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The Digestibility of Carotene by the Cow and the Goat as Affected by Thyroxine and Thiouracil

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It has been shown at this Institute that thyroxine affects the amounts of certain nutrients secreted in milk (Owen, 1948; Chanda & Owen, 1949; Chanda, McNaught & Owen, 1949) and that thiouracil produces opposite effects. Cama & Goodwin (1949) showed that thyroxine inhibited whilst thiouracil enhanced the loss of ingested carotene in the faeces of the rat. The present experiments were made to ascertain whether such effects were reproducible in the lactating cow and the lactating goat.

EXPERIMENTAL

(1) An experiment was carried out with six cows. It had already been shown in the goat (Chanda, Clapham, McNaught & Owen, 1951) that the Cr ratio method is as accurate as the direct method for determining the digestion of carotene. Cr_2O_3 was therefore mixed with the food fed, so as to measure the digestibility of carotene in the cows without removing them from the byre. Representative samples (1 to 2 lb.; 1 lb. = 0.45 kg.) of faeces voided between the morning milking and 11 a.m. were taken every 2nd day and analysed immediately for carotene. The cows ate only the experimental diet and were fed alternately on carotene-containing and carotene-free concentrate mixtures (compositions shown in Table 1) at the rate of 21 lb. of mixture/day

in two equal portions with 10 lb. oat straw/day as roughage, irrespective of which mixture was being fed. The sole source of carotene in the rations was the dried grass meal in mixture 1 which also contained 0.1% Cr_2O_3 dispersed, as described by Chanda *et al.* (1951). Mixture 2 (designed to be free from carotene) contained blood meal, potato starch and ground straw so as to simulate the content of protein, carbohydrate and fibre in the grass meal.

Table 1. Composition of carotene-containing and carotene-free diets for cows

Components	Parts by weight	
	Mixture 1* (containing carotene)	Mixture 2 (carotene-free)
Oats (bruised)	9	9
Bean meal	6	6
Grass meal	6	—
Potato starch	—	2.5
Blood meal	—	0.9
Ground straw	—	2.5

* Mixture 1 contained 2100 g. Cr_2O_3 in 31.5 cwt.

There were five periods. During periods 1 and 3 the carotene-free mixture was fed, and during periods 2, 4 and 5 the mixture containing grass meal was fed. During period 4

one pair of cows received thyroxine (10 mg./cow/day) by subcutaneous injection, a second pair similarly received thiouracil (20 mg./cow/day). The third pair were controls. The treatments will be apparent from Fig. 1.

(2) Six goats were kept in metabolism crates (Chanda *et al.* 1951), specially designed so that the animals could be readily milked. The crates were provided with attachments for quantitative separation of faeces from urine. The goats were on the same diet throughout the experiment. The concentrate mixture consisted of bruised oats (9 parts by wt.), dried grass meal (6 parts) and ground nut meal (6 parts). Each goat received 3 lb./day of this mixture in three equal portions. In addition 1 lb./goat/day oat glumes in two equal portions was given as carotene-free roughage. The grass meal was the sole source of carotene. There were three periods in the experiment; 1 and 3 acted as control periods. During period 2, one pair of goats received thyroxine by subcutaneous injection (10 mg./goat/day), a second pair received thiouracil injections (20 mg./goat/day), while the remaining pair served as controls.

Methods of analysis. β -Carotene was determined in the non-saponifiable fractions of the fat of 2 g. food or 10 g. moist faeces by the method of Seshan & Sen (1942). Extracted carotenoids were purified by chromatography in light petroleum (b.p. 40–60°) on 5 × 1 cm. columns of alumina (Savory and Moore Ltd.), β -carotene being eluted by 3% acetone in light petroleum. Eluates were transferred to *n*-hexane, and made up to 50 ml., prior to determination of their extinction in a Unicam spectrophotometer. β -Carotene was calculated by assuming $E_{1\text{ cm}}^{1\%} = 2500$ (Morton, 1942).

After washing through with 10 ml. light petroleum the alumina columns were used again for four or five more samples. Gentle suction from a filter pump was used during chromatography, but care was taken to replenish with solvent before the tops of the columns became exposed. To keep the columns free from traces of water a little anhydrous Na_2SO_4 was placed at the top.

Cr was estimated as described by Chanda *et al.* (1951).

RESULTS

Experiment 1

The average carotene and chromium contents of the concentrate mixtures fed to the cows are shown in Table 2. There was a gradual loss of carotene in the mixture as the experiment progressed, so a fresh mixture was made at the beginning of each period using dried grass which had been stored in brown-paper bags. The average carotene contents from the beginning to the end of periods 2, 4 and 5 respectively were 5.37–4.85, 5.30–5.03 and 5.20–4.78 mg./100 g. The higher concentrations corresponded to the beginning of each period. The coefficients of variation of chromium were 15.9, 10.2 and 4.2% in periods 2, 4 and 5 respectively. The three mixtures were made in numerical order as they were needed so that Table 2 shows that efficiency of mixing increased with experience. The carotene chromium ratios used in calculating the digestibilities in Table 4 are shown in Table 2.

Table 2. The carotene and chromium contents of carotene-containing diet for cows

	Period 2 Period 4 Period 5		
Carotene (mg./100 g.)	5.2	5.2	5.1
Coefficient of variation*	5.1	2.2	4.6
Chromium (mg./100 g.)	69.2	69.1	71.6
Coefficient of variation*	15.9	10.2	4.2
Carotene (mg.)/chromium (g.)	75.1	74.7	71.7

* Coefficient of variation = $100 \times \text{standard deviation} / \text{mean}$.

The faecal excretions of carotene of three of the cows, one receiving thyroxine, one receiving thiouracil and one receiving no hormonal treatment are shown in Fig. 1. The data for the remaining three

Fig. 1. Effect of thyroxine and thiouracil on the faecal excretion of carotene by cows.

cows were very similar and graphical illustrations have been omitted for brevity. Initially the concentration of carotene in the faeces of the cows varied from 2.5 to 3.8 mg./100 g. dry faeces. When the carotene-free ration was given the concentration dropped in 4 or 5 days to 100 $\mu\text{g.}/100$ g. dry matter. When the carotene diet was again given the faecal carotene rose rapidly and became relatively constant in 6–8 days. The variation of faecal carotene from one cow to another (5.2–5.7 mg./100 g.) was

not very great. In period 4, during which hormonal treatments were given immediately after the carotene-free period 3, the rates of reappearance of faecal carotene were markedly affected by thyroxine. Thiouracil caused an equally marked but opposite effect (Fig. 1). These rates of reappearance have been expressed in numerical form in Table 3 which records

Table 3. *The effect of carotene-containing diet subsequent to carotene-free diet on the rate of reappearance of faecal carotene in cows*

Cow no.	Treatment in experimental period 4	Rate of increase in the faecal carotene concentration ($\mu\text{g./day}$)	
		Pretreatment period 2	Experimental period 4
15	None	266	247
16	None	286	271
17	Thyroxine	272	158
18	Thyroxine	277	188
19	Thiouracil	279	353
20	Thiouracil	284	360

the slopes (expressed as $\mu\text{g.}$ increase of faecal carotene/day) of straight lines fitted by the method of least squares to the results for faecal excretion in periods 2 and 4. During period 2 the rates of reappearance of faecal carotene (Table 3) were comparable for all six cows, the average being $277 \mu\text{g./day}$ with a coefficient of variation of $\pm 2.7\%$. During treatment, these rates of reappearance of faecal carotene were reduced by thyroxine to

$158 \mu\text{g./day}$ with cow 17 and $188 \mu\text{g./day}$ with cow 18, and increased by thiouracil to $353 \mu\text{g./day}$ with cow 19 and $360 \mu\text{g./day}$ with cow 20.

When hormonal treatments were discontinued in period 5, the faecal carotene remained fairly constant in the controls. By contrast it increased rapidly in the cows which had received thyroxine and decreased rapidly in those which had received thiouracil (Fig. 1).

The average faecal carotene concentrations/100 g. dry faeces in periods 2, 4 and 5 of Exp. 1, when carotene was present in the diet, are shown in Table 4, together with carotene intakes and apparent digestibilities of carotene determined by the chromium sesquioxide method. The table clearly demonstrates that thyroxine increased while thiouracil diminished the digestibility of carotene by the cows.

Experiment 2

In the goat experiment, as in that with the cows, there was a progressive decrease in the concentration of carotene in the concentrate mixture which necessitated its being frequently analysed during the experiment. The concentration decreased progressively (Fig. 2), 24% of the original potency being lost in 40 days. One lot of concentrate mixture was used throughout the experiment and there were no changes of dietary regime.

The dose of thyroxine (10 mg./day) used by Meites & Turner (1948) was injected, but was too large and toxic effects supervened. One of the goats receiving

Table 4. *The effect of thyroxine and thiouracil on the faecal excretion and apparent digestibility of carotene by cows*

Cow no.	Period no.	Treatment in experimental period 4	Carotene intake (mg./2 days)	Faecal carotene (mg./100 g. dry matter)	Faecal Cr (mg./100 g. dry matter)	Carotene (mg./g. Cr in faeces)	Carotene excreted (mg./2 days)*	Apparent digestibility (%)
15	2	—	976.8	4.49	145.2	30.92	401.9	58.9
	4	None	978.3	4.25	134.6	31.58	413.8	57.7
	5	—	946.0	4.28	132.8	32.24	425.7	55.0
16	2	—	948.0	4.58	127.6	35.89	452.8	52.2
	4	None	947.4	4.29	133.6	32.11	407.4	57.0
	5	—	967.5	4.57	145.2	31.48	425.1	56.0
17	2	—	972.6	4.71	132.5	35.55	460.2	52.7
	4	Thyroxine	957.3	2.49	110.4	22.55	289.1	69.8
	5	—	957.3	4.65	153.8	30.24	404.0	57.8
18	2	—	963.4	4.79	136.3	35.14	450.5	53.2
	4	Thyroxine	980.4	2.45	122.4	20.02	262.8	73.2
	5	—	950.0	4.79	146.9	32.60	432.3	54.5
19	2	—	967.5	4.71	144.9	32.51	418.6	56.7
	4	Thiouracil	982.5	5.50	150.6	36.51	480.4	51.1
	5	—	928.5	4.85	145.9	33.24	430.8	53.6
20	2	—	928.5	4.60	137.0	33.58	414.9	55.3
	4	Thiouracil	973.2	5.35	137.2	38.98	508.0	47.8
	5	—	972.6	4.78	152.3	31.38	426.0	56.2

* Carotene intake $\times \frac{\text{mg. carotene/g. Cr in faeces}}{\text{mg. carotene/g. Cr in food (Table 2)}}$

Table 5. *The effect of thyroxine and thiouracil on the faecal excretion and apparent digestibility of carotene by goats*

Goat no.	Period no.	Treatment in period 2	Carotene, mg./100 g. food	Carotene intake (mg./2 days)	Faecal carotene (mg./100 g. dry matter)	Faecal carotene (mg./2 days)	Apparent digestibility (%)
1	1	—	3.14	83.3	2.54	30.8	63.0
	2	None	2.97	74.1	2.35	27.4	62.3
	3	—	2.71	59.6	2.09	20.9	64.5
2	1	—	3.14	56.1	2.18	21.1	62.9
	2	None	2.97	49.0	2.05	18.9	60.7
	3	—	2.71	27.1	1.39	10.2	61.3
3	1	—	3.14	72.9	2.05	26.8	62.9
	2	Thyroxine	2.97	41.8	0.99	10.3	74.1
	3	—	2.71	65.5	2.52	27.9	57.7
4*	1	—	3.14	69.9	1.97	23.8	66.0
	2	Thyroxine	2.97	23.9	1.31	6.2	73.9
5	1	—	3.14	85.5	2.24	26.8	68.4
	2	Thiouracil	2.97	79.6	3.42	36.8	53.8
	3	—	2.71	73.7	2.49	27.9	62.1
6	1	—	3.14	82.7	2.97	32.1	61.6
	2	Thiouracil	2.97	65.5	3.37	34.8	48.0
	3	—	2.71	55.6	2.20	19.6	61.3

* This animal died from hyperthyroidism.

thyroxine died of hyperthyroidism. Nevertheless, in the goats as in the cows, the effects of thyroxine on carotene metabolism were very marked and in the opposite direction to those of thiouracil. The changes in the digestibility of carotene in the goats were of the same kind as those in the cows. The average results of the experiment are shown in Table 5, in which the digestibilities of carotene obtained by the

Fig. 2. Loss of carotene on storage in the concentrate mixture fed to the goats.

direct method are recorded. Table 5 also shows that thyroxine enhanced whilst thiouracil diminished the digestibility of carotene. It also shows particularly well how thyroxine reduced and thiouracil increased not only the total amount, but also the concentration of carotene in the faeces of the goats.

DISCUSSION

Cama & Goodwin (1949) found that orally administered thiouracil increased whilst thyroxine diminished the faecal carotene of rats. It would be

reasonable to suppose that drugs so given would soon reach the intestinal mucosa and thus be more active in affecting an enzyme system in the intestinal wall (Glover, Goodwin & Morton, 1948; Thompson, Ganguly & Kon, 1949) than the same drugs subcutaneously injected. The present results, however, show that the effects of the drugs (1) are independent of the route of administration, (2) can be produced not only in the rat, but in both the lactating goat and the lactating cow, and (3) are not inhibited by the large fibre intake of the ruminant. If the sole site of conversion of carotene to vitamin A is the intestinal wall (Glover *et al.* 1948; Thompson *et al.* 1949) these results demonstrate hormonal control of an enzyme reaction occurring in the intestinal mucosa.

The present authors (Chanda *et al.* 1951) showed that the goat digests carotene better than does the cow. It is now found that thyroxine increases the power of the cows to digest carotene more than the power of the goats to digest carotene. On the other hand, thiouracil decreases the power of the goats to digest carotene more than the power of the cows to digest carotene. These findings are consistent with the view of Schultz & Turner (1945) that the goat thyroid gland is more active than that of the cow.

It could be argued that failure of carotene to appear in the faeces does not necessarily mean that more has been absorbed, but the observations of Seshan & Sen (1942) and of Goodwin & Gregory (1948) do not support such a criticism. Thus Goodwin & Gregory showed that carotene was not

destroyed by incubation with rabbit intestinal contents at 37°, whilst Seshan & Sen found no loss of carotene similarly incubated with cattle faeces.

The decreased concentrations of chromium in the faeces of the thyroxine-treated cows (Table 4) while intake of concentrates and roughages remained constant indicated that the digestibility of dry matter was decreased by thyroxine. A similar observation was also made earlier by Owen (1948). The digestibility of carotene was thus increased at a time when the digestibility of dry matter was reduced. This observation is consistent with the hypothesis (Glover *et al.* 1948) that carotene is converted to vitamin A in the intestinal wall.

The evidence presented here implies that the rate of conversion of carotene to vitamin A by the cow and by the goat depends on the thyroid status of the animal. Most experiments with rats indicate that 0.6 µg. β-carotene = 1 i.u. (0.3 µg.) of vitamin A (Rosenberg, 1945), but the present experiments suggest that the relationship will depend on the thyroid status of the animal.

Since the first detectable lesions of partial vitamin A deficiency in rats occur in the central nervous system (Irving & Richards, 1938), it is possible that cretinism in humans may be a result of suboptimal intake of carotene when the thyroid is damaged.

SUMMARY

The effects of thyroxine and thiouracil injections on the metabolism of carotene in the cow and the goat have been investigated with the following results.

1. Subjection of lactating cows to a diet free from carotene reduced faecal carotene to a trace in 6–8 days. Reinstatement of the carotene regime caused a rapid reappearance of faecal carotene.

2. Reappearance of faecal carotene after reinstatement of the carotene-containing diet was made markedly earlier by thiouracil and markedly later by thyroxine.

3. The digestibility of carotene by both cows and goats was markedly increased by thyroxine and markedly reduced by thiouracil.

4. Goats digested carotene more efficiently than did cows. The increase of digestibility of carotene resulting from thyroxine treatment was greater in the cows than in the goats. Conversely, the decrease due to thiouracil was greater in the goats than in the cows.

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