimize concentration differences of representative milk samples.

Excellent uniformity in calcium and radiomarker concentration is found in the serial samples collected after mixing for 90-95% of the accumulated milk volume. An abrupt change in concentration occurs at the point where oxytocin was injected. In unmixed milk samples, however, uniformity in marker concentration occurs in a smaller volume of accumulated milk while the uniformity is noticeably less than that found in mixed samples. Even the alveolar milk volume, that is milk ejected from the alveoli after oxytocin administration, showed only small variation in calcium concentration compared to unmixed milk. The higher calcium concentrations in alveolar milk reflect the richer concentration of calcium in new milk secreted by the epithelium.

Not only is calcium distributed uniformly over 90-95% of the total milk volume in the mixed gland, so is the radiomarker, ^{141}Ce . The fact that radio-cerium is able to distribute throughout the bulk of milk supports the reliability of this procedure as an isotopic dilution technique for estimating milk volume.

A major objective in this experiment was to determine the error in radio-calcium concentration in milk due to the extra ^{47}Ca secreted in the interval between the external count and milk sampling. This new milk secreted into the alveoli is part of the oxytocin milk, that is, milk available for sampling due to oxytocin administration. The difference in ^{47}Ca concentration of cisternal and alveolar milk is 0.076 % ml for the mixed gland. This excess ^{47}Ca in alveolar milk can produce an overestimate of ^{47}Ca in the total milk. The error contributed by this excess concentration in alveolar milk is contained

Figure 16. Distribution of ^{47}Ca and ^{141}Ce in serially collected milk samples from mixed and unmixed glands of goat 18 after a 24-hour equilibration of radiocerium milk marker.

Figure 17. Percent of ^{47}Ca dose recovered versus accumulated milk volume (grams) in goats 18 and 32 after a 2-hour equilibration period for radiocerium marker.

Table 16.

^{141}Ce -to- ^{47}Ca Ratios in Serial Samples of Milk
From Mixed and Unmixed Glands Collected After
a 2-Hour Waiting Period

		Unmixed Gland	Mixed Gland
Goat		18	18
Gland		R	L
Time Period		2	2
Milk Samples:	1	1.18	0.99
	2	1.03	1.00
	3	1.27	1.07
	4	1.10	1.04
	5	1.06	1.08
	6	1.05	0.97
	7	1.09	0.92
	8	1.11	0.94
	9	1.11	1.04
	10	1.18	1.07
	11	1.20	0.98
	12	1.12	1.05
	13	1.15	1.07
	14	0.58*	1.08
	15	0.55*	1.01
	16	0.66*	0.72*
	17	0.72*	0.70*
	18	0.81*	
	19	0.76*	
% Recovery ^{141}Ce		93.7	91.9

only in the relatively small volume of oxytocin milk collected. Table 18 shows only 15.4 ml or 5.33% of the total milk volume is alveolar milk.

An estimate of the excess, or error, of ^{47}Ca concentration is derived from the data in Table ____.

$$\text{Percent excess } ^{47}\text{Ca} \text{ in alveolar milk} = \frac{\% ^{47}\text{Ca}}{\text{ml alveolar milk}} - \frac{\% ^{47}\text{Ca}}{\text{ml cisternal milk}}$$

$$\text{Percent excess } ^{47}\text{Ca} \text{ in total milk} = \frac{\% ^{47}\text{Ca}}{\text{ml alveolar milk}} \times \text{ml alveolar milk}$$

Accordingly,

$$\text{Percent excess } ^{47}\text{Ca} \text{ in alveolar milk} = \frac{0.418 \% ^{47}\text{Ca}}{\text{ml alveolar milk}} - \frac{0.342 \% ^{47}\text{Ca}}{\text{ml cisternal milk}} = \frac{0.076 \% ^{47}\text{Ca}}{\text{ml alveolar milk}}$$

$$\text{Percent excess } ^{47}\text{Ca} \text{ in total milk} = \frac{0.076 \% ^{47}\text{Ca}}{\text{ml alveolar milk}} \times 15.4 \text{ ml alveolar milk} = 1.17\%$$

The error attributable to the ^{47}Ca accumulating in the alveoli after an external count may approximate 1% an error that is tolerable in the framework of the kinetic study.

Table 17 shows an irregular trend of increasing radiocalcium in alveolar milk with time while a steady decrease occurs in blood radiocalcium. This does not mean that the rate of ^{47}Ca increases with time since the third column of Table 16 shows a decrease in the rate of secretion of ^{47}Ca between oxytocin injections. The increase in ^{47}Ca concentration may be explained by the exchange of ionic ^{47}Ca with stable Ca in milk protein, thus increasing the cpm/ml even though the rate of secretion of ^{47}Ca appears to decrease. The decrease in secretion rate of ^{47}Ca may be due to the decreasing levels of ^{47}Ca in blood.

Table 17. Ca activity in alveolar milk collected after oxytocin injections at various time intervals.

Interval between oxytocin injections (min)	cpm/ml of milk sample	cpm/ml/min alveolar milk sample	cpm/ml of blood sample
5	693	138	744
15	709	47	737
25	664	26	699
45	876	20	663

Table 18. Comparison of average cisternal and alveolar concentrations of ^{141}Ce and ^{47}Ca in milk of the mixed gland.

Time period (hours)	Goat No.	Isotope	Ave. Cisternal milk (o/o/ml)	Oxytocin milk	% Diff.	Total milk vol.	Oxytocin milk vol.	% oxytocin milk
2	18	^{141}Ce	0.348	0.297	14.65	288.7	15.40	5.33
	18	^{47}Ca	0.342	0.418	22.22			

In summary, it appears that changes in ^{47}Ca content in milk that may arise during the period between external counting and milk sampling would be small and that the consequent errors in external udder count and ^{47}Ca concentration of milk at the time of external counting may be neglected.

1. Error in Estimation of Contained Milk Volume in the Dairy Goat with Cerium-141*

A basic requirement for the study of transfer rates of elements across the mammary gland epithelium is an accurate measurement of the contained-milk volume. In some kinetic studies of the mammary gland not only is time critical, but determination of milk volume without emptying the gland is often necessary. Studies involving absorption of materials infused into the milk, or secretion of milk constituents, demand knowledge of the total milk volume. Moreover, the sacrifice of experimental animals is often to be avoided in accumulating such data. An effective method currently used for milk volume determinations employs the dilution principle whereby a known amount of marker substance is infused into the udder. Samples are collected over time in a given experiment, and it is necessary to know the amount of milk in the gland at the start and the expansion or contraction of that volume at succeeding sampling intervals to estimate total change per unit of time in concentration of the element perturbing the system. Procedures based upon dilution of a known amount of nonabsorbable marker have been developed for this purpose. Substances advocated as markers are nonabsorbable and include polyethylene

* As published in J. Dairy Sci. 56: (1973) p. 935-938.

Subsection: Physiological Studies of Environmental
Pollutants

A brief review of work on transfer of elements into
milk carried on during the years 1970 to 1973.

30. Secretion of Mineral Elements and Contaminants Into Milk

F. W. Lengemann

Introduction

A common technique in ecological studies is to introduce a nutrient or contaminant into a particular stage of a system in order to chart its upward and downward traverse and to determine the harm that can be produced by the presence of the substance if it is foreign to the system or by an excess or insufficiency of a native material. When steps in the chain lead to concentration of the test material or are otherwise deemed to be especially significant, these steps are then isolated and studied separately in order to refine measurements and to learn how these particular steps can be manipulated.

In ecological studies with man as the focal point it has often been observed that milk is an important means of transferring environmental contaminants from vegetation not consumable by man to the human population. In the past much attention has been paid to the transfer of fission products, however, in recent years milk has received attention as a vehicle for the distribution to the human population of pesticides, drugs, corrosion products and other materials classified as environmental pollutants.

Having been identified as an especially important step in the ecological web, milk has been studied to determine the amount of various materials that can transfer, the degree of concentration that mammary secretion might add, and how the transfer through milk might be altered.

Our laboratory has been especially identified with the study of the transfer of radioiodine, radiocesium and radiostrontium into milk. One objective of our laboratory has been to quantitate the amounts of various

materials secreted into the milk of various species, to establish kinetic parameters and to manipulate the system to give an insight into the mammary transfer mechanisms involved. The species used have been goats, cows and guinea pigs. The technique employed for this basic study has been to give a small amount of labelled material to the lactating animal and to follow its appearance in milk, urine, and feces. This procedure is a basic first approach when beginning the study of materials likely to transfer to milk and has continuing usefulness when studying the transfer of substances across the mammary gland when the whole animal is subjected to various kinds of environmental stress.

A second objective is to develop and define kinetic models which can describe the transfer of various substances from blood to milk at the cellular level. It is strangely true that little is known about the transfer rates of materials across mammary epithelium.

The most definitive publications on transfer across the mammary gland, in addition to studies undertaken at this laboratory, are those of Linzell and Peaker (Physiol. 216: 683-734 (1971)) and Rasmussen (Studies on the Mammary Excretion and Absorption of Drugs, Mortensen, Copenhagen, 1966). These works provide some information on the movement across mammary epithelium of Na^+ , K^+ and Cl^- as well as some partially ionized and non-ionized organic molecules. These works are only a beginning in the elucidation of mammary gland transfer mechanisms. Our approach to the study of the transfer of materials across mammary epithelium has been to serially sacrifice lactating guinea pigs after I.V. injection of radioiodine or radiocalcium in order to obtain time sequence curves representing concentrations of these radioelements in blood, milk and secretory tissue. These data are then analyzed using a three compartment model

developed by Wollman and Reed (Am. J. Physiol. 196: 113 (1959)) to estimate transfer and clearance rates.

It is evident in studies on the transfer of radioiodine into milk is that large species differences exist in the degree of concentration of iodine from blood to milk. While this in no way implies that the basic transfer mechanisms are different between species it does suggest that cattle and goats be studied as well as laboratory animals such as the guinea pig. Since cost makes it impossible to use serial sacrifice techniques with large animals we are now in the process of developing an external counting technique to provide the needed time sequence data to be used in the computational analysis.

In the last three years we have progressed towards our stated objectives. A report has been published describing the transfer of iodine into the milk of goats and the effects of various chemicals on this transfer. In another study the action of perchlorate on reducing the transfer of iodide to the milk of goats was quantitated. We are reporting in detail on a comparable study on dairy cows which shows many similarities between cows and goats in response to perchlorate administration; however, some differences do exist between the two species. On a cellular level procedures have been developed to make quantitative studies of calcium transfer across the secretory epithelium of guinea pig mammary gland. Our present efforts include work leading to the development of an external counting procedure that may enable us to determine transfer rates using cows and goats. Under separate headings is presented a summary of work previously reported, a report on work not yet published, and a listing of papers published in this area.

Iodine Transfer to Milk

Within the past three years we have published the results of a study on the transfer of radioiodine into the milk of goats given the radionuclide twice daily for periods up to thirty days. The results of this study showed that a period of longer than 30 days would be required for a steady state to be attained. The rate of increase in milk radioiodine was greatest at the beginning of the experiment with a rather constant rate of increase being seen from day 6 and on. The plasma radioiodine increased sharply for about 10 days and then tended to increase at the same rate as milk iodine. The milk concentration of radioiodine was at least 5 times that of the plasma. The thyroids of these goats were quite active in sequestering iodine and tended to plateau only after 25 days with a thyroid content equivalent to 3.5 times the daily radioiodine intake. The radioiodine in the milk, plasma, thyroid, urine and feces of six of the goats is shown in Table 1. Milk is seen as the most important avenue of loss from the body of the goat being equal to the losses in the urine and feces combined. Four milligrams of supplemental iodide daily did not produce a statistically significant effect on the total amount of radioiodide secreted into the milk; the thyroid uptake, however, was markedly reduced. The concentration of radioiodide in milk appeared to be reduced by iodide administration, however; the high within-group variation prevented the difference from being statistically significant. Of interest is the high milk-plasma ^{131}I ratio of the goat when compared to the value of approximately 1 for the cow. Addition of stable iodide to the diet of the goat increased the M/P ^{131}I ratio significantly. This increase was not due to a change in the proportion of bound iodine in the milk since 80% of the radioiodine

Table 1. Radioiodine in milk, plasma, thyroid, urine, and feces of six goats maintained on the control diet and six goats on the control diet plus 4 mg of iodide (as NaI) per day. The data are the average of values obtained from 15 to 21 days after the first administration of radioiodide.

Iodide variable	Feces	Total daily recovery		Thyroid
		Urine	Milk	
	(%) ^a	(%)	(%)	(%)
No supplemental iodide	18 ± 2 ^b	18 ± 4	30 ± 13	322 ± 59
Iodide daily, 4 mg	19 ± 3	21 ± 3	33 ± 11	112 ± 24

	Milk	Concentration Plasma	M/P
	(%/liter)	(%/liter)	
No supplemental iodide	48 ± 21	8.2 ± 2	5.5 ± 1
Iodide daily, 4 mg	62 ± 13	5.9 ± 1	12.5 ± 3

^a Expressed as a percentage of a daily intake of radioiodine.

^b Mean ± standard error of the mean.

was in the form of iodide whether additional stable iodide was fed or not.

Table 2 shows the effect of various chemical treatments on radio-iodine partitioning. From these data it is evident that perchlorate, high levels of iodide and KBF_4 are effective inhibitors to the transfer of iodine into milk. Thyroxine, thiourea and chlorothyroid administration increased urinary ^{131}I and decreased milk ^{131}I . These data provided needed information for the goat for comparing to the data collected previously for the dairy cow. These data show a similarity between the cow and the goat except for the magnitude of the iodide-concentrating mechanisms. This suggests that the goat might be a suitable substitute for the dosed cow when studying milk secretion of iodine.

In further study of factors affecting secretion of iodine into milk goats were given twice daily doses of ^{125}I for extended periods of time. At stated intervals these goats were given single I.V. doses of sodium perchlorate at 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg of body weight. At least 2 days intervened between doses of perchlorate. In a second part of the study goats were given twice daily oral doses of perchlorate for periods of 7 days. The levels of perchlorate administered in this fashion were 1, 2 and 4 mg/kg body weight.

The parameter used to judge the effectiveness of perchlorate on iodide transfer to milk was the M/P ^{125}I ratio. The data of the primary experiment is shown in Fig. 1. In this figure the milk/plasma ratio of a particular treatment period is expressed as a percentage of the control period milk/plasma ratio plotted on the Y axis on a linear scale; the log of the single I.V. perchlorate dose is plotted on the X axis.

The regression of change in milk/plasma ^{125}I ratio was $Y = 49.5 - 32.0$

Table 2. Effect of various chemical treatments upon the radioiodine content of urine, milk, and blood of goats.

Goat	Period	Urine (%)	Milk (%/liter)	Blood (%/liter)	M/P
KClO_4 -1 g/day					
I-7 + I ^a	Control	12	94	8	11.8
I-8	Control	12	41	5	8.7
I-7 + I	Test	39	55	27	2.0
I-8	Test	35	12	14	0.9
KI-1.2 g/day					
I-13	Control	14	26	4	7.2
I-13	Test	45	8	11	0.7
KBF_4 -0.9 g/day					
I-14	Control	5	29	1	24
I-14	Test	20	6	12	0.5
Thyroxine-5 mg/day					
I-9	Control	27	31	8	4
I-10 + I	Control	10	84	5	18
I-9	Test	41	27	5	6
I-10 + I	Test	15	45	4	11
Thiourea-1 g/day					
I-11	Control	17	29	6	5
I-12 + I	Control	26	77	4	20
I-11	Test	45	18	10	2
I-12 + I	Test	40	59	6	11
Chlorothiazid-2 g/day					
I-3 + I	Control	29	72	7	10
I-4	Control	8	151	19	8
I-3 + I	Test	53	55	7	8
I-4	Test	36	85	22	4

^a These animals received 4 mg of iodide as NaI daily.

Fig. 1. Regression of milk/plasma ^{125}I ratio on administered perchlorate

log X. No difference was seen for intact and thyroidectomized animals which suggests that the action of perchlorate on the mammary gland iodide concentrating mechanism is independent of the thyroid status of the animal. Small doses of perchlorate were effective in reducing the transfer into milk and producing proportionately greater reductions than higher doses.

In previous studies it had been noted that iodide transport inhibitors increased plasma radioiodine. In this experiment the percentage change of plasma bound and nonbound iodine was plotted against the log of the administered single dose of perchlorate. The equation for the concentration of non-bound radioiodine for all goats was $Y = 32.3 + 61.2 \log X$ with no statistically significant differences between intact and thyroidectomized goats. Similar plots for the total radioiodine in plasma produced lines with regression of $Y = 20.3 + 28.1 \log X$ and $Y = 29.8 + 64.5 \log X$ for intact and thyroidectomized goats, respectively. These regressions show that for thyroidectomized goats the total iodine of plasma and the nonbound iodine are affected similarly by perchlorate administration. For intact goats the nonbound total iodine and the total iodine fractions diverged as the amount of administered perchlorate increased. The divergence of these curves was due to the presence of a bound iodine fraction of significant size in the plasma of the intact goats while there was little in the plasma of thyroidectomized goats. The bound iodine tended to decrease with increased perchlorate levels at the same time nonbound iodine fraction was increasing.

When 1 mg/kg of perchlorate was given twice daily to lactating goats, for 7 days the $M/P^{125}I$ ratio was about 28% of the same ratio during the control period. At 2 mg/kg perchlorate per day the M/P ratio decreased to 18% of the control and at a subsequent doubling of perchlorate intake to 12.9% of the control value. Comparison shows that 1 mg/kg of perchlorate twice daily was equivalent to the effect of a single I.V. dose of 4 mg/kg

of perchlorate. This greater efficiency of twice daily dosing when compared to the single perchlorate dose experiments may be due to the rapid metabolism of perchlorate. If this is the case, the maximal effect of perchlorate may easily be missed when a single dose is administered, particularly if milk sampling is carried out at the regular milking intervals. The metabolism of perchlorate, while rapid, might be slow enough so that low doses can build accumulations in the blood and other tissues that is equivalent to larger single doses of perchlorate.

31. Reduction of Iodine Transfer to Milk of Cows After Perchlorate Ingestion

F. W. Lengemann

These experiments quantitate the relation between the amount of perchlorate ingested and the radioiodine transferred to milk of the cow when the radioiodine is fed daily or after radioiodine administration ceases.

When radioiodine was given daily, graded doses of perchlorate, from 10 to 4000 mg/day, increased plasma iodine in accord with $Y = 88.1 X^{0.052}$. ($Y = \% \text{ of control value}$ and X is the daily dose of perchlorate in mg). Curves for ^{125}I in milk and the ratio of milk to plasma ^{125}I ($M/P \text{ } ^{125}\text{I}$) between the limits of 50 and 1000 mg ClO_4^- daily were fitted by $Y = 794.8 X^{-0.525}$ and $Y = 1046.5 X^{-0.601}$, respectively; these same curves between 10 and 50 mg ClO_4^- daily were fitted by $Y = 85.6 + 0.37 X$ and $Y = 82.3 + 0.37 X$. Between 1000 and 4000 mg ClO_4^- these curves had a slope of 0. These data indicate that perchlorate can inhibit the iodide-transfer mechanism of the mammary gland of the cow and that a $M/P \text{ I}$ ratio of 0.2 indicates complete blockage of this transfer.

Administration of 1 g of ClO_4^- daily leads to 55% less radioiodine being transferred to milk after administration of ^{125}I has ceased.

Anions that inhibit the uptake of iodide by the thyroid can also reduce the transfer of iodine into milk. Among the anions effective at the mammary gland are I^- , SCN^- , ClO_4^- , BF_4^- , IO_3^- , NO_3^- , BrO_3^- and Cl^- (1, 2, 3, 4, 5, 6, 7, 8, 9, 10). The relative effectiveness of these inhibitors is about the same for thyroid and mammary gland and suggests a similarity of iodide concentrating mechanisms (2, 11). Perchlorate is one of the most potent inhibitors and for this reason was chosen for this study.

The study of substances that can reduce transfer of iodine into milk can provide basic information about the transfer mechanisms and can lead to the development of procedures useful when pasture or other feedstuffs become contaminated with radioiodine. In most studies a single large dose of the inhibitor substance is given and so little information is available about the minimum and maximum amounts that can reduce iodide transfer to milk. One of the experiments reported here used a series of graded daily doses of NaClO_4 to determine the amounts needed to effect a reduction in radioiodine transfer into milk of cows.

In the second experiment cows that had attained a steady state of radioiodine in the milk from daily oral administration of ^{125}I were studied to determine the effectiveness of perchlorate in accelerating the decline of milk radioiodine after ingestion of radioiodine stopped.

Methods

The eight cows in this study were purebred Ayrshire, Holstein, Jersey, or Brown Swiss cows, nonpregnant, past midlactation, and free of all obvious diseases. The cows ranged in weight from 390 to 640 kg and produced from 7 to 22 kg of milk daily. Cows 161 and 163 were in the first and second experiments while the other six cows (157, 158, 162, 164, 165, and 167) were only in the second experiment.

For the first experiment cows 161 and 163 were kept in metabolism stalls for several days before and throughout the experiment. The animals were given water and alfalfa hay ad lib and 6.8 kg of a 16% crude protein commercial grain mixture daily. Radioiodine, as $\text{Na } ^{125}\text{I}$, was given twice daily in gelatin capsules for the entire experimental period; all capsules were made at the start of the experiment and contained 80 μCi of ^{125}I each.

Milk samples were collected each morning for the first 10 days and analyzed for ^{125}I in order to verify the establishment of steady state. Actual sampling began at 0800 on the 11th day. For five days, milk (twice a day) and blood samples were collected to represent the control state. On the evening of the 15th day the cows received the first of the twice-daily capsules containing sodium perchlorate. These capsules were given for 5 days. Sampling was as for the control period. After this perchlorate test period the animals were returned to a no perchlorate supplementation status. The perchlorate-free periods served as the control period for the subsequent perchlorate period. The levels of perchlorate were 10, 50, 100, 300, 500, 1000, 2000, and 4000 mg per cow per day. Data of only the last 3 days of each 5 day period were averaged; any changes in milk and plasma as a result of perchlorate supplementation were judged to be complete by this time. Milk and plasma ^{125}I of a particular perchlorate feeding period were calculated as a percentage of the respective control period.

For the second experiment cows were given ^{125}I twice daily from 20 to 60 days. After ^{125}I administration stopped, milk collections continued from 11 to 15 days. Four cows (157, 158, 161, and 163) were given twice daily doses of 0.5 g ClO_4^- (as sodium perchlorate) during the phase of declining milk ^{125}I . The milk ^{125}I concentrations were expressed as the percent of the steady state radioiodine in milk. Data for cows receiving perchlorate were compared with those of cows not so supplemented.

Results

The data of the first experiment are in Fig. 2. This is a log-log plot of percent change in ^{125}I concentration in plasma and milk vs. the daily intake of perchlorate. Previous experiments have shown that

Fig. 2. Log-log plot of the regression of the percent change in ^{125}I concentration vs. the daily intake of perchlorate for plasma and milk ^{125}I , and the M/P ^{125}I ratio. For plasma the data can be expressed by the equation $Y = 88.1 X^{0.052}$. The portions of the milk ^{125}I and the M/P ^{125}I curves between 50 and 1000 mg ClO_4^- can be expressed as $Y = 794.8 X^{-0.525}$ and $Y = 1046.5 X^{-0.601}$, respectively. The portions of these curves between 10 and 50 mg ClO_4^- can be expressed as $Y = 85.6 + 0.37 X$ and $Y = 82.3 + 0.28 X$ for milk and M/P ^{125}I . The portions of the curves from 1000 to 4000 mg have no slopes as determined by a simple linear regression program. The areas, bracketing some of the curves represent one standard deviation.

inhibitors of iodide transfer increase plasma iodide at the same time that of milk iodide decreases (3, 5, 6). In agreement, plasma iodine of the cows in this study increased steadily as perchlorate intake increased. The equation for plasma iodine is $Y = 88.1 X^{0.052}$ where Y is the percent of the control and X is the daily dose of perchlorate in mg. Plasma iodine consists of both bound and non-bound (mostly iodide) iodine. The major action of perchlorate on plasma is to increase the nonbound iodine rather than the bound fraction (3,5). No attempt was made in this study to distinguish between bound and nonbound iodine. It is reasonable to expect that bound-iodine would increase with time as more and more iodide is organified by the thyroid and released into blood. This increase of bound-iodine has been minimized by expressing the data of a perchlorate test period as the percent of the preceding control period, and so the rise in Fig. 2 mostly represents an increase in nonbound iodine. Total plasma iodine underestimates the change that is occurring in plasma nonbound iodine (3).

It was not possible to express the changes of milk ^{125}I as influenced by perchlorate as a single curve. The curve is triphasic with little or no response of milk ^{125}I to perchlorate doses below 50 mg and no further response to amounts above 1000 mg per day. Daily doses of perchlorate ranging from 50 to 1000 mg reduced ^{125}I in milk; the data required a log-log plot to be expressed as a straight line. The equation for the function is $Y = 794.8 X^{-0.525}$.

As noted, increasing dose size of perchlorate resulted in an increase of plasma nonbound radioiodine, most of which was probably in the form of inorganic iodine (3). Increases in plasma iodide can lead to more radioiodine moving into milk by diffusion. As a result the decrease in milk

radioiodine from perchlorate dosing could understate the effectiveness of perchlorate on the concentration mechanism. To correct for this the ratio of milk to plasma ^{125}I (M/P ^{125}I ratio) is also plotted in Fig. 2. This second plot is similar to the plot of the decrease in ^{125}I concentration of milk. The efficiency of the M/P ratio is shown by the closer clustering of the data points around the line $Y = 1046.5 X^{-0.601}$ when contrasted to the milk ^{125}I concentration data; moreover, the slope of the line for M/P ^{125}I is steeper because the effect of increase in plasma iodine with time has been eliminated.

The actual values for M/P ^{125}I ranged from 1.65 for the control portion of the 10 mg ClO_4^- test period to 0.20 for the test period when the cows were given $4000 \text{ mg of ClO}_4^-$ per day. For ^{125}I in milk the actual values for the respective test periods were 1.30 and 0.26% of the daily dose per liter of milk and for plasma 0.80 and 1.30% of the daily dose per liter.

The data for the second experiment, the effect of perchlorate on the rate of falloff of ^{125}I in milk after long term dosing has ceased, are in Fig. 3. The upper curve presents the data of six cows not given perchlorate. The lower curve is the data of four cows that began to receive perchlorate at the time radioiodine administration ceased. Perchlorate administration accelerated falloff of milk iodine. Over the 15 day period only 55% as much radioiodine appeared in the milk when perchlorate was given to the cows as when the animals were not so supplemented; by day 15 both groups had achieved the same radioiodine in milk. Since the cows had been receiving radioiodine for a long time, this effect of perchlorate is the maximum that can be expected.

Fig. 3. The decline of ^{125}I in the milk of cows after cessation of radioiodine intake. The lower curve is for cows receiving 1 g of perchlorate daily (2 x 0.5 g) while the upper curve is for cows not receiving perchlorate.

Discussion

The use of twice daily dosing for extended periods is an attempt to simulate the long term radionuclide ingestion pattern after contamination of pastures by fallout. With long term ingestion of radionuclides the level of these in milk rises for several days and then declines with a half-time dependent upon the half-life of the radioactive nuclide and the residence half-time of the nuclide on pasture grasses. The first experiment shows that perchlorate supplementation reduces the amount of radioiodine transferred to milk by up to 80% even though the amount of radionuclide ingested has not changed. When ingestion of the radionuclide ceases and the only source of the radioiodine of milk is that stored in the tissues of the cow, perchlorate can accelerate the rate of falloff of radioiodine in milk. The success of perchlorate administration suggests its use as a countermeasure in the event of pasture contamination by radioiodine. Before such an action can be recommended, however, the transfer of perchlorate to the milk and its effect upon the health of the human population consuming the milk must be considered. Little is known about the transfer of perchlorate into milk of cows and goats.

A study of the effect of perchlorate upon the transfer of radioiodide into goats' milk has been reported from this laboratory (3), and so it is possible to compare the cow and goat. For both species perchlorate exerts its effect within 12 hours. The site of action of perchlorate is the mammary gland as judged by the fact that the reduction in the M/P radioiodine ratio is able to account for all of the changes in milk radioiodine. If the weight of the average cow is 500 kg, the 1000 mg of ClO_4^- daily is equivalent to 2 mg/kg/day; this amount of perchlorate reduced the M/P ^{125}I ratio to 18% of the control value for the cows and to 28% for the

goats. Four or 8 mg ClO_4^- /kg/day had no additional effect in the cow whereas in the goat the M/P ^{125}I ratio decreased to 18% and 13% of the control value (3).

The different milk iodine levels achieved would suggest a greater sensitivity of the cow than the goat to perchlorate. If the comparison is based upon the dose of perchlorate per kg of metabolic body weight ($\text{mg/kg } W^{3/4}$), however, the cow and goat show equal responses. Using metabolic body weight, an intake by the cow of 2 mg of perchlorate/kg/actual body weight/day is equivalent to 4 mg/kg/day for the 50 kg goat. At these levels the iodine transferred to milk by both species was equal to 18% of the control value. A difference between the cow and goat is evident in that the iodine in the milk of the goat could be reduced still further by increasing these perchlorate levels whereas this was not true for the cow (3).

The effect of perchlorate supplementation is to cause increases in plasma radioiodine in both cow and goat. This is probably due to an increase in iodide as in the goat study (3). Increased plasma iodine is probably the result of major reductions in the clearance of radioiodide by thyroid and mammary gland without compensatory increases in excretion of radioiodine by the kidney and intestinal tract. This present paper demonstrated that plasma radioiodine increased before milk radioiodine starts decreasing which suggests that the iodide concentrating mechanism of the thyroid of the cow is more sensitive to perchlorate than is that of the mammary gland. The plasma ^{125}I curve suggests that for perchlorate levels less than 10 mg/day, plasma radioiodine might be depressed; this would be in accord with observations on the goat (3).

From curves for ^{125}I in milk and M/P ^{125}I (Fig. 2) it is obvious that at least two different mechanisms are operative in the transfer of iodine into milk. The amount of iodine transferred to milk is an inverse function of the amount of perchlorate when the daily intake of perchlorate ranges from 50 to 1000 mg. When perchlorate reaches 1000 mg per day, there are no further reductions. Since perchlorate is a recognized inhibitor of the thyroid iodide concentrating mechanism, the descending portion of the milk iodine curve can be attributed to inhibition of a concentrating mechanism in the mammary gland. The flat portion of the curve, from 1000 to 4000 mg daily, could be interpreted as that portion of milk iodine that enters via diffusion. This suggests that in studies of the transfer of iodine into milk, a M/P ratio greater than 0.2 rather than 1 should be regarded as indicating activity of an iodide concentrating mechanism.

The first portion of the curve in Fig. 2 is flat between 10 and 50 mg ClO_4^- /day. This suggests that perchlorate was not interfering with iodide transfer. There is no obvious explanation for this except for the possibility that while perchlorate can successfully compete with iodide for the transfer mechanism, there may be sufficient sites of transfer available so that the amount of iodide moving into milk is not curtailed. It is useful to note that the levels of perchlorate necessary to reduce iodine transfer into milk are from three or more times greater than the amount of iodine commonly supplied in the diet of the cow. There is a lack of knowledge of the absorption of perchlorate from the intestine and its proportionate transfer to milk. More will be known about the iodide transfer mechanism when the metabolism of perchlorate is determined.

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32. Transfer of Mercury through Milk.

F. W. Lengemann

Mercury is among the environmental pollutants considered to be significant hazards to man and other species. While the levels of mercury in some part of the food web have been determined, little is known of its transit in the food web or the processes that can result in high concentrations being available to man through his food. Because of the importance of milk in the human diet, we set out to study how lactating animals metabolize mercury, not by administration of gram quantities of mercury as in the earliest experiments but at environmental levels.

Three mixed-breed lactating goats, about 60 kg in weight, were maintained in collection stalls throughout the experiment and were milked, fed and watered twice daily. One goat was dosed orally with $^{203}\text{HgCl}_2$ and studied for only 9 days; the other two goats were given an oral dose and collections were made for 16 days, and they were then dosed intravenously and collections made for 18 days. The data are shown in Fig. 4.

The recovery of the radiomercury in the feces of the goats after oral dosing averaged 95% and implies very poor absorption of mercuric ion and is consistent with evidence for other species. The total recovery in urine ranged from 0.4 to 4.5% while only 0.006 to 0.039% was in the milk.

After an I.V. dose of $^{203}\text{HgCl}_2$ about 25% was excreted in the urine and in the feces for a total of about 50% of the administered dose. The total recovery from milk was about 0.9% which is about 2.5 times higher

than expected from oral dose data of these goats. At 9 days after I.V. dosing there was about a 100-fold difference between blood and milk levels as compared to a 10-fold difference when the HgCl_2 was given orally.

Ten minutes after an I.V. dose the red blood cells contained no detectable ^{203}Hg but at 24 hours and on, they contained more radiomercury than an equal volume of plasma. The loss of mercury from the blood cells could be described by a simple exponential function with a $t_{1/2}$ of 5.5 days. Loss from the plasma was multi-exponential with a $t_{1/2}$ of 0.5 days for the first component and 7.2 days for the second. After oral dosing the red blood cells contained more radiomercury than the plasma at all sampling periods up to 7 days; after that the plasma ^{203}Hg exceeded that of the red blood cells. This crossover was apparently due to a greater rate of loss from the cells.

Analysis of hemolyzed red blood cells showed that ^{203}Hg was associated with the cellular contents and not with membranes.

The differing mercury distribution in blood and loss of mercury after oral and I.V. doses suggest that different forms of mercury may be being compared. The bacterial flora of the rumen, intestine, and caecum could transform a portion of the inorganic mercury given orally into an organically bound form of mercury. This could produce a pattern in blood that would be unlike that when the HgCl_2 is given I.V. and result in a greater proportion of the absorbed mercury being transferred to milk. The formation of methylmercury from dietary inorganic mercury has been observed in some animals and membranes have been noted to block the transfer of inorganic mercury while allowing some forms of organic mercury to pass.

The present data indicate that inorganic mercury is poorly absorbed from the digestive tract and only a small fraction of that absorbed is transferred to milk. In terms of the food web the lactating animal acts to reduce the transfer of environmental mercury to man.

Figure 4. The patterns of distribution of radiomercury, given as HgCl_2 , in milk, urine, feces and blood after oral and intravenous dosing.

33. Kinetic Studies on the Transfer of Elements Across the Mammary Gland Epithelium.

F. W. Lengemann

1. Guinea Pig Studies

The previously described researches have been carried out using the classical approach of feeding or injecting a radioelement and then estimating the amount being returned in the milk. This technique has been rewarding in providing data of use in appreciation to practical situations, however, to understand how substances are secreted into milk, it is necessary to use more refined techniques. A basic first step then is to determine the rate at which an element or compound is transferred from blood across mammary epithelium into milk and the return. When coupled with information about the form of the compound being transferred, the transmembrane electropotential, and the effect of binding to proteins and other substances, it may be possible to decide the kind of transfer mechanism that is operative and how it can be manipulated.

The mammary gland can be considered to consist of three compartments, the blood plasma, the secretory tissue together with its connective tissue components, and milk. The basic model is shown in Figure 5. Note that the mammary gland tissue compartment has been subdivided into the secretory epithelium (E), those cells which are directly involved in milk formation, and the supportive tissue (S) which includes connective tissue, erythrocytes and all other tissues not included in other categories. The kinetic model chosen was the open three compartment model developed by Wallman and Reed (Am. J. Physiol. 196: 113-120 (1959); Am. J. Physiol. 202: 182-188 (1962)) for the thyroid gland. The assumptions are a) that the

Figure 5. The basic model for the mammary gland; B = blood plasma,
 E - secretory epithelium, S = supportive tissue and M - milk.

element being studied can move in both directions between plasma and epithelial cells and between epithelial cells and milk and b) there is no direct movement between plasma and milk. The pertinent equations for the movement of calcium in this three compartment model are:

$$d Ca_M/dt = K_{EM} (Ca_E E)/M - K_{ME} Ca_M \quad (1)$$

and

$$d Ca_E/dt = C(Ca_B/E) + K_{ME} M Ca_M /E - K_{EB} Ca_E - K_{EM} Ca_E \quad (2)$$

where

$d Ca_M/dt$ and $d Ca_E/dt$ = net radioactive calcium movement in milk and epithelial cells, respectively

C = mg of plasma cleared of calcium by epithelial cells/min

$V_G + V_M$ = mg of epithelial cells and milk, respectively

Ca_B , Ca_M , and Ca_E = percent radioactive calcium dose per mg for plasma, milk and secretory epithelium respectively

K_{ME} , K_{EB} , K_{EM} = rate constants for calcium transfer from milk to epithelium, epithelium to plasma, and epithelium to milk in fraction per minute.

E , M and B = secretory epithelium, milk, and blood plasma (mg).

Solution of the equation is a two-step process whereby the exchange of Ca between epithelial cells and milk only is considered (a closed, 2-compartment model). The experimental values for Ca_M and Ca_E are plotted on linear paper and the curves integrated between various time intervals using a planimeter. Next values for $(Ca_{MB} - Ca_{Ma}) / S Ca_E dt$ vs $S Ca_M dt / S Ca_E dt$ are plotted on linear paper. The slope of this line is K_{ME} , the Y intercept is $K_{EM} (E/M)$ and the X intercept is $K_{EM} (E/M) / K_{ME}$.

The second step included solving equation (1) for $K_{EM} C_{aE}$ and substituting this into equation (2). This equation was then integrated as above and values for C/E and K_{EB} were determined using graphical procedures as above.

From this brief description it is clear that the samples needed over time are estimates of the radionuclide concentration in plasma, milk and secretory epithelium. Blood sampling is easily carried out and so this data is readily available. It is unnecessary to determine the volume of the blood plasma compartment since the movement from blood into cells is a clearance value estimate.

While milk is more readily sampled than the blood, it is necessary to measure the volume of milk within the mammary gland since the constants sought are transfer coefficients. Measurement of contained milk volume is an old problem in dairy science that had not been satisfactorily answered. While techniques were developed that produced reasonable answers the procedures were time consuming or difficult. For example, one of the more recent, more successful, techniques used the dilution of polyethylene glycol-4000 (PEG-4000) by the contained milk of the mammary gland (Knutsson, P. G. and I. Sperber, Kungl. Lantbrukshogsk Ann. 27: 33-40 (1961). While this was successful, the turbidometric assay required extreme precision in timing. For this reason, we embarked on an effort to increase the dependability of the milk volume estimates by using either ^{14}C - PEG - 4000 or ^{141}Ce as the unabsorbable marker in lactating guinea pigs.

Figure 6 presents the label distribution results for five glands in which both ^{141}Ce and ^{14}C -PEG were used. Only 45 to 70% of the total contained-milk (initial estimate) was recoverable by machine milking, even with the use of oxytocin. Data for percent of dose per milligram of

Figure 6. ^{14}C polyethylene glycol -4000 and ^{141}Ce concentrations in serial samples of milk from the glands of 5 guinea pigs.

milk were similar for ^{14}C -PEG and ^{141}Ce with the exception that values for ^{141}Ce were always larger than the corresponding values for ^{14}C -PEG. Also, the concentrations of ^{14}C -PEG and ^{141}Ce were relatively constant for about the first 40% of the estimated total milk volume and then tended to decrease, indicating non-uniform penetration of the marker substances into remote compartments of the glands. Results from a second experiment indicated that part of the ^{14}C -PEG dose was being absorbed. The range for recovered percent dose of ^{14}C -PEG among six mammary glands was 79.8 to 95.5% with a mean and standard deviation of $86.6 \pm 7.0\%$; recovery of ^{141}Ce was $98.0 \pm 1.6\%$ S.D. This more than accounted for the 6.9% difference between the volume estimates for ^{14}C -PEG and ^{141}Ce since absorption of a portion of a label yields a larger estimate for the volume of distribution.

If the milk volume indicator is uniformly distributed, a plot of percent of accumulated dose of the indicator as a function of the percent of the "initial" estimate for total milk should be a straight line with a slope equal to one. This line is drawn in Figure 7 and is labeled "initial" slope equal to 1.0.

When using isotope dilution principles, non-uniform penetration of a leveled dilution indicator into the remote spaces of the gland will always result in a low estimate for total milk if the estimate is based upon the initial milk sample. If the contained-milk estimate is low, then the actual volumes of milk collected in a serial sampling trial will be too high when expressed as a percentage of the estimated total volume. Under such conditions, when the percent of the estimated volume collected is plotted against the percent of accumulated dose of indicator, the plot will have a slope greater than one. The ^{141}Ce data plotted in this manner (Figure 7), show a slope greater than one, thus verifying that the initial estimate of the contained-milk volume was low.

Figure 7. The correlation between percent of ^{141}Ce dose and percent of "initial" and "revised" estimates for the total milk content of five guinea pig mammary glands.

A method to correct for the underestimate of contained-milk volume can be developed if the difference between the actual slope and the "slope equals one" is considered to represent the error of the total milk estimate. The appropriate equations for determining the percent error of the total milk estimate are:

$(\% \text{ of initial volume}) / (\% \text{ of accumulated dose}) - \text{error of initial estimate} = 1.0$, which can be rearranged to:

$$\frac{(\% \text{ of initial volume}) - (\% \text{ of accumulated dose})}{\% \text{ accumulated dose}} \times 100 = \% \text{ error}$$

of the initial estimate.

The data from Figure 7 were used to calculate the percent error data for ^{141}Ce over the range of 20 to 60% recovery of the ^{141}Ce indicator. Since the error estimates could be described well by straight lines the total error of the milk volume estimate was obtained by extrapolating these lines to 100 percent of the accumulated ^{141}Ce dose. The mean $(12.8 \pm 3.8\% \text{ S.D.})$ of these maximum error intercept values was then used to revise the initial estimate for the contained-milk. As indicated in Figure 7 the axis for percent revised total milk estimate has a range of 0 to 100% that is 12.8% longer than the axis for percent initial estimate. A new slope of one has been plotted for the revised estimate.

Data obtained for an additional five mammary glands using only ^{141}Ce had an average error of underestimate for the milk volume of $0.6 \pm 2.6\% \text{ S.D.}$ There was no statistically significant difference between the mean error of underestimate of 12.8% for the first five glands and the 9.6% for the second five glands. The percent error of the total milk estimate for all 10 glands was plotted as a function of the corresponding initial estimate for total milk (Figure 8). Linear regression analysis yielded the line equation: $Y = 1.5 + 0.00195 X$ where X = the initial estimate for total

Figure 8. Percent error as a function of the initial estimate for total milk using ^{141}Ce as the volume indicator.

milk, and Y = the percent error for the total milk estimate. The error of underestimate tends to increase as a function of milk yield.

The results of these studies indicated that estimation of the size of the milk volume compartment of the guinea pig is not a simple isotope dilution procedure. Cerium-141 can successfully be employed as a contained-milk volume indicator. For this purpose a small sample of milk is used to determine the initial contained-milk volume using the equation: $(^{141}\text{Ce DPM infused}) / (^{141}\text{Ce DPM/mg of milk}) = \text{initial estimate (mg) of total milk}$.

This estimate can then be revised using the formula: $(\text{initial estimate}) (1 + \text{error of underestimate}) = \text{revised estimate for total milk}$.

Knowing the amount of milk in the lactating gland permits the determination of the amount of radionuclide in the tissues of the gland. For this the intact gland is excised, weighed and macerated and a count made on an aliquot. Knowing the amount of milk in the total milk radionuclide it can be subtracted from the total nuclide of the intact gland. Subtracting the weight of milk from the weight of the gland provides the weight of the tissue alone.

The tissue concentration determined in this manner is misleading since all of the tissue is not secretory tissue. To put the data in relation to secretory tissue we employed the Delessee principle. According to this principle, the average fractional area of a random tissue section of negligible thickness occupied by a morphological component of tissue approximates the fractional volume occupied by that component in the intact tissue. Therefore, it is possible to sample and stain a tissue for histological examination, divide the tissue area into fractions that can be readily identified and make an estimate of the fractional composition of the tissue.

Partially-milked mammary glands were randomly sampled to yield 10 tissue samples per gland. The tissue samples were fixed in phosphate-buffered 4% glutaraldehyde solution (pH 7.15) for 24 hours, then rinsed and stored temporarily in additional phosphate buffer. The samples were trimmed to 2 x 4 x 5 mm, embedded in glycol methacrylate and sectioned at 2 microns; the sections were mounted on 1 x 3 inch glass slides, stained with acid fuchsin toluidine blue and cover-slipped for microscopic examination.

A random series of 10 black-and-white 35 mm transparencies was made for each slide at 100x magnification for a total of 100 transparencies per mammary gland. Each transparency was projected onto a 1.5 cm grid containing a total of 88 points of intersection. The tissue component projected at each point of intersection was classified as milk (M), secretory tissue (E), or supportive tissue plus blood plasma (S'). These data provided estimate of f_E and f_S , (fraction of secretory epithelial tissue and supportive tissue).

The fraction of blood (f_B) in the mammary glands was estimated by use of radioiodinated serum albumin (^{125}I). The Risa was injected into the common carotid artery of anesthetized guinea pigs and 15 minutes later a blood sample was obtained and the animals immersed in liquid nitrogen. The mammary glands were dissected and analyzed for ^{125}I . Other necessary data were obtained at this same time and the equation:

$$f_B = \frac{(^{125}\text{I DPM per gland}) - \text{mg of milk } (^{125}\text{I DPM per mg of milk})}{^{125}\text{I DPM per mg of blood plasma}}$$

The data for the initial stereological examination of 14 mammary gland tissues gave the fraction of milk in the glands as 0.388 with a standard deviation of 0.050. Since the quantity of milk in a gland depends upon many factors it was necessary to express the fractions of secretory

and supportive tissue on the basis of a milk-free gland. To do this, an assumption was made that each of the various divisions of mammary components had the same specific gravity. Once the fraction of milk (f_M) in a gland was known it was possible to correct the gland to a milk free basis. In this way the sum $f_E + f_S$, became equal to 1. For the first determination f_E was $0.557 \pm$ a standard deviation of 0.023 and f_S was 0.443 ± 0.023 .

An analysis of variance for the mammary glands indicated that the greatest variation was among animals and between glands. Within gland variation was quite small so it was concluded that sample size could be much reduced in subsequent work.

A total of 16 mammary glands were used for the determination of f_B . The range for total milk in the glands was 2681 to 8379 milligrams; eight glands were partially milked and eight glands were essentially filled (non-milked) at the time of experimental sampling. Using the equations presented above, it was calculated that 0.045 ± 0.01 was a reasonable estimate of the mean and standard deviation of the fractional blood volume of the 16 lactating glands. There was no statistical difference in blood content attributable to the amount of contained-milk in the glands at the time the experiment was performed.

There was no correlation between contained milk content of the mammary gland and fractional distribution of the tissue components. This result indicates that the experimentally quantitated values for f_E , f_S and f_B can serve as useful approximations under any degree of distension of the milk spaces.

Having accepted a kinetic model and developed procedures for the needed measurements it was possible to study the transfer of calcium across the secretory epithelium into milk and blood. Two physiological conditions

of the mammary gland were included in the experiment. One gland was milked 90 minutes prior to dosing the animal with ^{45}Ca ; this was followed by an infusion of 0.1 ml of a solution containing ^{141}Ce volume indicator label.

To provide evidence for or against a high specific activity fraction of ^{45}Ca in plasma, total calcium and ^{45}Ca were measured in blood plasma and blood plasma ultrafiltrate.

Figure 9 presents the results of measurements for total and ultrafiltrable calcium (mg%) in blood plasma and blood plasma ultrafiltrate; it also includes the data for specific activity for blood plasma ultrafiltrate for each time period of the kinetic experiment. Stable calcium levels in blood plasma and blood plasma ultrafiltrate remained essentially constant among all animals; the values for mean and standard deviation for total and ultrafiltrable calcium in blood plasma were 9.3 ± 0.6 mg% and 7.3 ± 0.6 mg%, respectively. The percent ultrafiltrable calcium was $78 \pm \text{S.D.}$

Stable calcium measurements in blood plasma by atomic absorption spectrometry were probably low due to the interference caused by large amounts of plasma proteins, the samples were diluted only 20X and contained approximately 300 mg of protein per 100 ml of solution. Examination of Figure 8 presented by Zettner and Seligson (Clin. Chem. 10: 869-890 (1964)) supports the idea that protein content of the blood plasma samples could have reduced values for total calcium. If the measured values are increased by 15% there is agreement with the literature values of 10.7 mg%. The proportion for ultrafiltrable calcium in plasma is now 68%.

Figure 9. Milligrams percent calcium in blood plasma and blood plasma ultrafiltrate, percent ultrafiltrable calcium for blood plasma and specific activity for ^{45}Ca in blood plasma and blood plasma ultrafiltrate.

An increase of 15% for total calcium in blood plasma also accounts for the observed differences in specific activities seen in Figure 9.

The mean and standard deviation values for total, ultrafiltrable and percent ultrafiltrable calcium in milk were 163 ± 12 mg%, 21 ± 4 mg% and $19 \pm 3\%$, respectively. The value for total calcium in milk agrees with reported values for total calcium in milk for the cow and goat. The value of $31 \pm 4\%$ SD for calcium in guinea pig milk ultrafiltrate agrees well with the value of 34.7 mg% reported for the cow.

Linear regression analysis showed that milk production was $9.49 \times 10^{-4}\%$ body weight per minute and $8.99 \times 10^{-4}\%$ body weight per minute for non-milked and pre-milked mammary glands, respectively. The mean animal weight was 790 grams; therefore, the milk production rate was 7.5 mg/minute for the non-milked and 7.1 mg/minute for the milked gland, respectively. This represents the production of 1168 mg of milk per mammary gland during 160 minutes, assuming 7.3 mg of milk is produced by each gland per minute. This milk production rate is plausible and will be assumed a reasonable estimate. For the non-milked gland, this production rate yielded a 21% increase in the average milk content of each gland after 160 minutes relative to the milk content at time zero. Analogous results for the milked glands indicated a 34% increase in milk content over the same time period. These changes were considered in the calculations of the kinetic parameters.

The kinetic parameters for calcium transfer between the three mammary gland compartments was estimated using the techniques described earlier. These data are shown in Table 3. Overall, the kinetic parameters were comparable for both the milked and non-milked physiological conditions of the mammary gland. The value for the kinetic parameter C indicated that an amount of calcium equivalent to that present in 875 mg of blood

Table 3. Summary of kinetic parameters for calcium movement
in the guinea pig mammary gland.

<u>Kinetic Parameter</u>	<u>Condition of Mammary Gland</u>	
	<u>Not Milked</u>	<u>Milked</u>
C/E	0.190	0.220
C ^a	840 mg	911 mg
K _{EB}	0	0
K _{ME}	0.116	0.100
K _{EM} (time 0) (160 min.)	0.446	0.277
	0.538	0.371
K _{EM} /gram of milk	0.0774	0.0825

^a The mean values for E of 4420 mg and 4142 mg were used for the not-milked and milked glands, respectively.

plasma moves in the direction from blood plasma to mammary secretory epithelium per minute.

Values for K_{ME} were 0.116 and 0.100 for the non-milked and milked glands, respectively. These values indicated a flux per minute of calcium from milk to mammary epithelial tissue equivalent to 10 to 12% of the total calcium present in milk. As the milk volume increased, the value for K_{ME} remained constant. This implied an increase in the total milligrams of calcium moving from milk to mammary epithelium per minute. The K_{EM} values were proportionate to the amount of total milk, suggesting that K_{EM} was variable. If the total calcium in mammary epithelial tissue remained constant and the calcium flux increased from milk into secretory tissue, K_{EM} had to increase; otherwise, the two compartments could not have maintained their calcium balance. When calculated as a volume-dependent kinetic parameter, K_{EM} has a value of 0.0774 per gram of milk and 0.085 per gram of milk for the non-milked gland, respectively. These values are similar and indicate that K_{EM} is constant for a constant volume. However, K_{EM} depends on the total amount of milk; as milk volume increases, K_{EM} increases. The value for K_{EB} (the fraction of total calcium in mammary secretory epithelial tissue transferred to blood plasma per minute) had an apparent value of zero.

A value for K_{EB} near zero would imply the movement from mammary epithelial cells to blood plasma of a small fraction of a large storage "pool" of calcium in epithelial tissue. The small fraction of the total calcium "pool" would be equivalent to a flux of 0.08 mg of calcium per minute so the pool would be quite large. Data for Ca_E are inconsistent with the existence of a "pool" of calcium in mammary epithelial tissue of the guinea pig. Consideration of factors involved in quantitating this parameter yield the conclusion that K_{EB} is variable and can essentially

equal any value sufficient to maintain a balance of calcium in mammary epithelial cells; the value of K_{EB} apparently depends on how much of the calcium which moves from blood plasma to secretory epithelium can be used in the production of milk. Calcium utilization in the production of new milk can account for only 10% of the calcium flux from blood plasma to mammary epithelial tissue and loss of calcium in lymph can account for only 0.2%; the unused 90% must return to blood plasma. This conclusion is based on the probability that $Ca_{E-} < Ca_B$ and the ratio of Ca_E/Ca_B equals some constant value less than or equal to 1.0 for all time periods after dosing the animal with ^{45}Ca .

The results are consistent with the hypothesis that the movement of calcium between blood plasma and mammary secretory epithelial tissue is relatively unhindered in the guinea pig mammary gland; a high rate of calcium flux occurs between these two compartments. Ten times as much calcium moves from blood plasma per minute as is needed for use in the formation of new milk; an amount of calcium equal to the unused 90% returns to blood plasma. Factors which determine the accumulation of ^{45}Ca by milk are the total calcium content of milk and the ability of milk protein to adsorb calcium or "trap" calcium by other mechanisms (these may be the same mechanisms responsible for the accumulation of stable calcium in milk). The 10- to 15- fold difference in mg% calcium between milk and blood dictates that at equilibrium the Ca_M/Ca_B value will be 10 to 15 (specific activities) for ^{45}Ca in blood plasma and milk should be equal, however). Finally, the rate of calcium flux among blood plasma, mammary epithelial tissue, and milk depends on the relative amounts of ionizing and/or filterable calcium in the three compartments and on the ease with which calcium can pass through the pertinent biological barriers. The chemical composition of blood plasma, mammary secretory epithelium, and milk determines the

fraction of the total calcium present in each compartment which is available for free movement between compartments; mass action is probably the governing factor for calcium distribution in each compartment. Mammary discrimination among other inorganic substances present in blood plasma and secreted into milk may be dependent upon these same physical factors.

2. Goat Studies

It is well recognized that marked species differences do exist in the composition of milk. In the case of organic constituents this implies that the synthetic pathways utilized differ. For inorganic materials, however, the implication is that transfer mechanisms either may differ in their characteristics or their degree of function. For this reason, it is necessary to develop techniques that allow the study of milk transfer mechanisms using large animals such as the goat and cow. These methods, to be practical must not depend upon serial sacrifice or even tissue sampling. A parallel effort in our laboratory has been to attempt to develop such a procedure.

The Wollman and Reed model discussed earlier is the model we are considering at this time. The data input needed is again milk, blood and secretory epithelial concentrations over time of the radionuclide being studied. As before, milk and blood are easily sampled as needed. The problem resides in determining the amount of radionuclide associated with the secretory tissue. We are attempting to make this estimate using external counting of the mammary gland tissue.

Basically, the idea is to remove enough milk at the end of an experimental time period so as to cause a significant drop in the external udder count of the labeled material under study. A second isotope (Ce-141), counted separately, is to be used as a marker substance which will allow the estimate of total milk volume in the gland. Subtracting

the count of the nuclide under study in the total milk volume from the total udder count will yield the residual count of the blood and tissue.

If the blood volume in the mammary gland full of milk is a small fraction of the total volume (around 5%) it may be possible to ascribe all of the residual count to tissue and to accept the error in the final answer. However, if the blood volume must be accounted for, then a possible approach would be to utilize a pre-calibration procedure in which udder blood count (external) is related to circulating blood isotope concentration. A major assumption here is that the blood volume does not change significantly during filling of the udder with milk.

Once the external count at the end of the experiment is able to be partitioned into milk, blood and tissue, in that order, then data collected at several time periods during the experiment can be divided in the same way. This would involve an estimate of the milk external count and the blood external count, then subtracting these contributions from the total external (probe) count to arrive at the external tissue count.

This procedure follows the outline below.

- a. At the end of an experiment an external probe count is made, for example, of Ca-47 with the udder full of milk (QP_{Un}). The udder is then milked sufficiently to reduce the probe count a reasonable amount without appreciably changing the counting geometry. A second count (QP_{Una}) and the volume of milk removed (V_{Mn}) then provide data to calculate the external count of Ca-47 for that volume of milk per ml.

$$\frac{QP_{Un} - QP_{Una}}{V_{Mn}} = P_{Mn} \quad (1)$$

b. To produce a factor to correlate Ca-47 well counts per minute (W) to external probe counts per minute (P) the milk collected above can be used.

$$\frac{W_{Mn}}{P_{Mn}} = Y \quad (\text{correction factor}) \quad (2)$$

c. At this point, it is necessary to determine the amount of milk left in the udder (V_{Mna}^*) after the last milk sampling. Cerium-141 can be used as the contained-milk volume indicator.

$$\frac{Ce_i - \sum Ce_{r \rightarrow n}}{Ce_{rn}/V_{Mn}} = V_{Mn}^* \quad (3)$$

Where Ce_i equals cpm Ce-141 injected into the udder at time 0

Ce_{rn} equals cpm Ce-141 collected at time "n"

$Ce_{r \rightarrow n}$ equals total cpm Ce-141 collected during entire experiment

V_{Mn} equals ml milk removed from gland at time "n"

d. The milk collected at time "n" has been counted in a well counter.

So the total well count of activity for Ca-47 in the milk can be calculated and then converted to external counts.

$$P_{MT} = \left[(V_{(M + M^*)n}) \frac{W_M}{Y} \right] \quad (4)$$

e. Subtraction of the value in (4) from the total udder external count at time "n" gives the external count due to the sum of blood and glandular components.

$$Q_{Un}^P - (V_{(M + M^*)n}) \frac{W_{Mn}}{Y} = Q_{Gn}^P + Q_{Bn}^P \quad (5)$$

f. At this point further resolution depends upon being able to determine the volume of blood in the gland (V_B). This may be difficult in practice so other possible solutions are sought.

One solution depends upon the blood being such a small fraction of the mammary tissue that consideration of it can be overlooked. In such a case equation (5) reduces to

$$Q^P_{Un} - (V_{(M + M^*)} n) \frac{W_M}{Y} = Q^P_{Gn} \quad (6a)$$

The body blood count needed for the calculation of transfer coefficients in the Wollman-Reed equation can come directly from the venous or arterial blood samples.

If the blood is a significant fraction of the mammary tissue, a possible way to take account of it is to run a calibration trial with the experimental animal before (or after) performing the complete kinetic experiment. At that time, since only one radionuclide is present (in the blood) and is of a form which will not transfer into milk, simplifying assumptions can be made that blood volume of the gland will not change significantly during the experiment and that activity in the udder blood has the same geometry and degree of attenuation that is true for milk.

$$\frac{Q^P_{B'}}{W_{B*}} = X \text{ (ml)} \quad (6b)$$

"X" is a correlation between concentration of blood marker isotope in the blood and in the mammary gland.

(i) During the actual experiment, measurement of blood levels of the radionuclide investigated will give an estimate of the probe count due to blood in the udder.

$$Q^P_B = W_{B*} \cdot X \quad (6b_1)$$

(ii) Note that the total udder count is equal to

$Q^P_{Un} = Q^P_{Gn} + Q^P_{Bn} + Q^P_{Mn}$. Therefore, substituting results from 6b' in equation (5) gives

$$Q^P_{Un} - (V_{(M+M^*)n}) \frac{WM_n}{Y} - W_{B^*n} X = Q^P_{Gn} \quad (6b_{ii})$$

(iii) At this point it is worth noting that the blood volume of the udder can be determined.

$$\frac{Q^P_B \cdot Y}{W_{B^*}} = \frac{(P_B \cdot V_B) \frac{W_M}{P_M}}{W_{B^*}} = V_B \quad (6b_{iii})$$

(iv) The relationship in 6b_{iii} is essentially

$$XY = V_B \quad (6b_{iv})$$

A third means of arriving at an estimate of the udder blood volume is to measure the amount of blood in an excised mammary gland. The blood vessels are tied off and the total activity in the blood is measured in a whole gland or in a sample of the gland homogenate. Udder blood volume will be related to a dose standard of the isotope. This determination of udder blood volume will be used for comparing results in either "a" or "b" above.

g. The volume of milk and blood can be determined easily; however, V_G and V_U are more difficult. The measurement of V_U is the easier of the two. Determination of V_U can be accomplished by displacement techniques, cast, etc. If this is done V_G can be determined by subtraction.

$$V_G = V_U - V_M - V_B \quad (7)$$

h. We now have enough information to proceed to work with the samples collected in time intervals from $0 \rightarrow n$; we have

Milk counted in well counter (W_M)

Blood counted in well counter (W_{B*})

The equivalent isotope concentration for tissue can be computed

$$W_G = \frac{QP_G}{V_G} Y \quad (8a)$$

Or, for a specific interval

$$W_G = \frac{(QP_{U_1}) Y - \left[\left(\frac{Ce_i - Ce_{ro \rightarrow n}}{Ce_{rl} / V_{M_1}} \right) W_{M_1} \right] - (W_{B*1} X)}{V_U - \frac{Ce_i - Ce_{ro \rightarrow n}}{Ce_{rl} / V_{M_1}} - XY} \quad (8b)$$

This is repeated for each sample time. The resulting contribution can be plotted along with milk and blood.

As with the guinea pig study it was necessary to develop an isotope dilution procedure to estimate the volume of milk in the mammary gland of the goat. For this purpose we again utilized the polyethylene glycol-4000 of Knutsson and Sperber replacing the turbidometric measurement with a radiotracer to improve the precision. In addition we tested ^{141}Ce as a marker substance. Both ^{141}Ce and ^{14}C -PEG-4000 yielded equivalent results; the labeled PEG could reasonably be expected to distribute similar to the unlabeled PEG. It was found that the sampling of milk to determine the volume in the gland can be done almost immediately after infusion of either of the labeled markers or after a predetermined waiting period. Before sampling, an intravenous injection of oxytocin is given to the animal and the udder is kneaded gently for a few minutes to ensure mixing

of the marker into all of the milk. At this point, a small sample of milk can be taken for determination of its marker concentration. Dividing the amount of marker infused into the udder by the concentration of marker per milliliter of milk gives the volume of milk in the udder at the time of oxytocin administration.

In exploring other facets of the technique, we observed that the results were likely to be erratic if the label was not mixed with the milk within the udder.

Figure 10 is a presentation of the data if the data of an experiment in which one half of the gland was not kneaded after ^{141}Ce was injected and where the other half was mixed gently for 10 minutes after the ^{141}Ce was infused. To be noted is that the ^{141}Ce was very uniformly distributed among serial samples when mixing took place and in the unmixed glands the concentration tended to fall off with time, particularly with milk recovered after oxytocin was injected to obtain residual milk. Recovery of ^{141}Ce or ^{14}C PEG was essentially 100% in a three day period after dosing and indicates no absorption into the body counter to the results with the guinea pig.

Figure 10. Mammary gland-contained milk volume approximation by extrapolation of a plot of percent of dose versus milk volume to the 100% dose intercept. Arrows indicate oxytocin administration; A represents the results of facilitated udder mixing, part B represents the results of the non-facilitated udder mixing.

34. Magnitude of the Error Associated with Estimation of
Contained Milk Volume in the Dairy Goat Using Cerium-141

C. E. Sagan, M. B. Snipes, and F. W. Lengemann

Abstract: Cerium-141 was used in nine separate trials with two goats as a non-absorbable radiomarker to determine milk volume by isotopic dilution techniques. A correction procedure, developed for guinea pigs, showed an average underestimate of contained milk volume of 1.1% (range - 0.61 to 4.06%) for goats having from 353 to 1202 ml of milk per udder half. A correction for incomplete penetration of ^{141}Ce into the alveolar spaces of the mammary gland is not needed in the goat.

Introduction

A basic requirement for the study of transfer rates of elements across the mammary gland epithelium is an accurate measurement of the contained-milk volume. In some kinetic studies of the mammary gland not only is the time factor critical but the determination of milk volume without emptying the gland is often necessary. Studies involving absorption of materials infused into the milk, or secretion of milk constituents, demand knowledge of the total milk volume. Moreover, the sacrifice of experimental animals is often to be avoided in accumulating such data. An effective method currently used for milk volume determinations employs the dilution principle whereby a known amount of marker substance is infused into the udder. Samples are collected over time in a given experiment and it is necessary to know the amount of milk in the gland at the start and the expansion or contraction of that volume at succeeding sampling intervals to arrive at an estimate of the total change per unit of time in the concentration of the element perturbing the system. Procedures based upon the dilution of a known amount of a non-absorbable marker have

been developed for this purpose. Substances advocated as markers are non-absorbable and include polyethylene glycol-4000, ^{14}C -polyethylene glycol-4000 and ^{141}Ce (1,3).

Recently Snipes and Lengemann (4) reported on the use of ^{141}Ce as milk volume estimator of the guinea pig mammary gland. Contained milk volume was estimated using the first gram of milk removed from each gland and compared to the milk volume estimated from analysis of the entire gland. Less than 0.3% of ^{141}Ce was absorbed into the body. However, the constancy of ^{141}Ce concentrations in milk was maintained only in the first 40% of the estimated volume. To improve the accuracy of the estimate of the total milk volume a correction procedure was developed based on the deviation of the ratio of % initial milk volume to % accumulated dose from a theoretical ratio of 1. The average error of their milk volume estimates in the guinea pig was 12.8%. The % error was found to increase as the milk volume increased.

No determination has been made of the need for the use of such a correction procedure for the milk volume estimate (MVE) for other species when small samples are the basis of the estimate. The purpose of this study was to determine the necessity of such a correction procedure for the goat.

Experimental Procedure

Nine separate trials were performed in two goats over a 24-month period to determine the MVE based on the initial milk sample and the uniformity of marker distribution in the milk. A minimum of two weeks elapsed between experiments performed on the same animals. Procedures for the study were adapted from those described by Snipes and Lengemann (3,4).

In each trial 2.0 ml of ^{141}Ce dosing solution, containing 2 to 20 μCi radiocerium, was injected into the mammary gland and the gland manipulated for one minute to disperse the isotope into the udder. Shortly

before the end of the waiting periods of 2 or 16 hours, 2 IU of oxytocin were injected into the jugular vein and the gland kneaded by hand for five minutes. Successive milk samples of approximately 50 ml were then collected by teat catheterization. When the flow of milk stopped, another 2 IU dose of oxytocin was administered. Milk collections were then continued as soon as milk ejection occurred. At the end of the collection the mammary gland was gently stripped in an attempt to recover as much milk as possible; milk collection was completed within 20 minutes. Quantitation of ^{141}Ce in the milk was made by gamma counting, and the data were expressed as the % of the ^{141}Ce dose per milliliter.

Total milk volume was calculated separately from each of the milk samples approximating the first 20% of the total collected volume. Dividing % ^{141}Ce dose per ml into 100 produced an estimate of the total milk volume for each sample and the average of these was used as the 100% initial MVE. Plots were then made of % dose per ml of milk versus % milk volume so as to follow the uniformity of distribution of the marker. Plots of % accumulated dose versus accumulated milk volume were also made to check for any deviation from linearity of marker concentration for a particular volume of milk.

The % error of the MVE for each milk sample was calculated in the following way:

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

A line was fitted to a plot of accumulated %error versus % accumulated dose using a linear regression analysis. Extrapolation of these lines beyond the last sample to 100% volume gave the total % error of the MVE.

Results and Discussion

Plots of % dose per ml of milk versus % milk volume similar to graphs presented by Knutsson and Sperber (1) and Snipes and Lengemann (3) indicated the relative constancy of marker concentrations in the samples up to 90-97% of the milk removed. Figures 11 and 12 are plots of the regression lines for all 9 trials for accumulated percent error versus accumulated % dose ^{141}Ce of successive milk samples. (These plots are presented in two figures in order to present the data clearly). These values were calculated according to equation 1 using the MVE based on the first 20% of the milk collected. A positive error is indicative of an underestimate of the milk volume while a negative value indicates that the initial sample overestimated the actual volume. To obtain the true volume the MVE would need to be corrected for the error. For the goats in this study the error ranged from -0.61 to 4.06%. (These values are tabulated for the individual trials in Table 4). The average error of the MVE was 1.1%. In comparison, the guinea pig showed a range in the error of estimate from 8.9 to 17.1% with an average of 12.8%. All of the guinea pigs studied showed underestimates whereas the milk volume was slightly overestimated in 2 of the nine trials with the goats.

Figures 11 and 12 show the points generally well distributed about the linear regression lines indicating that the choice of a linear function was correct. Extrapolation beyond the last data point to the 100% value was made assuming that the linearity of the function would continue. At worst, this assumption introduces only a small error because of the large proportion of the estimated milk volume ($AV = 94\%$) collected and the large proportion of the marker dose collected (93%).

Combining the data of Figures 11 and 12 and Table 4, it can be noted that there is a tendency for the % error to vary directly with the amount

Figure 11. A plot of Accumulated Percent Error versus Accumulated Percent Dose for trials 2, 4, 7 and 8 showing regression line fits of the data of each trial.

Figure 12. A plot of Accumulated Percent Error versus Accumulated Percent Dose for trials 1, 3, 5, 6 and 9 showing regression line fits of the data of each trial.

of milk estimated to be in the udder. A dependence of % error on milk volume has been shown for guinea pigs. Linear regression analysis of the present data suggests such a relationship in the goat and a line fitting these points is described by the equation $Y = -.567 + .003X$.

However, examination of the data in Table 4 shows that animals subjected to the 2 hour equilibration period have a mean error in MVE of less than .2%, which is notably less than the 2.44% mean error in animals given a 16 hour equilibration period. Linear regression on the 16 hour data indicates the tendency for % error to vary with contained milk volume and can be described by the line equation $Y = .143 + .003X$. The increase of the error with increasing milk volume suggests that distention of the gland may restrict access of the marker to the alveolar spaces during mixing. The magnitude of this phenomenon is much greater in the guinea pig than in the goat and may be related to differences in average size of glandular area, lobes, ducts and cisterns between the species. The earlier study with guinea pigs presented a procedure for correcting for incomplete penetration of the marker. Knowing that errors of volume estimation exist is especially important in kinetic experiments on the mammary gland. For, if the error of estimation arises with exogenous markers diffusing from teat to alveolar spaces then just as serious errors can be expected when measuring constituents moving from alveoli to the teat. It would be better if no correction procedure was necessary. This paper shows such a possibility exists for the goat where the average underestimate of volume was deduced to be 1.1% as opposed to the 12.8% observed for guinea pigs. Since the size of the error may be associated

with milk volume the investigator can select his experimental animals on the basis of production, or plan his experiment so as to keep the udder milk volume relatively low and in so doing minimize the amount of error associated with the contained milk volume estimate.

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Table 4

Milk Volume Estimates (MVE)* and Associated Percent Errors for the Goat as
Determined by ^{141}Ce Milk Volume Indicator

Trial	Goat No.	Time Between ^{141}Ce Injection And Milk Sampling (Hours)	Recovered Ce- 141 (%)	Initial Estimate of Total Milk Volume (ml)	Error of Total Milk Volume Estimate (%)
1	30	2	92.3	395	0.41
2	32	2	94.4	785	0.63
3	30	16	91.8	353	1.04
4	32	2	90.2	300	1.05
5	30	16	96.0	512	1.36
6	32	16	88.6	725	2.49
7	32	16	95.0	1202	4.06
8	30	2	95.0	362	-0.56
9	30	2	91.3	356	-0.61
Mean			92.7	554	1.10
S.D.			2.5	289	1.46

* Based on first 20% of milk collected.

35. Comparison of 24-Hour Secretion Rates in Emptied and Partially Full Mammary Glands of the Goat.

C. E. Sagan and F. W. Lengemann

Abstract

Using ^{141}Ce as a contained-milk volume indicator the secretion of milk in emptied and partially full glands of lactating goats was followed over a 24-hour period. In these goats, the slopes of the milk accumulation curves were essentially linear over the 24-hour period. The slopes were essentially parallel for both glands indicating little effect of the degree of fill on milk secretion.

Introduction

Another important factor in attempting to quantitate the secretion rate of calcium in milk over an extended time interval is the necessity of establishing that formed milk is secreted at a uniform rate. For our planned studies, it is contemplated that the time period to be encompassed will be a 3-4 hour interval.

This experiment was designed to determine if the secretion rate changes over a 24-hour period and to compare the milk secretion in the completely emptied gland with the partially emptied gland. Two approaches were applied to each goat to determine the relationship between milk secretion over time. In the first approach one of the glands was emptied completely at zero time and its milk yield followed over a 24-hour period. In the second approach the gland was only partially emptied at zero time and its volume also followed for the 24-hour period. If secretion is constant during part or all of the time period, a straight line should be observed. Moreover, if both glands of each animal are secreting at the same rate, a plot of the partially emptied gland would be expected to show

the same slope as the completely emptied gland.

Milk volumes were measured in vivo through the application of radio-cerium as a marker in isotopic dilution methods. The equation used to determine repeated milk volume measurements over the 24-hour period after a single dose of marker is:

$$V_{Mn}^* = \frac{Ce_0 - \sum Ce_r \text{ } 1 \rightarrow n}{Ce_{rn}/V_{Mn}}$$

where Ce_0 equals the cpm of ^{141}Ce injected into the gland, $Ce_{r1 \rightarrow n}$ is the total ^{141}Ce removed from the gland during the experiment, Ce_{rn} is the cpm of ^{141}Ce collected at time n , and V_{Mn} is the ml of milk removed from the gland at time n . V_{Mn}^* equals the volume of milk estimated to be found within the gland at any time n .

Procedure

On the morning of the experiment, 25 ml of milk were withdrawn from the goat. Approximately 3 μCi of ^{141}Ce stock solution were added to the milk. This labelled milk solution served as the dosing solution to be injected into the udder for milk volume determinations.

About 15 minutes before dosing the udder, one of the glands was completely emptied while the second gland only partially emptied. The volume of milk to be withdrawn from the second gland was estimated from the 16-hour milk collection of the previous day. At the time milk was removed from the glands, blood and urine were sampled to determine if radiocerium was being absorbed from the mammary gland.

Before dosing the animal, 5 ml of the labelled milk dosing solution was transferred via a 5 ml syringe and PE-90 cannula to a 100 ml volumetric flask. The flask was filled to volume with distilled water and this diluted solution served as a standard.

At zero time, approximately 8:00 AM, the first gland was injected with 5 ml of the labelled milk dosing solution. Five minutes later another 5 ml dose was injected into the second gland. The gland was kneaded by hand for three minutes to disperse the radiocerium within the glands. A second standard was prepared in the manner of the first preparation. Aliquots of both standards were removed to be counted later with the milk samples.

About 55 minutes after administering the radiocerium dose 2 IU of oxytocin were injected into the jugular vein of the animal and the udder manipulated for 3 minutes. The teats were washed with 0.5% Zephiran solution to help remove possible contamination. At one hour after dosing approximately 3-5 ml of milk were collected in a preweighed glass vial from the first gland. Five minutes later a milk sample was extracted from the second gland. The sampling procedure just described was repeated at intervals of 2, 4, 6, 8, 12 and 24 hours. Milk volume estimations of the glands at these sampling times were determined by the isotopic dilution method. Blood and urine samples were collected during the 24 hour period and also at 2 and 3 days post dose.

After the 24 hour period both glands were milked out completely then given an injection of Unico antibiotic to prevent mastitis. The antibiotic treatment was repeated following the evening milking.

The total milk yields for each gland over the next two days were recorded and samples analyzed for radiomarker activity to determine the percent recovery of marker.

Each milk sample was weighed to determine the amount of milk and radiocerium withdrawn from the gland. Duplicate aliquots of 1 ml were taken from each standard and milk sample, transferred to a gamma counting vial, diluted to 20 ml and counted in an automatic gamma counter.

The total milk volume estimate for each time interval was calculated. The activity remaining in the gland was determined by subtracting the total activity removed from the gland at each time interval from the initial dose injected into the gland. The volume of milk in the gland at each interval was computed by dividing the total remaining activity in the gland at the particular interval by the cpm/ml that interval's sample. The total volume for the interval equalled the volume estimate for the interval plus the total volume withdrawn from the beginning of the experiment.

Finally, the milk volume estimate for each time interval was plotted against time to determine how milk secretion varied over the 24-hour period.

Results and Discussion

The accumulation of milk in the emptied and partially emptied glands of one of the goats used in the study is shown in Figure 13. This curve is representative of that for all of the goats used in this study. To be noted is that the lines for both glands tend to be linear at least over the first 16 hour period and are essentially parallel for the two conditions of fill. In only one of the four animals (32) did the two lines tend not to be parallel and this could be accounted for by the observation that in the seven days preceding the experiment the left gland consistently produced 48% more milk than the right gland.

Figure 13. The accumulation of milk in empty and partly emptied glands over a 24-hour period.

Table 5 presents the data for regression lines calculated for the milk secretion lines shown in Figure 13. The values are calculated for the percent of the 24-hour milk volume versus time.

This table shows the secretion rate and the goodness of fit of the data to the regression line. To be noted is the high "r" values indicating little deviation over this period of time. Thus the milk secretion rate can be judged to be constant and will not change over the time periods projected for use in our external counting experiments. Little difference was seen between the secretion rates of emptied and partly full glands.

Table 5

Slopes of Regression Lines for Percent of
24-hour Milk Volume Versus Tissue

<u>Goat</u>	<u>Emptied Glands</u>		<u>Partially Emptied</u>	
	<u>% of 24 H vol/hr</u>	<u>r</u>	<u>% of 24 H vol/hr</u>	<u>r</u>
183	3.95	1.0	3.95	0.998
170	3.76	0.996	3.97	0.969
166	3.83	0.996	4.05	0.998
32	3.67	0.999	4.40	0.997
AV	3.82	0.996	4.07	0.984
All. glands	3.94	0.987		

36. Preliminary In Vitro Studies of the Udder.

C. E. Sagan and F. W. Lengemann

In attempting to study transfer of materials across the mammary epithelium into milk and blood of large animals, it is necessary to use a technique such as external counting to provide the same data that serial sacrifice provides in a small animal experiment. Problems of geometry arise in attempting to determine the calcium transfer constants in the mammary gland of the goat by external means. One phase of the problem involves geometrical variations due to blood and milk volume changes during the experiment. Another involves the positioning of the probe in relation to the udder. Since the glandular anatomy is somewhat irregular, care must be exercised to include all portions of the udder in the detection field of the probe.

The inverse square law and self absorption of the udder make the detector more sensitive to radiation from superficial tissues than from the depths of the mammary gland. In addition, the non-symmetrical nature of the udder complicates evaluation of activity distributed within the mammary gland. The following experiments were performed, therefore, on in vitro simulations of the udder in order to gain insight into the practical problem of external detection before proceeding with the less symmetrical real udder.

1. Relation of external count to volume of ^{141}Ce solution.

A single rubber balloon suspended in a nylon stocking was placed in the center of a wax-coated paper bucket. The distance from the face of the probe to the center of the bucket was maintained constant at 20 or 40 cm. Volume increments of ^{141}Ce (0.14 mev) were pumped into the balloon from a reservoir of ^{141}Ce solution located approximately 40 cm from the

balloon. Two-inch thick lead bricks were positioned between the reservoir and the balloon to shield out activity from the reservoir. Thirteen separate counts were made on volume increments of 25 ml to 100 ml for a total volume of 1165 ml. The corrected counts were plotted against volume.

At the distances of both 20 cm and 40 cm from the face of the probe to the center of the balloon the total count registered by the counting system increased linearly with increasing volume of labelled aqueous solution. This means that increasing volumes of labelled blood or labelled milk ought to behave similarly in the live goat.

2. Relation of external counts to volume of ^{141}Ce solution in a polyethylene tube simulated udder compartment.

In this experiment an attempt was made to relate external counts to increasing volume of ^{141}Ce solution contained in a length of PE 320 tubing. First, a known length of tubing was filled with a known volume of water to establish the average volume/cm length of tubing. Next, a length of tubing was graduated in 5 cm divisions then filled with ^{141}Ce solution. The tubing was inserted spirally inside a wax-coated bucket and counts were recorded for the known lengths of tubing inserted into the bucket. The tubing was placed as symmetrically as possible about the vertical axis of the bucket.

A straight line relationship between counts and length of tubing, or volume of solution, added to the bucket was obtained for tubing lengths of 20 to 155 cm, or 1.06 to 8.22 ml of labelled solution. Linearity was also obtained when the ratio of external counts per ml of solution was plotted against tubing length.

3. Effect of water on the attenuation of external counts.

When a fixed length of latex tubing containing ^{141}Ce was placed inside a balloon filled with water and positioned 30 cm from the probe, it was

found that water attenuated little of the activity within the latex tubing. The balloon was fitted to a rubber stopper through which the latex tubing filled with ^{141}Ce entered the balloon. The balloon was then filled with 1200 ml of distilled water counted. Subsequent counts were made after each 100 ml of water were withdrawn from the balloon. These continued until the balloon was emptied. The counts after each withdrawal were compared to the initial count of the full balloon and expressed as the percent of initial count. Values obtained for 12 withdrawals ranged from 99.1 to 108.7%, indicating that the water did not interfere with the detection of activity contained in a separate compartment. It appears reasonable that milk in the gland of the goat would also provide minimal attenuation to activity located in the blood or tissue since it is approximately 90% water.

4. External counting efficiency of a point source of ^{141}Ce .

In making external counts of the mammary gland it is assumed that the entire udder will lie within the detection field of the probe. Along the probe to udder axis the counting efficiency is expected to be maximal. The question arises, therefore, as to the efficiency of detection at the periphery of the gland. In this experiment counting efficiency was determined for a point source at varying peripheral distances by comparing peripheral counts to midline counts at the midline of the probe's counting field.

The probe was supported on a steel frame and positioned parallel to the top of a laboratory bench. The support was placed over a piece of heavy white paper taped to the table top. A center line was drawn from the center of the probe's detector crystal and perpendicular to the face of the probe. Lines two centimeters apart were drawn parallel to the left and right of the center line. Next an arc was marked off at a distance

30 cm from the center of the face of the probe, intersecting the series of parallel lines. A glass vial containing a small volume of ^{141}Ce solution was then clamped to a ring stand so that the ^{141}Ce volume was vertically centered to the face of the probe.

After a 10 minute background count, 10 minute counts of the ^{141}Ce source were made along the 30 cm arc at the intersections of the arc and the parallel lines. Detection efficiency was expressed as the percent of the center line count, which was set at 100%. Counts were made at equal distances to the right and left of the center line for comparisons.

It was found that the counting efficiency of the point source decreased only slightly over distances up to 6 cm from the center line at this distance the efficiency was 97% that of the center line count. The efficiency was 90% at 8 cm, 79% at 10 cm and 58% at 12 cm. These results indicate that distances over 6 cm from the center line fall outside the maximally efficient counting field of the detector when placed 30 cm from the face of the probe.

This information, obtained with the probe on an in vitro system, is useful in estimating the distance at which the probe should be placed from the goat udder. In the udder one can assume that the radius of the longest plane encompassing the udder that lies within the field of the probe where counting is maximally efficient is that vertical distance from the center of the udder to the radial distance of the probe field intersecting that longest plane of the udder (Figure 14). In most cases the longest radius of this udder plane is the distance from the center of the udder to the tip of the teat.

In order to estimate the minimum distance that the udder should be located from the probe the angle of the field of maximum counting efficiency should be known. This minimum distance can be readily calculated from in vitro study and is given in the radial distance from the center of

Figure 14. Counting set up used to estimate minimum distances of udder from probe.

the probe to the tip of the udder. The radial distance of the field can be computed from the equation

$$\text{Radial distance} = y / \sin \theta$$

where y is $\frac{1}{2}$ the vertical distance, or the radius of the longest plane, of the udder and θ equals one half of the field angle. The distance from the probe to the center of the udder along the center line is given by the equation

$$\text{Centerline distance to center of udder} = y / \tan \theta$$

where y is again the udder radius and θ is one half the field angle.

Using the data from the simulated udder one can calculate the necessary parameters for the real udder. From the simulated udder the following information may be determined:

- a. The angle of the field of maximal counting efficiency.
- b. The radial distance from probe to the tip of the teat.
- c. The center line distance from probe to the center of the mammary gland.

The angle of the field which gives 97% counting efficiency was calculated in the following way:

$$\sin \theta = y / \text{radial distance from probe to } ^{141}\text{Ce source}$$

$$\sin \theta = \frac{6 \text{ cm}}{30 \text{ cm}} = 0.200$$

$$\theta = 11^{\circ} 32'$$

$$\text{Angle of field} = 20$$

$$\text{Angle of field} = 2 \times 11^{\circ} 32' = 23^{\circ}$$

In most cases the radius of the udder from its center to the tip of the teat will be 12 cm or less. Using 12 cm for the radius of the udder, the minimum radial distance from probe to the tip of the udder

giving 97% counting efficiency at the periphery of the udder was calculated as follows:

$$\begin{aligned}
 \text{Radial distance from probe to udder} &= y/\sin \theta \\
 &= \frac{12 \text{ cm}}{\sin 11^{\circ} 32'} \\
 &= \frac{12 \text{ cm}}{0.200} \\
 &= 60 \text{ cm}
 \end{aligned}$$

The midline distance from the probe to the center of the gland was calculated as follows:

$$\begin{aligned}
 \text{Midline distance to center of gland} &= y/\tan \theta \\
 &= \frac{12 \text{ cm}}{\tan 11^{\circ} 32'} \\
 &= \frac{12 \text{ cm}}{0.20406} \\
 &= 59 \text{ cm}
 \end{aligned}$$

Inasmuch as the center line distance from the probe to the center of the udder and the radial distance from the center of the probe to the tip of the teat differ by only 2% the latter value may be used for approximating the center line distance in positioning the udder relative to the face of the probe. Furthermore, since most of the goats to be used in this study have udder radii approximating 12 cm the probe-to-udder distance may vary between 60 to 70 cm to assure a counting efficiency of the periphery of the udder being 97% of that of the center of the udder.

5. Estimation of the Blood Volume of a Simulated Udder.

The Wollman-Reed three compartment model being employed to describe the movement of calcium in the mammary gland of the goat contains a factor for the calcium concentration in the blood. In order to evaluate the contribution of blood to the total external count in the goat's udder the blood volume of the udder at the time of counting must be determined.

The experiments discussed here were attempted in a simulated udder before applying the procedure to the live, unanesthetized animal.

Simulation of the goat mammary gland was attempted using a balloon within a balloon assembly. Such a set-up appeared to be justifiable in that the separate compartments of milk and blood thus formed were uniform and symmetrical within the field of the detector. While the compartments did not intermingle in a way more analogous to in vivo conditions it was felt that the artificial arrangement at least approximated the uniform distribution of each compartment, which is assumed for the real situation.

The simulated udder was constructed of a plastic 2½ liter cylindrical can. Positioned inside the cylinder were two balloons, one inside the other, and fitted to valves and polyethylene or rubber tubing that entered the balloons separately through rubber stoppers. The inner balloon represented the milk compartment, the outer balloon the blood compartment and the water-filled space between the outer balloon and the cylinder represented the tissue compartment. Since a quantitative estimation of the blood compartment was the point of interest the tissue compartment was disregarded in the simulation, except for maintaining a fixed distance from the external probe.

The probe was kept in a fixed position while the plastic cylinder was placed at measured distances from the probe along its center line. It was assumed that the uniform distribution of material in each compartment was analogous to that found in the udder. Geometry changes in the size and distance of the "udder" were assumed to be compensated proportionately for each compartment.

Two different solutions of ^{141}Ce were prepared in volumetric flasks to represent milk and blood storage reservoirs. Blood solution concentrations were about ten times that of the milk solution concentrations.

For each trial known volumes of cerium-labelled milk and blood solutions were transferred to the proper compartment from the reservoirs of each solution.

The blood volume determination used the following procedure: First, ten minute background counts were made of the udder at the proper distance to ensure that the periphery of the udder was counted at 97% or better of the efficiency of the midline. Approximately 1 liter of milk solution was then pumped from the milk solution reservoir to the inner balloon and counted several times for one minute. The background count was subtracted from the second count to give the external cpm for milk. Next, from 40 to 240 ml of the blood solution were pumped from the blood reservoir to the second balloon, that is, to the space between the inner and outer balloons. The udder now containing both solutions, was then counted, and from this count the second count was subtracted to give the net external count due to blood.

Well counts were also made on the milk and blood solutions. One ml aliquots were transferred from the milk and blood storage flasks, prior to pumping, into separate gamma counting vials, diluted to 20 ml, then counted in an automatic gamma counter.

External counts per ml of milk were calculated by dividing the total external count of the udder milk by the total volume of milk solution added to the balloon.

A correlation factor relating well counts to external counts was computed by dividing external milk counts per ml into well milk counts per ml. Dividing the blood well counts by the correlation factor converted well cpm/ml to blood external cpm/ml. When this calculated value is divided into the measured total external blood count, the quotient represents an

estimation of the total blood volume of the udder at the time of external counting.

Since the volume of blood pumped into the balloon was known from the specific gravity of the solution and the weight measurement of the blood storage flask before and after pumping blood solution into the blood compartment it was possible to compare the experimental blood volume estimate with the known blood volume. Differences were calculated as percent error of the known value.

In 27 trials the percent error ranged from 9.6 to -20.4 for blood volumes of from 40.2 to 243.5 ml. These results were computed from the average count of several external counts. The average percent error for the 27 trials was -0.96 with a standard deviation of ± 7.4 .

It appears that a similar procedure can be applied to the goat udder for making reasonable estimates of the udder blood volume. It should be noted that the guinea pig data suggests that about 10% of the udder volume is blood. Errors in estimating the size of this compartment should not have a large effect upon estimates of the milk or tissue radionuclide content.

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