FACTORS AFFECTING IODINE CONCENTRATION IN BOVINE MILK[1]

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ABSTRACT

The purpose of this study was to investigate the effect upon the secretion of iodine into cows' milk of various compounds demonstrated to alter thyroid function. Nine cows were given radioiodine twice daily to achieve a steady state condition. After a control sampling period the cows were given $KClO_4$, NaI, NaCl, and L-thyroxine, D-thyroxine, or 2-4 dinitrophenol. $KClO_4$, NaI, NaCl, and L-thyroxine reduced the accumulation of radioiodine in milk and reduced the milk-I^{131}/plasma-I^{131} ratio; whereas, D-thyroxine and 2-4 dinitrophenol did not. L-thyroxine, although probably acting directly upon the mammary gland, was limited in its ability to reduce I^{131} accumulation in milk. It was concluded that transfer of iodide into milk was in part an active process and that thyroxine could affect the transfer of iodide from plasma to milk and from milk to plasma.

The transfer of iodine across the mammary gland has been of interest to many investigators over the years and interesting observations have been made. The ability of the mammary gland to concentrate iodine has been observed (using laboratory animals) to bear a marked similarity to the iodide concentration mechanism of the thyroid (2). In both glands anions such as thiocyanate, nitrate, perchlorate, and excess iodide interfere with concentration of iodide. Compounds that block thyroid function by affecting the binding of iodine in organic complexes do not, however, seem to affect the iodide-concentrating mechanism of the mammary gland. Further information is needed to determine if the iodide-concentrating mechanism in the mammary gland is identical with that of the thyroid.

While the mammary iodide-concentrating mechanisms are likely to be similar in laboratory animals and large ruminants, quantitative differences may exist that may make it more meaningful to examine milk secretion in species kept primarily for milk production. The recognition that radioiodine from fallout enters the food chain of man through milk provides an additional reason for using economically important species.

It was with these points in mind that the present experiments were done on the transfer of iodide from plasma to milk. Whereas these studies represent a gross approach to the subject, they can serve as a basis for more precise studies on how iodine is secreted into the milk of dairy animals.

METHODS

A total of nine milking cows was used for this study; in several instances a particular cow was used for more than one experiment. The animals were from three to seven years old, had no diseases, were not pregnant, and had been milking at least four months. Five of the cows, three Guernseys and two Holsteins, were purchased from local farms and weighed from 370 to 425 kg. The four other cattle were Holsteins raised at the laboratory, weighing from 500 to 600 kg. During the experimental period the animals were fed from 4.6 to 9.2 kg of a commercial dairy ration (18% protein) and all the mixed orchard grass–alfalfa hay they would consume. Milking, feeding, and radioiodine administration were done at 8 AM and 4 PM every day. The animals were confined in metabolism stalls to facilitate collection of samples and were given an I^{131} capsule per os after each milking during the experiment. This capsule was prepared by pipetting 0.5 ml of an alcoholic solution containing about 100 μc of KI^{131} (carrier-free) into a gelatin capsule, then drying under low heat. All capsules were prepared before the start of an experiment.

Taking of samples began after the animals had received I^{131} for seven days. Milk was collected at each milking; blood samples were taken and thyroid counts made about 10 AM each day. In some instances urine and feces

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also were collected; when this was done, sampling took place at 9 AM.

After the control collection period the cows were given various chemicals, orally or intravenously, to determine their effect upon the transfer of radioiodine into milk. Among those tested were $KClO_4$, NaI, NaCl, L-thyroxine, D-thyroxine, and 2-4 dinitrophenol. The L-thyroxine and D-thyroxine were administered by intravenous injection about 10 AM each day. The other chemicals were weighed into gelatin capsules and given orally twice daily to the cows at the same time radioiodine was administered. Total amounts of each chemical given to the animals are shown in Table 1. Sampling was continued as usual during the five-day test period. Data presented in the tables represent the average amount of radioiodine detected in a particular sample during the last three days of the test period.

To determine I^{131} in the samples, 3 ml of the liquids were pipetted into 16 by 150-mm test tubes, counted in a well-scintillation counter, and compared to a suitable standard handled in the same manner. An alternate procedure was to fill a quart screw-cap jar with the sample and place these jars 30 cm from the crystal face of a scintillation probe. These samples were compared to a standard made by dissolving one of the I^{131} capsules in a quart of water. Results were expressed as per cent of a daily dose of I^{131}.

Thyroid counts were made using a scintillation probe held 38 cm from the thyroid of the cow. The count-rate meter readings were corrected for body backgrounds and compared to a standard consisting of one of the I^{131} capsules contained in a plastic phantom that simulated the neck.

Bound iodine of milk and plasma was determined by adding 3 ml of a 1:1 slurry of Dowex 1-8x resin (Cl^- form) to 3 ml of the sample. After 10 min of gentle mixing, the mixture was centrifuged to precipitate the resin. This resin was counted after washing and recentrifuging. The count was taken to represent the nonbound I^{131} and subtraction of this count from the total count for the milk or plasma sample gave a count due to the bound iodine in the sample.

RESULTS

During the control periods of these experiments the nine cows averaged 79% of a daily dose of I^{131} in their thyroids, about 1% of the daily dose per liter of milk, and a milk-I^{131}/plasma-I^{131} ratio of 2.3. About 5% of the I^{131} in the milk was found to be bound to the milk proteins (5).

Table 1 presents the data of the control periods and contrasts this to periods when various chemicals were given to the cows. For $KClO_4$ it can be seen that when this chemical was given at a level of 10 g per day the milk I^{131} concentration dropped from 1.7 to 0.4% of a daily dose per liter. This decrease is approximately a factor of four; however, the milk/plasma I^{131} ratio decreased by a factor of eight. This greater decrease in the milk/plasma ratio was due in part to a 1.7 times increase in plasma I^{131} during the period of perchlorate administration. Levels of protein-bound I^{131}

TABLE 1

Effect of various chemicals upon accumulation of radioiodine in milk

Test substance	Amount/day	No. animals	Plasma	Milk	m/p
			(% daily dose/liter)		
$KClO_4$					
Control	0	2	0.7	1.7	2.5
Test	10 g	2	1.2	0.4	0.3
NaI					
Control	0	2	0.8	1.2	1.5
Test	10 g	2	1.2	0.4	0.3
NaCl					
Control	0	1	1.1	1.2	1.0
Test	60 g	1	1.2	0.8	0.7
L-thyroxine					
Control	0	4	0.5 ± 0.05[a]	1.1 ± 0.05	2.6 ± 0.4
Test	10 mg	4	0.5 ± 0.1	0.6 ± 0.15	1.3 ± .45
D-thyroxine					
Control	0	1	0.4	0.5	1.3
Test	10 mg	1	0.6	1.0	1.7
2-4 Dinitrophenol					
Control	0	1	0.7	0.7	1.0
Test	0.75 g	1	0.9	0.8	0.9

[a] Mean ± standard error of the mean.

were 0.06 and 0.09% of a daily dose per liter of plasma and milk during the control period. Administration of perchlorate did not change these concentrations. Because of the changes in the nonprotein-bound I^{131} the proportion of protein-bound I^{131} decreased from 9.1 to 7.2% of the total I^{131} in the plasma and increased from 6 to 23% in the milk.

The administration of 10 g of NaI per day to milking cows resulted in an increase of plasma I^{131} of 1.5 times, a decrease in milk I^{131} by a factor of three, and a lowering of the milk plasma ratio by five. Again, the plasma I^{131} increased markedly during the period of administration of the test substance; therefore, the decrease in milk I^{131} was less than expected on the basis of the change observed in the milk/plasma ratio. These data are in general accord with observations made with cows given 2 and 4 g of NaI daily (6). No fractionation of milk or plasma I^{131} was made in this experiment.

It was of interest to compare the effect of another halide upon the transfer of I^{131} into milk. For this, 60 g of NaCl were given daily to a cow in addition to the 50 g customarily supplied in the commercial mixed feed. Data in Table 1 show that the high level of sodium chloride decreased the amount of radioiodine in milk. This decrease was not as marked as that observed with 10 g of NaI. The milk/plasma ratio was lowered, however, and thus suggests that sodium chloride can affect the iodide-concentrating mechanism of the mammary gland.

An increase in the levels of thyroxine circulating in the blood can reduce the uptake of I^{131} by the thyroid gland. Premachandra and Turner (7), moreover, have shown that increasing of the thyroxine levels in blood decreases the amount of I^{131} found in milk; no explanation of the mechanism was offered. With this in mind, four cows were given daily intravenous injections of L-thyroxine and the blood and milk levels of I^{131} followed (Table 1). The data show that 10 mg of exogenous L-thyroxine per day reduced both the concentration of I^{131} in milk and the milk/plasma I^{131} ratio by about two. Since no change in the blood I^{131} level was noted while the milk I^{131} level decreased, it is possible that this action of the thyroxine was directly upon the mammary gland.

In these experiments the average levels of protein-bound I^{131} were 0.088 and 0.059% of a daily dose per liter for plasma and milk, respectively, during the control period. When the 10 mg of thyroxine were being given daily, these levels decreased to 0.032 and 0.027% of a daily dose per liter for the plasma and milk, in that order. Expressed on the basis of proportions the plasma protein-bound I^{131} decreased from 17.6 to 6.4%, whereas the milk protein-bound I^{131} changed from 5.4 to 4.5% as the result of the administration of thyroxine. Thyroxine administration thus reduced plasma protein I^{131} proportions by a factor of 2.8, but had relatively little effect on milk protein-bound I^{131}. During the thyroxine studies it was noted that levels of 5 to 10 mg of thyroxine per day increased milk production and provided evidence that the thyroxine preparation was active.

To supplement these observations two cows were given daily doses of D-thyroxine and 2-4 dinitrophenol intravenously (Table 1) and the levels of milk and plasma I^{131} followed. The D-thyroxine did not cause a decrease in the transfer of I^{131} to the milk; in fact, an increase was evident. Similarly, 2-4 dinitrophenol did not produce a decrease in the milk I^{131} levels.

Figure 1 plots the changes in milk, plasma, urine, and thyroid I^{131} of two cows before and after the start of daily oral administration of 10 g of $KClO_4$. It should be noted that the ordinate on the left refers to the I^{131} concentration in morning milk and blood plasma, whereas the ordinate on the right is the per cent of I^{131} in the thyroid and in the urine.

In these experiments $KClO_4$ was first administered at 4 PM; the next morning's milk sample showed the reduced I^{131} concentration and the

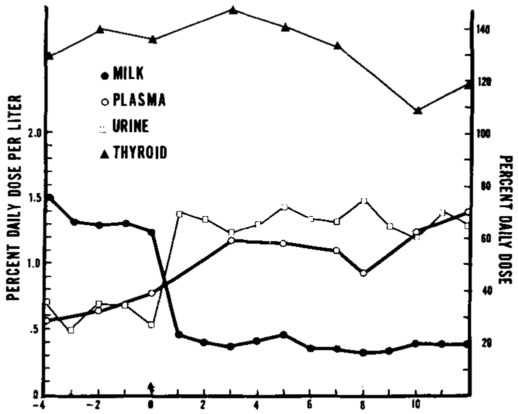

FIG. 1. Effect of $KClO_4$ upon the milk, plasma, urine, and thyroid I^{131} levels. The left ordinate refers to the I^{131} concentration in milk plasma and urine. The right ordinate indicates the I^{131} accumulation in the thyroid. All values are expressed as a percentage of the daily intake of radioiodine. Day 0 on the baseline is the day administration of perchlorate began.

urinary I^{131} was increased. These new levels were maintained during the entire course of perchlorate administration and attest to the rapidity and completeness of action of this level of perchlorate. Due to a loss of samples, it is uncertain how quickly the plasma I^{131} level rose. The rapid increase in urinary I^{131}, however, suggests that the plasma I^{131} may have risen much more rapidly than depicted in the graph.

From the graph (Figure 1) it is apparent that thyroid I^{131} did not show a noticeable decrease until some time after the start of perchlorate dosing.

Figure 2 is a plot of the data obtained for one of the four cows given L-thyroxine (Table 1). This animal was somewhat atypical, because the milk I^{131} varied widely and showed a downward trend during the control period. Data are presented, however, because they show that administration of L-thyroxine reduced the milk I^{131} levels and that upon stopping the thyroxine a rapid return of the radioiodine concentrations to that of the start of the experiment was noted. In agreement with the results of the other cows, it took about two days for the effect of thyroxine to become apparent. Increasing the amount of L-thyroxine from 5 to 10 mg resulted in only a slight further decrease in milk radioiodine. Twenty milligrams of thyroxine per day had no greater effect than 10 mg in another of these cows; moreover, at this high level the cow stopped eating and showed a marked drop in milk production. Figure 2 also shows that L-thyroxine reduced the milk/plasma I^{131} ratio. This ratio returned to near control levels when thyroxine dosing stopped.

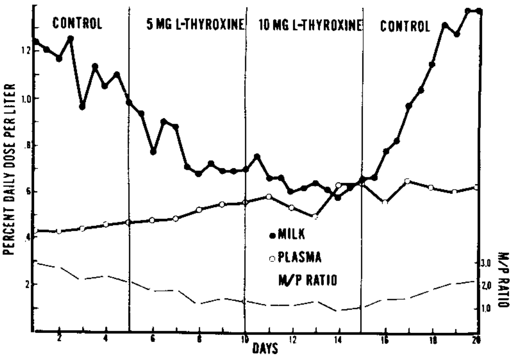

FIG. 2. Effect of daily injections of L-thyroxine upon the milk and plasma radioiodine concentrations.

In another experiment I^{130} was injected into one quarter of the mammary gland of a cow on the last day of the control period and again on the last day of a period of intravenous thyroxine administration and the blood I^{130} concentrations were followed. Each experiment was started about 2 hr after the morning milking. As seen from Figure 3, the I^{130} levels of blood rose more steeply and to greater heights during the control phase of the experiment than during the period when the cow was receiving 5 mg of thyroxine per day. Since thyroxine administration would tend to reduce thyroid I^{131} uptake, the lower blood values during the thyroxine-period are indicative of a lesser transfer of iodine from the mammary gland back to the body.

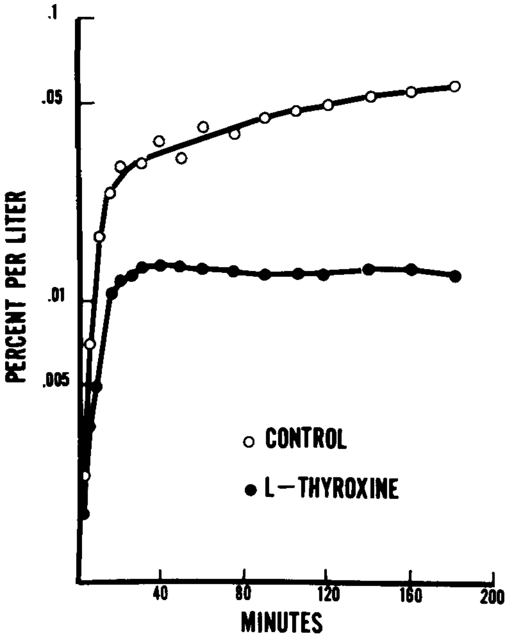

FIG. 3. Blood levels of I^{130} as a function of time after injection of I^{130} into the mammary gland of a cow.

DISCUSSION

At the outset it is important to restate that the radioiodine was given to these cows in twice-daily oral doses. In this way the levels of radioiodine in the various tissues tended to approach a steady state condition and any differences between milk and plasma were real and not artifacts due to time differences in production of milk or collection of samples.

It has been demonstrated in this paper that $KClO_4$ NaI, and L-thyroxine can produce marked decreases in the transfer of radioiodine from plasma to milk. Garner et al. (3) have shown that thiocyanate ion can act in the same manner. Concurrent with the decrease in milk radioiodine concentration was a decrease in the milk-I^{131}/plasma I^{131} ratio. Two possibilities suggest themselves as explanations for these observations. One is that the chemicals given to the cows had an effect upon the iodide-concentrating mechanism of the mammary gland. Another is that these chemicals induced the production of a new form of iodine not available for use in formation of milk.

An earlier study comparing the blood and milk levels of radioiodine after a single dose produced results that hinted at the presence of forms of iodine in blood which have different availabilities for milk production (4). This was a possibility only for cows kept on low-iodine diets for a long time; for cows on high levels of dietary iodine there was no evidence that any form of iodine other than plasma iodide contributed detectable amounts of radioiodine for milk formation. Even for the cows on the low-iodine diet the deviation was not marked; therefore, a new form of iodine could be present only in small amounts. To produce changes of the magnitude observed in the experiments reported in this paper, marked changes in the composition of blood iodine would have to be present. Data on blood iodine fractionation for the $KClO_4$ experiment of this paper and of a NaI experiment carried out previously (6) do not support this possibility.

Using $KClO_4$ (Figure 1), NaI (6), and NaSCN (3) the milk and blood levels of dairy cows were altered within 12 hr of administration of the chemical. Experiments by Brown-Grant using rabbits have shown that KSCN, $KClO_4$, and KI can alter the m/p ratio within 1 hr (1). It is not likely that the appearance of a new form of labeled iodine could take place so rapidly. Another factor that minimizes the probability of a new form of iodine is that this new form must transfer with difficulty across the cells of mammary gland and the thyroid, but must be able to be readily filtered by the kidney.

It has been well accepted that KI, KSCN, and $KClO_4$ act to interfere with the iodide-concentrating mechanism of the thyroid gland. Therefore, in addition to the qualifications detailed above, there is little reason to try to explain the action of these chemicals other than as an effect on the iodide-concentration mechanism of the mammary gland.

Data of Table 1 show that the effect of a substance such as $KClO_4$ is not adequately described simply by indicating how much milk radioiodine was decreased. Blood levels of radioiodine might increase and so minimize the magnitude of the change. When milk I^{131} values are divided by plasma I^{131} values to give a milk/plasma ratio, an index is achieved which described the over-all ability of the mammary gland to concentrate iodine during production of milk. Such a device has been used in expressing results in this paper. To be more precise, however, the ratio should be the milk I^{131}/plasma-nonbound I^{131} (4). If the proportion of plasma bound-I^{131} is small, and does not change markedly as a stress is applied to the system, then the milk-I^{131}/plasma-I^{131} is sufficient to chart the magnitude of changes, even though it underestimates, to some degree, the ability of the gland to concentrate radioiodine. Data in this and other publications have indicated that the milk/plasma I^{131} ratios in dairy cows can vary from 0.3 to somewhat more than 5. This upper value, however, is markedly less than the 20 or 30 observed in other species (2).

Changes in the milk/plasma ratios strongly suggest that the manner in which radioiodine concentration in milk was reduced was a direct action of the chemicals at the mammary gland. The depression of the iodide accumulation seems similar to that exhibited by the iodide-trap of the thyroid gland when acted upon by these substances. That the milk/plasma radioiodine ratio was severely depressed by these chemicals suggests that the accumulation of iodide in milk is at least in part an active process.

In the presence of the inorganic substances that inhibited iodide transfer the milk/plasma ratio was observed to be as low as 0.3. The presence of this radioiodine in milk under these conditions implies that a passive transfer of iodide into milk also takes place. That the milk/plasma ratio can be much less than one suggests that iodide does not readily diffuse across the membranes of the mammary gland during passive transfer. Milk/plasma ratios above 0.3, and certainly those above 1, may indicate that the active accumulation is operating to raise milk iodide levels up to or above that of the plasma. When the ratios are above 1, it can be assumed that the rate of transfer from plasma to milk exceeds that of the reverse movement. While it is evident that perchlorate, thiocyanate, and excess iodide can inhibit the active transfer from plasma to milk, it is not possible at this time to estimate their influence upon the reverse movement.

Of interest was the demonstration that thyroxine could bring about a reduction in the ability of the mammary gland to concentrate iodide in milk.

That the thyroxine took approximately two days to produce its effect upon milk radioiodine is evidence that its mechanism of action may differ in some way from that of the inorganic anions. This action of thyroxine is unexplained at this time, but preliminary experiments upon three cows seem to indicate that it may not be mediated through thyroid-stimulating hormone. Daily injection of up to ten units of TSH had no consistent effect upon the milk/plasma I^{131} ratio in these cows. The lack of effect of 2-4 dinitrophenol means that more than a phenolic group is necessary to elicit the thyroxine response. That D-thyroxine increased the milk/plasma ratio, in contrast to the action of L-thyroxine, suggests a specificity for L-thyroxine.

Data of Figure 3 show that thyroxine can reduce the movement of iodide from milk back to plasma. For thyroxine to produce an overall reduction in the accumulation of iodide in milk, it is also necessary for the transfer of iodide from plasma to milk to be reduced. This evidence is only qualitative; quantitative measurements have yet to be made.

An interesting observation during the thyroxine experiments was that there was a definite maximum response that could be elicited by the exogenous thyroxine. The data show that the per cent of I^{131} per liter of milk and the milk/plasma I^{131} ratio could be decreased to only about 50% of the control value. In view of the low milk/plasma ratios sometimes encountered in normal cows, and in cows given substances such as perchlorate, the limited effect of thyroxine suggests that the hormone is not the sole control of the mammary iodide-concentration mechanism.

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