pretreatment level, and the graphs for the yields of milk and milk fat showed distinct minima. During this last period of the experiments tissue anabolism evidently took precedence over milk secretion in its claim for available nutrients.

The foodstuffs used in these experiments were, with the exception of groundnut, such as can readily be grown in Britain. It might, therefore, at times be economic to obtain a temporary increase of the milk supply by the feeding of iodinated casein, the action of which is very similar to that of thyroxine. Although such feeding would temporarily increase catabolism, any consequent ill effects would be largely overcome by the stimulus to anabolism which occurs when the treatment is discontinued. Any subsequent loss of efficiency by the cow would have to be balanced against the temporary gain of milk yield without any increase in the number of cows in the herd, in the man-hours or in the materials other than foodstuffs required for its production.

SUMMARY

1. The effect of injecting 10 mg. thyroxine/day for 4 weeks into cows at three different levels of food intake has been investigated. The resultant increase in milk yield was accompanied by an increase in pulse rate, by loss of weight, by markedly negative nitrogen balance and by an increase in the excretion of water and creatine in the urine. The percentage of fat in the milk was increased and small but statistically significant increases were observed in the percentage of solids and of nitrogen in the fat-free milk.

2. Thyroxine appeared to stimulate lactation by increasing the cows' catabolism, and this resulted in negative nitrogen balances.

3. The negative nitrogen balances could be inhibited by increasing the intake of food.

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The Effect of Thyroxine on the Metabolism of Lactating Cows

2. CALCIUM AND PHOSPHORUS METABOLISM

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In the previous paper (Owen, 1948), experiments have been described on the effect of thyroxine injections on the nitrogen metabolism of lactating cows. During these experiments, data for the calculation of calcium balances were obtained for all the cows, and data for phosphorus balances for two of them. The purpose of the present communication is to report the effect of thyroxine on the calcium and phosphorus contents of the milk, and also on the

calcium and phosphorus metabolism of the cows when thyroxine was administered with the object of increasing the milk yield.

EXPERIMENTAL

The general plan of the experiments and the nature of the rations have been described already (Owen, 1948). There were three experiments, each comprising three periods. The intention was to have three cows in each experiment, but in

the last experiment only two of the three completed the required period in the metabolism house. There were, therefore, eight animals in all. In the first and third periods of each experiment, no thyroxine was given, but in the second period two of the cows were injected with 10 mg. thyroxine daily for at least 28 days. The third cow acted as a control and received no thyroxine in any of the periods. For all eight animals Ca was determined in the food, milk, urine and faeces, and from these data the Ca balances were calculated for each period. For the two animals in the third experiment the same was done for P.

Methods. Owing to the amount of work involved in metabolism studies of this type with large animals, the Ca and P analyses had to be carried out after the main experimental work was finished. It was necessary, therefore, to store samples of the food, milk, urine and faeces. Milk samples were preserved with formaldehyde, faeces were

RESULTS

Milk composition. The average figures for the content of calcium and phosphorus in the fat-free milk are shown in Table 1. Inspection and statistical analysis of these results showed that the variations which occurred in the calcium content could not be attributed to the administration of thyroxine. Thus, with cows 1, 3, 6 and 7, there was a slight increase in the calcium content during thyroxine treatment as compared with the preceding period; whereas, with cows 4 and 8, there was a slight decrease. Of the control cows, no. 2 showed a slight decrease and no. 5 a slight increase in calcium content during period 2 as compared with period 1.

Table 1. *The calcium and phosphorus contents of the fat-free milk before, during and after thyroxine treatment*

(Thyroxine was given during period 2, except to the control cows 2 and 5.)

Exp. no.	Cow no.	Calcium (g./100 ml.)			Phosphorus (g./100 ml.)		
		Period			Period		
		1	2	3	1	2	3
1	1	0.104	0.115	0.130	—	—	—
	2 (control)	0.114	0.109	0.127	—	—	—
	3	0.107	0.115	0.117	—	—	—
2	4	0.125	0.119	0.125	—	—	—
	5 (control)	0.090	0.094	0.095	—	—	—
	6	0.130	0.138	0.142	—	—	—
3	7	0.128	0.142	0.130	0.0945	0.1089	0.0966
	8	0.141	0.133	0.134	0.0946	0.1106	0.1008

stored dry in stoppered bottles and urine samples were kept in a refrigerator. Before analysis the urine samples were made acid to dimethylaminoazobenzene (Töpfer's reagent, pH 2.9) by additions of 6N-HCl. By this means any deposits of Ca and phosphate were dissolved, and copious precipitation of uric acid occurred. Analysis of this precipitate, however, showed that it did not contain any Ca or P. Every second 2-day sample of food, milk and excreta was analyzed.

Analytical methods used were identical with those employed previously in human balance experiments (Owen, 1939). The samples were ashed in vitreosil basins in an electric muffle furnace which was thermostatically controlled so that its temperature did not exceed 600°. 10% Mg(NO₃)₂ was added to food, milk and faeces to prevent loss of P by volatilization, and the samples were dried on a sand bath before being placed in the muffle furnace. The ash was dissolved in warm 6N-HCl and made up to a known volume. After silica had been allowed to settle overnight, samples were taken for analysis. With urine it was found impracticable to estimate Ca and P on the same sample of ash. This was because, on using Mg(NO₃)₂ to bind the P, serious deflagration occurred when the mixture was dried, whilst with MgCl₂, the resulting glassy ash caused the basins to crack on cooling. For estimating P, therefore, the urine was digested with H₂SO₄ and 30% H₂O₂ (Horecker, Ma & Haas, 1940), and for the estimation of Ca the urine was ashed in the muffle furnace without addition of magnesium salts. The final stage in the estimation of calcium was the titration of Ca oxalate with KMnO₄. In the P estimation the strengths of the coloured solutions were determined in a Spekker absorptiometer.

The phosphorus results were obtained for two cows only, but with both of them, there was a marked increase in the phosphorus content of the fat-free milk during thyroxine treatment; and after treatment ceased the values tended to return towards their pre-thyroxine level. The increases of 15 and 17%, which occurred during hormone treatment as compared with the pre-thyroxine level, were statistically significant.

Calcium metabolism. During all periods of the experiments (Table 2), the cows were in negative calcium balance, except for cow 4 which showed a small positive balance of 0.9 g. Ca/day in the period after thyroxine treatment ceased. The type of fluctuation occurring from one 4-day period to another, as found in Exp. 3, can be seen from Fig. 1. It is clear that the output of calcium in the urine did not vary appreciably, and that it was not affected by thyroxine treatment.

It will be noted that a minimum occurred in the graph of calcium intake (Fig. 1). This resulted from the fact that the chief source of calcium in the diet was the hay, and during that particular period of Exp. 3 the calcium content of the hay was low. The apparent digestibility of the calcium is shown in Table 2. The values became negative in periods 2 and 3 of the third experiment.

Table 2. *The apparent digestibility of calcium and a summary of the results required to obtain the calcium balances*

(Thyroxine was given during period 2, except to the control cows 2 and 5. All results which are average figures are given in g. Ca/day.)

Exp. no.	Cow no.	Period	Ca intake	Output of Ca			Total output	Balance	Apparent* digestibility of Ca
				Milk	Urine	Faeces			
1	1	1	14.6	9.6	0.6	11.1	21.3	- 6.7	24.1
		2	14.6	8.3	0.4	12.5	21.2	- 6.4	15.0
		3	14.6	5.1	0.9	11.9	17.9	- 3.3	19.0
	2 (control)	1	15.2	13.2	0.9	11.0	25.1	- 9.9	27.8
		2	15.2	11.8	0.8	9.1	21.7	- 6.5	40.5
		3	15.2	11.5	0.6	9.8	21.9	- 6.7	35.6
	3	1	14.9	12.4	1.2	9.4	23.0	- 8.1	37.2
		2	14.9	13.1	1.8	9.1	24.0	- 9.1	38.9
		3	14.9	7.3	2.6	7.3	17.2	- 2.3	51.0
2	4	1	18.3	10.4	0.7	12.8	23.9	- 5.6	30.4
		2	19.3	10.0	1.1	16.0	27.1	- 7.8	16.9
		3	19.2	5.5	1.2	11.6	18.3	+ 0.9	39.8
	5 (control)	1	19.6	8.9	1.5	12.7	23.1	- 3.5	34.8
		2	19.1	7.6	2.0	14.4	24.0	- 4.9	24.6
		3	18.9	7.4	2.0	13.9	23.3	- 4.4	26.8
	6	1	20.3	14.8	1.5	13.5	29.8	- 9.5	33.5
		2	20.3	15.4	0.7	14.2	30.3	- 10.0	29.7
		3	20.2	8.2	1.2	13.2	22.6	- 2.4	35.0
3	7	1	41.0	21.6	1.8	33.8	57.2	- 16.2	17.2
		2	40.0	26.8	1.5	40.9	69.2	- 29.1	- 2.2
		3	31.4	15.1	1.5	36.8	53.4	- 22.0	- 17.2
	8	1	39.4	20.6	1.8	31.4	53.8	- 14.4	20.3
		2	36.2	18.3	1.2	38.7	58.2	- 22.0	- 6.9
		3	25.6	7.5	1.7	35.1	44.3	- 18.7	- 37.1

$$* 100 \frac{(\text{Intake of Ca} - \text{faecal Ca})}{\text{Intake of Ca}}$$

Fig. 1. The effect of thyroxine on the output of calcium in milk, urine and faeces. The calcium intake is also shown.

Phosphorus metabolism. The data obtained for calculating the phosphorus balances for cows 7 and 8 are shown in Table 3. The balances were all positive throughout the whole of Exp. 3, and in this respect they resembled the nitrogen balances rather than the calcium balances.

Table 3. *The apparent digestibility of phosphorus and a summary of the results required to obtain the phosphorus balances in Exp. 3*

(Thyroxine was given during period 2. All results which are average figures are given in g. P/day.)

Cow no.	Period	Intake of P	Output of P			Total output	Balance	Apparent* digestibility of P
			Milk	Urine	Faeces			
7	1	51.3	15.8	0.25	30.5	46.6	+ 4.7	40.6
	2	66.2	20.1	0.62	35.4	56.1	+ 10.1	46.6
	3	51.2	11.1	0.41	31.6	43.1	+ 8.1	38.3
8	1	45.7	14.2	0.18	27.3	41.7	+ 4.0	40.3
	2	55.0	15.0	0.58	29.3	44.9	+ 10.1	46.7
	3	35.9	5.8	0.44	23.4	29.6	+ 6.3	34.8

$$* 100 \frac{(\text{Intake of P} - \text{faecal P})}{\text{Intake of P}}$$

The outputs of phosphorus in the milk, faeces and urine were all greater in the thyroxine period than in the periods preceding and following hormone treatment. Unlike the corresponding results for calcium, the urinary output of phosphorus was very markedly increased, but this made little difference to the total excretion since the proportion of the output accountable to the urine was very small. In spite of the fact that the output of phosphorus was increased during the thyroxine period, the balances showed that the amount of phosphorus retained by the cows was also greatest in that period. It was possible to have increases occurring simultaneously in both output and retention in period 2, since during that period extra rations were fed according to the increase in milk production, and this led to a greater intake of phosphorus. The apparent digestibility of the phosphorus (Table 3), unlike that of calcium (Table 2), was higher during the thyroxine period than in the other two periods.

DISCUSSION

Negative calcium balances in lactating animals in early or mid-lactation are quite commonly observed (Mackintosh, 1938; Garry & Wood, 1946). It is not surprising to find, therefore, that with the animals used in the present work, the calcium balances were almost all negative, the only exception being the value for cow 4 in the third period of Exp. 2 which was slightly positive. In the first two experiments the balance in the first period was on the whole very similar to that during thyroxine treatment, but a big decrease in negative balance occurred after thyroxine administration had ceased. This can be

attributed largely to the decrease in milk yield, which resulted in a diminished output of calcium in the milk during the third period. In the third experiment, the negative balances were more marked during thyroxine treatment than either before or after treatment. This was due mainly to an increase

in the output of faecal calcium during the thyroxine period. Indeed, it can be seen from Fig. 1 that faecal output of calcium was closely paralleled by total output. Before these results were obtained, it was expected, from the work of Hunter (1930) on humans, that thyroxine treatment would cause all the cows to exhibit a marked increase in the amount of calcium excreted in the urine. This, however, did not occur. Owing to the bulkiness and relative indigestibility of their food as compared with that of non-vegetarian animals, cows void more water in their faeces than in their urine, and also, their urine tends to be more alkaline. Possibly it is due to both these facts that the faecal but not the urinary excretions of calcium were increased. The absence of effect of thyroxine on the urinary excretion of calcium, and its effect on the faecal calcium, is well shown with cow 7 in Fig. 1. The corresponding graphs for cow 8 were very similar. The only cow which showed an increased output of urinary calcium during thyroxine treatment, the increase continuing after thyroxine injections ceased, was cow 3, and it was this cow which excreted an exceptionally large volume of urine in the latter part of period 2 and the early part of period 3 (Owen, 1948).

The results for the apparent digestibility of the dietary calcium, which are shown in Table 2, are very low. Indeed with cows 7 and 8, negative values were obtained in the second and third periods of Exp. 3, more calcium having been voided in the faeces than had been taken in in the food. Hunter (1930) records that Aub observed this same phenomenon in humans. In these two cows, therefore, thyroxine must have brought about a large increase in the production of faecal metabolic calcium, and

even after thyroxine treatment had ceased, the excretion of calcium relative to the amount taken in still remained very high.

These negative calcium balances raise the question as to whether cows on winter rations, to whom thyroxine is being administered either as the hormone itself or as iodinated casein, should be given some additional source of readily assimilable calcium. Even if negative calcium balances could not be entirely prevented by dietary measures, it is reasonable to expect that extra dietary calcium would help to replace the loss engendered by increased excretion in the faeces. If this extra loss of calcium in cows treated with thyroxine were not replaced, the cows might have to start their next lactation with depleted stores of mobilizable calcium. Progressive chronic depletion of the skeleton by successive reproductive cycles of pregnancy and lactation on diets containing suboptimal amounts of calcium has been recorded in so many species of animals by many observers, that an extra drain caused by the administration of thyroxine should not be allowed to go uncompensated. Some source of extra readily assimilable calcium such as steamed bone flour or defluorinated rock phosphate, or fish meal, could with advantage be included in the rations of any cow to which iodinated casein or other source of exogenous thyroxine is to be fed.

Since some 99 % of the calcium and some 70–80 % of phosphorus in the animal body are in the skeleton (McCollum, Orent-Keiles & Day, 1939), it is perhaps surprising to find that the phosphorus metabolism was so different from that of the calcium. The phosphorus balances in Table 3 were all positive, and they were more strongly positive in the thyroxine period than in either of the other two periods. Unlike that of the calcium, the apparent digestibility of the

phosphorus was increased during thyroxine treatment.

It would appear from these results that phosphorus deficiency is not likely to occur in cows which are receiving thyroxine or iodinated casein, but this conclusion can be put forward only tentatively since it is realized that the results on which it is based were obtained with only two cows.

SUMMARY

1. Eight Ayrshire cows in mid-lactation were used to determine the effect of thyroxine administration on the metabolism of calcium and phosphorus. With one exception, the calcium balances were negative both for the treated and for the control animals throughout all the experiments.

2. With two of the cows which were fed more liberally than the others, the output of calcium was greatest in the thyroxine period, and this was attributable to an increase in the faecal metabolic calcium resulting from the hormone treatment. There was no increased output of calcium in the urine.

3. The apparent digestibility of the dietary calcium was found to be very low. This, together with the negative balances, might well result in thyroxine treatment leading to a depletion of calcium reserves which would be dangerously excessive unless dietary measures were taken to make good the loss.

4. Unlike those of calcium, the phosphorus balances (obtained for two cows only) were positive throughout the whole experiment, thyroxine actually tending to increase the amount of phosphorus retained.

5. The calcium content of the milk was not affected by thyroxine, but the phosphorus content was significantly increased.

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