SUMMARY

1. Rats have been made deficient in riboflavin on a diet free from riboflavin and containing 1% succinylsulphathiazole and the amine oxidase and D-amino-acid oxidase of liver extracts have been determined.

2. It has been confirmed that the D-amino-acid oxidase of the liver is rapidly and severely reduced

in riboflavin deficiency and that it is rapidly and fully restored upon the administration of riboflavin.

3. The amine oxidase activity of the liver is about halved in riboflavin deficiency. There is no rapid restoration of enzymic activity on giving riboflavin. If inositol is present in the diet, some restoration of amine oxidase occurs when riboflavin is administered.

REFERENCES

- Axelrod, A. E., Sober, H. A. & Elvehjem, C. A. (1939). *Nature, Lond.*, **143**, 281.
 Axelrod, A. E., Sober, H. A. & Elvehjem, C. A. (1940). *J. biol. Chem.* **134**, 749.
 Best, C. H., Lucas, C. C., Patterson, J. M. & Ridout, J. H. (1951). *Biochem. J.* **48**, 452.
 Carpenter, K. J., Harris, L. J. & Kodicek, E. (1948). *Brit. J. Nutrit.* **2**, vii.
 Kapeller-Adler, R. (1949). *Biochem. J.* **44**, 70.
 McFarland, M. L. & McHenry, E. W. (1948). *J. biol. Chem.* **176**, 429.
 Ochoa, S. & Rossiter, R. J. (1939). *Biochem. J.* **33**, 2008.
 Rossiter, R. J. (1940). *J. biol. Chem.* **135**, 431.
 Warburg, O. & Christian, W. (1938). *Biochem. Z.* **298**, 150.
 Zeller, E. A., Stern, R. & Wenk, M. (1940). *Helv. chim. Acta*, **23**, 3.

The Effect of Thyroxine and Thiouracil on the Composition of Milk

2. THE CAROTENE AND VITAMIN A CONTENT

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The interactions between the utilization of carotene as a provitamin A and the activity of the thyroid gland have been much studied both for animals and for man (Kunde, 1926; Lindquist, 1938; Thomson *et al.* 1939; Drill, 1943). Experiments with rats provide a basis for the connexion (long known to clinicians and quoted by Lindquist and by Drill) between carotene and the thyroid in certain types of human illnesses. Thus it has been shown in rats (Johnson & Baumann, 1947) that thyroxine accelerates repletion of hepatic stores of vitamin A from dietary carotene, whilst thiourea has the reverse effect. Thyroxine also accelerates the depletion of hepatic stores of rats deprived of dietary carotene whilst thiourea again has the reverse effect (Kelley & Day, 1948). Furthermore, Cama & Goodwin (1949) have correlated these effects with those produced by the hormone and by a goitrogen on the faecal excretion of dietary carotene in the rat.

For many years effects of thyroxine injections on the composition of milk have been studied at this Institute (Smith & Dastur, 1940; Owen, 1948; Chanda & Owen, 1949) and it was thought that the influence of thyroxine on the liver reserves of vitamin A of animals and humans might have a parallel in its effects on the concentration of vitamin

A and carotene in milk. Fellenberg & Grüter (1932) and Fasold & Heidemann (1933) claimed that thyroidectomy caused carotene to appear in goat's milk, but Kon and his co-workers (1944-6) and Smith, Niedermeier & Schultz (1948) could not confirm these observations. The purpose of the present paper is to report experiments which were begun in 1948 on lactating cows and goats in which effects of thyroxine and of the goitrogen thiouracil on the composition of milk were investigated.

EXPERIMENTAL

Experiment 1

This experiment was divided into seven periods. Six lactating Ayrshire cows were used. In period 1 all were deprived of carotene and in period 2 they all received a carotene-containing diet. In period 3 all the cows were again deprived of carotene. In period 4, the diet containing carotene was fed again to all, but cows 17 and 18 were also given subcutaneously 10 mg. DL-thyroxine/day each, while cows 19 and 20 each received 20 mg. thiouracil/day by the same route. The remaining two cows served as controls. In period 5, the carotene-containing diet was continued, but the hormonal treatments were stopped. The experiment was continued into periods 6 and 7, during which the cows all received the carotene-free diet. During period 6 each cow was given the same 'hormonal' treatment as it had received

previously in period 4. In period 7 the hormonal treatments were discontinued, while all six cows were still being deprived of carotene. The duration of each period can be seen from Fig. 2.

Diets in Experiment 1. The preparation of the diets for Exp. 1 was as described by Chanda, Clapham, McNaught & Owen (1951 *a, b*). In both carotene-containing and carotene-free diets the roughage was 10 lb. oat straw/cow/day fed in two equal portions, one in the morning and one in the afternoon.

The concentrate mixture of the carotene-containing diet consisted of 9 parts whole oats, 6 parts beans and 6 parts dried grass (all by weight).

Experiment 2

Eight lactating goats were used, one British Alpine (no. 5) and the rest British Saanens. The experiment was divided into three periods, and the hormonal treatments were confined to period 2. The goats received throughout a daily ration of 3 lb. of a concentrate mixture consisting of 9 parts by weight of oats, 6 parts of groundnut cake and 6 parts of dried grass. A carotene-free roughage was provided by oat glumes. Each goat received 1 lb. oat glumes per day in two equal portions. Water was given *ad lib*. The goats were divided into four pairs for treatment. During period 2 each goat of one pair received daily 10 mg. thy-

The carotene-free diet was designed to have the same content of digestible nutrients and digestible protein as the carotene-containing diet. Accordingly it contained, instead of dried grass, a mixture of whole blood, dried at low temperature (W. and J. Dunlop, Dumfries) 0.90 parts, ground straw 2.55 parts, and dry potato starch 2.55 parts (all by weight), making in all 6 parts to replace the 6 parts of dried grass in the other diet. This mixture, together with oats (9 parts) and beans (6 parts), formed the concentrate for the carotene-free rations. Each cow received 21 lb. of mixture each day in three equal portions, one in the morning, another at mid-day and the third in the evening.

roxine, each of a second pair 20 mg. thiouracil and each of a third pair 10 mg. thyroxine, together with 1 mg. stilboestrol. The remaining pair were untreated controls. Thyroxine and thiouracil were subcutaneously injected in aqueous solution. The stilboestrol was given by the same route in solution in 1 ml. olive oil (Malpress & Owen, 1947). The dose of thyroxine proved rather too large, for the goats developed symptoms of hyperthyroidism. They rapidly lost weight, developed a wrinkling of the palpebrae and their breathing became rapid but shallow. Their appetites were adversely affected, with simultaneous polyuria. Polyuria, resulting from thyroxine treatment, has been noted before

in cows by Owen (1948). The dose of thyroxine was, however, based on the experiments of Meites & Turner (1948). Possibly the humidity of the Scottish summer made it more difficult for our goats to dispose of the heat burden which thyroxine treatment necessarily causes. In continental America, where Meites & Turner worked, the average humidity was perhaps not so high.

On alternate days fat and vitamin A were estimated in goat's milk. Cow's milk from alternate evening milkings was analysed for vitamin A and carotene. The goats' milk was expressed by hand, and that of the cows was taken from the recorder vessel.

Chemical methods

Separation of carotene, lutein, vitamin A alcohol and vitamin A ester present in milk fat. Milk fat was extracted by the method of Olson, Hegstedt & Peterson (1939). The carotene, lutein, vitamin A alcohol and vitamin A ester were separated as described by Chanda, Owen & Cramond (1951). The method was based on that of Ganguly, Kon & Thompson (1947), and is summarized in the scheme shown on p. 405, which gives an outline of the chromatographic treatment of the fat.

(1933) was used to extract vitamin A and carotenoids from liver and to extract vitamin A from kidneys. These extracts were then chromatographed by the procedure used for milk.

RESULTS

Differences between the compositions of cow's and goat's milk

The reliability of the scheme of analysis outlined on p. 405 was tested by measuring the recoveries of vitamin A alcohol and ester which had been added to milk fat. Duplicate samples of milk fat were dissolved in *n*-hexane, and measured amounts of pure vitamin A were added to one of each pair of samples of fat. To some samples vitamin A was added as the alcohol, to others as the ester. The results of spectrophotometric measurements are shown in Table 1 from which it will be seen that with 30 i.u. vitamin A, as ester or as alcohol, 93 % could be recovered when added to milk fat of either the cow or the goat. Even with as little as 5 i.u., 80 % could be recovered (Table 1).

Table 1. *The recovery of different forms of vitamin A added to milk fat*

Source of milk fat	Vitamin A in original milk fat from 100 ml. milk (i.u.)		Vitamin A added (i.u.) in the form of		Vitamin A in fortified milk fat (i.u.)					
					Ester			Alcohol		
							Recovery of added vitamin (%) $100(e-a)$ <i>c</i>			Recovery of added vitamin (%) $100(f-b)$ <i>d</i>
					Expected (a+c)	Found (e)		Expected (b+d)	Found (f)	
	Ester (a)	Alcohol (b)	Ester (c)	Alcohol (d)						
Cow 1	71	6	50	—	121	120	98	6	5	—
2	67	4	30	—	97	95	93	4	5	—
3	99	7	—	10	99	96	—	17	15	80
4	88	5	—	20	88	92	—	25	23	90
5	77	5	—	30	77	80	—	35	34	97
6	84	4	—	50	84	81	—	54	52	96
Goat 1	184	—	30	—	214	212	93	—	—	—
2	122	—	50	—	172	170	96	—	—	—
3	106	—	—	5	106	105	—	5	4	80
4	132	—	—	10	132	129	—	10	8	80
5	115	—	—	20	115	118	—	20	18	90
6	155	—	—	30	155	159	—	30	29	97
7	165	—	—	40	165	161	—	40	38	95
8	141	—	—	50	141	138	—	50	47	94

Optical measurements were made in a Unicam spectrophotometer. Total vitamin A was also determined as vitamin A alcohol in a separate sample of fat after saponification and chromatography. All the spectrophotometric readings were corrected for irrelevant absorption by the three-point method of Morton & Stubbs (1946), as previously reported by Chanda, Owen & Cramond (1951). By this means Glover, Goodwin & Morton (1948) showed that it is possible to determine vitamin A in the presence of a preponderant irrelevant absorption.

Analysis of liver and kidney. The procedure of Davies

The method was applied to cow's and goat's milks with the results shown in Tables 2 and 3 in which milk yields and percentages of fat are also recorded. The cow's milk contained appreciable amounts of carotene (Table 2), but no carotene could be found in the goat's milk. Cow's milk contained about 6 % of its vitamin A as alcohol, but in goat's milk no measurable amount of vitamin A alcohol was present. Goat's milk contained more vitamin A per g. fat (39 i.u.) than cow's milk (21 i.u.).

Table 2. *Carotene and vitamin A in cow's milk*
(6-8 weeks post partum.)

	Cow no.						Mean	c.v.*
	1	2	3	4	5	6		
Milk yield (kg./2 days)	30.2	28.6	28.8	28.9	32.0	29.7	29.7	4.3
Fat in milk (%)	4.1	4.4	3.9	4.2	4.7	3.9	4.2	7.4
β -Carotene (μ g./100 ml.)	18.5	12.1	19.5	17.6	19.4	16.5	17.3	16.0
Total vitamin A (a) (i.u./100 ml.)	79.5	71.4	83.8	89.7	107.9	93.5	87.6	10.2
Vitamin A ester as % of (a)	89.4	93.7	91.9	93.5	92.5	94.0	92.5	1.9
Vitamin A alcohol as % of (a)	7.5	4.7	5.9	4.6	6.5	5.4	5.8	19.2
β -Carotene (μ g./g. fat)	4.5	2.8	5.0	4.2	4.1	4.2	4.1	18.2
Vitamin A (i.u./g. fat)	19.4	16.2	21.5	21.4	23.0	24.0	20.9	13.3

* c.v. (coefficient of variation) = $\frac{\text{standard deviation}}{\text{mean}} \times 100$.

Table 3. *Vitamin A in goat's milk*
(6-8 weeks post partum.)

Goat no.	Milk yield (ml./2 days)	Fat (%)	Vitamin A		Ester* (%)
			(i.u./100 ml.)	(i.u./g. fat)	
1	2670	3.1	174	56.0	98.5
2	1940	4.1	120	29.2	97.9
3	1330	3.8	128	33.7	98.6
4	2270	3.0	142	47.2	98.1
5	4600	4.3	165	38.3	99.2
6	1780	4.2	151	35.9	97.8
7	2730	4.0	110	27.5	98.9
8	3760	3.4	150	44.2	98.2
Mean	2635	3.7	142	39.0	98.4
c.v.	41.0	13.6	15.4	24.7	0.5

* The figures in this column show the proportion of the total vitamin A which was present as ester, and indicate that no measurable amount of vitamin A was present in the goat's milk.

Table 4. *Vitamin A in liver and kidneys and β -carotene in liver and colostrum of goats*

Goat no.	Cause of death and date	Thyroxine (mg./day)	Liver		Kidney vitamin A (i.u./g.)	β -Carotene in the first colostrum (μ g./100 ml.)
			Vitamin A (i.u./g.)	β -Carotene (μ g./g.)		
4	Hyperthyroidism (1950)	10	369	0.34	38	28
8	Hyperthyroidism* (1950)	10	253	0.00	9	12
9	Unknown (1950)	None	471	0.67	81	35
10	Bloat (1950)	None	452	0.09	77	19
5	† (1951)	3	176	0.84	8	46
7	† (1951)	3	272	Trace	33	24

* This animal received also 1 mg. stilboestrol per day.

† Killed after treatment with 3 mg. DL-thyroxine per day.

The occurrence of β -carotene along with vitamin A in the colostrum and liver of the goat

Cow colostrum is richer in carotene than the milk which is secreted later. It was noticed in the present experiments that the first colostrum of the goats was orange-yellow in colour. The immediately following samples of the secretion were yellow and were succeeded by the normal white milk of the goat. By analogy with the cow it was thought that yellow pigment of colostrum should contain β -carotene. Chromatography followed by spectrophotometry showed that the pigment contained

β -carotene. The amount in the colostrum varied from goat to goat as shown in Table 4. It was further thought that this colostrum carotene probably came from the goat's liver. Accordingly, the livers of six goats were examined post-mortem for β -carotene. The results in Table 4 show that β -carotene was demonstrable in most of the livers, and that the amount in the colostrum was probably determined by the amount in the liver. In view of the facts that the livers of goats examined in Liverpool by Goodwin & Gregory (1948) did not contain carotene and that one of the livers which we examined (Table 4) also contained none, the yellow pigment of

the liver was subjected to chromatography on alumina and then measured spectrophotometrically. In Fig. 1 the absorption spectra of a sample of the pigment from goat liver and of a sample of β -carotene (British Drug Houses Ltd.), both purified by chromatography on alumina, are shown. The figure demonstrates that the maxima of absorption for both the pigment from the goat liver and pure β -carotene were 423, 451 and 475 $m\mu$. in n -hexane. These maxima are concordant with those quoted by Karrer & Jucker (1950) which were also measured in n -hexane. They also agree closely with the values obtained in petrol solution by Morton (1942). The absorption spectrum of β -carotene isolated from goat colostrum had the same absorption maxima but has been omitted for the sake of brevity. These figures leave little doubt that the pigments of both goat liver and goat colostrum contain β -carotene.

Fig. 1. Absorption spectrum of β -carotene and of the pigment isolated from goat's liver. Both pigments were dissolved in n -hexane after purification by chromatography on alumina. ●—●, pigment from goat's liver (goat no. 5); —, pure β -carotene.

In both liver and colostrum the β -carotene was accompanied by another yellow pigment which from its chromatographic properties on alumina, and by analogy with cow's milk was presumed to be lutein (xanthophyll), but this latter pigment was not investigated spectrophotometrically. Our goats therefore resembled the ewes investigated by Ganguly & Deuel (1951) which also had both carotene and lutein in the liver. The kidneys differed from the liver (Table 4) in not containing carotenoids or other yellow lipochromes. The figures in Table 4 show that the livers and kidneys

of the goats which had been treated with thyroxine contained less vitamin A than those of the untreated animals, nos. 9 and 10, in which the hepatic vitamin A was of the same order as that of the sheep examined by Moore & Payne (1942). The varying liver reserves listed in Table 4 will be referred to again later.

One of the goats was rather exceptional, for, within 2 days of parturition, the lactation resulting from the previous parturition had not completely ceased. The milk (which is obviously not to be confused with the preparturient colostrum which may, for experimental purposes, be expressed from the cow's udder), did not contain carotene or any other carotenoid. When this same goat came again into milk after parturition the new secretion was colostrum and was yellow. The presence of β -carotene in this colostrum was also confirmed by chromatography followed by spectrophotometry.

The effect of thyroxine and thiouracil on the carotene in cow's milk (Experiment 1)

From Figs. 2 and 3 and Table 5 it will be seen that in period 1 the removal of carotene from the pre-experimental diet was followed by a drop in the yield of carotene in the milk and in the percentage of carotene in the milk fat of all the cows. That this drop was more precipitate than the natural decline of the yield of milk is shown by the fact that in all cases the concentration of carotene in the milk decreased after the institution of the carotene-free diet. In period 2, when carotene was replaced in the diet, the trends of the yield of carotene and percentage of carotene in the milk and the trend of carotene concentration in the milk fat were all reversed.

In period 3, carotene in the milks of all the cows decreased in much the same way as it had already done in period 1. In period 4 the two control cows behaved, with respect to carotene, much as all six cows had done in period 2, for carotene in their milk increased. In the two cows which received thyroxine in period 4 the increases in the yield of carotene per cow were noticeably larger than the increases shown by the control cows. In the two cows which were treated with thiouracil no such increases occurred at all. The carotene content of the milk fat increased more rapidly in the two thyroxine-treated cows than in the two controls. Thiouracil had the reverse effect. The effect of thyroxine in facilitating the transfer of carotene to the milk is shown by the fact that in both the thyroxine-treated cows the concentration of carotene in the milk increased at the same time as the yield of milk was increasing.

In period 5, when all the cows continued to ingest carotene, the effects of the discontinuance of the two drugs on carotene in the milk were observed. The

Fig. 2. The effect on the carotene of cow's milk of superimposition of treatment with thyroxine upon diets with and without carotene.

Fig. 3. The effect on the carotene of cow's milk of superimposition of treatment with thiouracil upon diets with and without carotene.

Table 5. *Effect of thyroxine and thiouracil on the mean content and the mean yield of β -carotene in the milk of cows both with and without β -carotene in the diet. Experiment 1*

(For details of dietary regimen see p. 404.)

Cow no.	Period	Treatment in periods 4 and 6	β -Carotene intake (mg./2 days)	Fat in milk (%)	β -Carotene in the milk		
					$\mu\text{g./100 ml.}$	$\mu\text{g./g. fat}$	$\mu\text{g.} \times 10^{-3}$ secreted/2 days
15	1	—	Trace*	4.2	15.1	3.6	4.2
	2	—	978	3.9	17.5	4.5	4.8
	3	—	Trace	3.6	9.3	2.6	2.3
	4	None	978	3.9	15.0	3.8	3.4
	5	—	948	3.8	16.5	4.3	4.1
	6	None	Trace	3.7	11.1	3.0	2.3
	7	—	Trace	3.6	2.9	0.8	0.6
16	1	—	Trace	4.1	9.0	2.2	2.4
	2	—	948	3.6	14.8	4.1	3.9
	3	—	Trace	3.7	10.1	2.7	2.5
	4	None	948	3.8	12.8	3.4	3.5
	5	—	972	4.0	15.7	3.9	3.5
	6	None	Trace	3.8	6.6	1.7	1.4
	7	—	Trace	4.0	4.2	1.1	0.8
17	1	—	Trace	3.9	12.7	3.3	3.6
	2	—	912	3.8	17.4	4.6	4.0
	3	—	Trace	3.5	7.7	2.2	1.5
	4	Thyroxine	960	4.2	23.6	5.6	5.4
	5	—	960	4.4	18.8	4.3	3.6
	6	Thyroxine	Trace	4.4	8.9	2.0	1.5
	7	—	Trace	4.3	3.6	0.8	0.8
18	1	—	Trace	4.0	11.8	3.0	2.9
	2	—	966	4.2	16.2	3.9	3.2
	3	—	Trace	4.1	8.2	2.0	1.3
	4	Thyroxine	984	4.3	22.5	5.2	4.0
	5	—	954	4.2	17.5	4.2	2.2
	6	Thyroxine	Trace	4.0	9.7	2.4	1.0
	7	—	Trace	4.1	7.2	1.8	0.9
19	1	—	Trace	4.2	15.1	3.3	4.6
	2	—	972	4.1	18.8	4.6	4.9
	3	—	Trace	3.8	7.9	2.1	1.7
	4	Thiouracil	984	3.4	10.6	3.1	1.9
	5	—	930	3.9	17.0	4.4	2.6
	6	Thiouracil	Trace	3.6	6.7	1.9	0.8
	7	—	Trace	3.9	6.1	1.6	0.5
20	1	—	Trace	4.2	12.1	2.9	3.4
	2	—	930	3.8	15.4	4.1	4.0
	3	—	Trace	3.6	8.7	2.4	2.0
	4	Thiouracil	978	3.9	8.2	2.1	1.5
	5	—	972	3.4	14.1	4.1	2.4
	6	Thiouracil	Trace	3.3	9.5	2.9	1.5
	7	—	Trace	3.5	3.5	1.0	0.5

* Approx. 2 mg./2 days from the straw.

concentration of carotene in the milk was maintained in each of the two control animals, whilst in the two which had received thyroxine it showed a marked diminution. By contrast, the two which had received thiouracil showed increases in carotene yield and in the percentage of carotene in both milk and fat. As was expected, neither thyroxine nor thiouracil appeared to affect the carotene content of the milk produced during periods 6 and 7 when the cows were receiving no carotene in their diet.

Effect of thyroxine and thiouracil on vitamin A in cow's milk (Experiment 1)

In all six cows, replacing the pre-experimental diet by one not containing carotene was followed by a fall in the yield of vitamin A in the milk, and this fall was greater than the natural decline of the milk yield because the concentration of vitamin A in the milk was also decreased (Figs. 4, 5 and Table 6). The vitamin A per g. of fat was likewise decreased in all six cows. All these three effects were reversed when

Fig. 4. The effect on the vitamin A of cow's milk of superimposition of treatment with thyroxine upon diets with and without carotene.

Fig. 5. The effect on the vitamin A of cow's milk of superimposition of treatment with thiouracil upon diets with and without carotene.

the cows returned in period 2 to a diet containing carotene. The increases in the vitamin A per g. fat and per 100 ml. milk were very marked in period 2. In period 3, when carotene was again withheld, a repetition of the results obtained in period 1 was observed, except that some of the cows reverted to figures for milk vitamin A which were less than those of period 1.

vitamin A in the milk of the two control cows were much enhanced by reversion to the carotene-containing diet in period 4. These increases were magnified in cows treated with thyroxine and nullified in cows treated with thiouracil.

The removal of thyroxine while the cows were still ingesting carotene (period 5) caused a sudden fall of vitamin A in both milk and fat and a sudden fall of

Table 6. *Effect of thyroxine and thiouracil on the mean content and the mean yield of total vitamin A and on the partition of vitamin A in the milk of cows both with and without β -carotene in the diet. Experiment 1*

(For details of dietary regimen see p. 404.)

Cow no.	Period	Treatment in periods 4 and 6	β -Carotene intake (mg./2 days)	Vitamin A in the milk				
				i.u./100 ml.	i.u./g. fat	Ester (%)	Alcohol (%)	i.u. $\times 10^{-3}$ secreted/2 days
15	1	—	Trace*	64	15.3	89	9.3	18.3
	2	—	978	90	26.3	91	6.8	24.7
	3	—	Trace	60	16.8	90	9.2	14.8
	4	None	978	72	18.7	93	7.2	16.4
	5	—	948	70	18.6	91	6.1	15.8
	6	None	Trace	52	14.1	89	8.2	10.9
	7	—	Trace	52	14.4	89	11.3	10.5
16	1	—	Trace	59	13.8	91	6.3	15.8
	2	—	948	77	21.7	94	4.4	20.2
	3	—	Trace	51	13.4	89	8.0	12.5
	4	None	948	70	18.4	93	4.7	16.0
	5	—	972	70	17.5	94	4.0	15.7
	6	None	Trace	45	11.8	89	8.6	9.6
	7	—	Trace	48	12.1	87	10.6	11.4
17	1	—	Trace	69	17.1	90	7.1	19.3
	2	—	912	88	23.2	93	5.5	20.7
	3	—	Trace	54	15.2	91	8.3	10.8
	4	Thyroxine	960	103	24.2	93	5.5	21.9
	5	—	960	80	18.6	94	4.9	15.2
	6	Thyroxine	Trace	95	21.2	83	15.1	26.3
	7	—	Trace	53	12.2	87	11.2	10.8
18	1	—	Trace	62	15.4	91	6.4	15.3
	2	—	966	95	22.6	94	4.4	18.4
	3	—	Trace	55	13.5	90	7.8	8.7
	4	Thyroxine	984	104	24.1	94	4.4	17.8
	5	—	954	83	20.0	94	3.9	10.5
	6	Thyroxine	Trace	110	27.2	80	18.3	12.4
	7	—	Trace	73	18.0	88	9.1	9.0
19	1	—	Trace	79	18.1	91	7.4	23.6
	2	—	972	102	25.5	91	6.3	27.0
	3	—	Trace	55	14.5	91	7.7	12.0
	4	Thiouracil	984	62	18.3	89	8.6	11.1
	5	—	930	98	25.4	92	6.2	15.0
	6	Thiouracil	Trace	49	13.4	90	7.3	5.8
	7	—	Trace	70	18.4	88	8.1	6.6
20	1	—	Trace	66	15.5	92	7.4	18.5
	2	—	930	95	27.6	92	5.5	24.2
	3	—	Trace	53	14.4	90	7.2	11.9
	4	Thiouracil	978	61	15.9	89	8.0	11.4
	5	—	972	92	27.7	93	5.1	16.0
	6	Thiouracil	Trace	45	13.7	87	8.2	7.0
	7	—	Trace	36	10.2	89	8.9	5.0

* Approx. 2 mg./2 days from the straw.

In period 4 the effects of the hormonal treatments were evident. Thyroxine caused a very marked rise in the vitamin A per g. fat and per 100 ml. milk of both the cows which were treated with it, while the behaviour of the two cows which received thiouracil resembled that of the two controls. The yields of

the vitamin A yield. Discontinuance of thiouracil caused effects of the reverse kind which were, however, not quite so marked (Fig. 5).

An interesting feature of Tables 5 and 6 is the effect, on the concentration of carotene and vitamin A in the fat, of discontinuance of thiouracil while

carotene was being ingested. This effect was strikingly similar to that of giving thyroxine. Conversely, it is true to say that the effect of discontinuing thyroxine in cows nos. 17 and 18 was similar to that of giving thiouracil to cows nos. 19 and 20.

Partition of vitamin A between alcoholic and ester forms in cow's milk

Fig. 6 and Table 6 show that when, during periods 1, 3, 6 and 7 the cows were deprived of carotene, the proportion of vitamin A present in the

The vitamin A in goat's milk was found to be all in the esterified form, so that free vitamin A was absent. For this reason, therefore, and also because the goats were never deprived of carotene, no attempt was made to partition vitamin A in this experiment. In general the effects were similar to those already reported for total vitamin A in cow's milk. Thus thyroxine caused an increase in the vitamin A both per 100 ml. milk and per g. fat, even although treatment with thyroxine caused an increase of the percentage of fat in the milk (Fig. 7 and Table 7). In goat no. 3, which received thy-

Fig. 6. The effect on the vitamin A alcohol of cow's milk of superimposition of thyroxine treatment (lower two curves) upon diets with and without carotene. Of the two upper curves, one shows the effect of dietary treatment alone and the other shows the lack of effect of superimposition of thiouracil treatment upon each diet. ●—●, cow no. 20; ■—■, cow no. 16; ▲—▲, cow no. 17; —, cow no. 18.

milk in the alcohol form increased. When carotene was being ingested, reversion to the initial percentage occurred. During period 6, the two cows which received thyroxine simultaneously with a carotene-free diet exhibited very large increases in the proportion of vitamin A present in the form of alcohol in the milk. These increases were spasmodic and coincided with increases of the total concentration of vitamin A in the milk; in these cases most of the increase of the total vitamin A was due to the increase of the alcohol fraction.

Effects of thyroxine, thiouracil and stilboestrol on the vitamin A in goat's milk (Experiment 2)

Numerous analyses of milk from many goats confirmed the well known observation that carotenoids occur only in minute traces in goat's milk.

roxine alone, these increases were accompanied by a transient increase in the milk yield. In each goat which received thyroxine, whether with or without stilboestrol, there were marked increases in the vitamin A per 100 ml. milk and in the vitamin A per g. fat. Of two goats, nos. 4 and 8, which died of hyperthyroidism, one (no. 8) had received stilboestrol as well as thyroxine. In each of these two animals the secretion of the watery phase of the milk regressed much faster than the secretion of fat, so that average fat percentages for period 2 were 8.3 and 9.9 compared with 4.8 and 4.4 in the other two goats which received thyroxine. In goat no. 7 stilboestrol administration caused lactation to cease at the end of period 2 (Fig. 8), but a week later (6 days after the discontinuance of the stilboestrol) she came back into milk at the same yield as

Fig. 7. Milk yield, percentage fat in the milk and the vitamin A content of milk and fat of a thyroxine-treated goat. —, vitamin A (i.u./100 ml. milk); ■—■, vitamin A (i.u./g. fat); ▲—▲, fat (%); ●—●, milk yield (l./2 days).

Table 7. *The effect of thyroxine, thiouracil and thyroxine plus stilboestrol on the mean fat and the mean vitamin A contents of goat's milk. Experiment 2*

(For details of dietary regimen see p. 405.)

Goat no.	Period	Treatment in period 2	Dietary carotene (mg./2 days)	Fat (%)	Vitamin A		
					i.u./100 ml. milk	i.u./g. fat	i.u. $\times 10^{-3}$ secreted/2 days
1	1	None	139	3.1	179	57	4.4
	2		124	3.3	172	53	3.9
	3		100	3.3	168	52	2.7
2	1	None	94	4.3	114	27	1.8
	2		82	4.6	107	23	1.2
	3		45	4.2	97	23	2.2
5	1	Thiouracil	143	4.1	163	40	7.3
	2		133	3.2	104	33	4.5
	3		123	3.6	108	30	4.1
6	1	Thiouracil	138	4.3	156	36	2.6
	2		109	3.8	107	30	1.5
	3		93	4.1	120	29	1.0
3	1	Thyroxine	122	3.7	119	32	1.6
	2		70	4.8	261	55	3.5
	3		109	3.7	136	36	1.3
4	1	Thyroxine	117	3.1	134	43	2.8
	2		40	4.4	225	51	1.3
	3		—	—	—	—	—
7	1	Thyroxine + stilboestrol	142	3.9	112	29	3.4
	2		87	8.3	285	34	5.5
	3		111	7.6	238	30	1.0
8	1	Thyroxine + stilboestrol	114	3.4	138	41	3.1
	2		12	9.9	441	45	2.2
	3		—	—	—	—	—

previously. In this goat, as she rapidly dried off, both the percentage of fat in the milk and the percentage of vitamin A in the milk increased rapidly. When she resumed lactation both the fat and vitamin A were of about the same magnitude as they had been when lactation ceased (Fig. 8). Neither when she ceased nor when she resumed milking did this goat show any evidence of carotenoids in the milk.

supposed, from the work of Hoch & Hoch (1946), to be in the alcohol form. However, Chanda (1951) has found that cows at grass have some 16–30 % of their blood plasma vitamin A in the ester form. Glover, Goodwin & Morton (1947) have adduced evidence that reserves of vitamin A are present in the liver as the ester but are in equilibrium with vitamin A alcohol in the blood. On their hypothesis, if a cow is deprived of carotene and thereby forced to mobilize

Fig. 8. Milk yield, percentage fat in the milk and the vitamin A content of milk and fat of a goat treated with thyroxine and stilboestrol. The breaks in the curves indicate that the treatment completely inhibited lactation just before the end of period 2 but that it was resumed in period 3. —, vitamin A (i.u./100 ml. milk); ■—■, vitamin A (i.u./g. fat); ▲—▲, fat (%); ●—●, milk yield (l./2 days).

Thiouracil depressed the milk yield slightly, but at the same time it noticeably reduced the percentage of fat in the milk. This drug also depressed the amount of vitamin A per g. fat so that the yield of vitamin A in the milk was depressed (Table 7).

DISCUSSION

The results of the present experiments may be explained by supposing that when dietary carotene is absorbed it is transformed into the ester form of vitamin A (Thompson *et al.* 1950) and passes via the lymph, the thoracic duct and the blood stream to the mammary gland. In the cow some carotene probably accompanies the vitamin A ester, since both will be constituents of the chylomicrons. In the goat the accompanying carotene will be negligible. Most of the vitamin A in the blood is usually

its hepatic reserves, an increase in vitamin A alcohol in the milk would not be unexpected and this is what was found in the present experiments. Experiments by Greeves & Schmidt (1936) have shown that thyroxine accelerates the release of reserves of vitamin A from the livers of rats kept on diets deficient in the vitamin, thus explaining the effect of thyroxine in the present experiments in causing enhanced increases of vitamin A alcohol in the milk when, in period 6, the cows were deprived of carotene. The fact that vitamin A in the milk of cows was less when thiouracil was given with a carotene-free diet than it had been on the same diet without thiouracil (Table 6), can be explained on the basis of the observations of Kelley & Day (1948) who found thiouracil to retard the rate of depletion of hepatic stores of vitamin A in rats on a diet deficient in the vitamin.

Johnson & Baumann (1947) showed that, in rats, thyroxine greatly enhanced hepatic storage of vitamin A from dietary carotene but had little effect on storage from preformed dietary vitamin A. They also found that thiourea inhibited storage from dietary carotene but not from dietary vitamin A. It is therefore reasonable to suppose that the effect of thyroxine in the cow in stimulating the apparent digestibility of carotene (Chanda *et al.* 1951*b*) was due rather to the stimulation by the hormone of conversion of carotene to vitamin A in the intestinal mucosa (Glover *et al.* 1948; Thompson, Ganguly & Kon, 1949) than to any effect it may have had in increasing the absorption of carotene as such. Nevertheless, the figures in the last column of Table 5 do give reason for supposing that the increase caused by thyroxine in the output of milk carotene itself was chiefly from dietary carotene. Thus, in the control cows 15 and 16, the output of carotene in the milk was smaller in period 4 than in period 2, both of these being carotene periods. When thyroxine was given in period 4, however, cows 17 and 18 show that thyroxine caused the carotene output to be bigger in period 4 than in period 2. That this was an effect of thyroxine is shown by its reversal in cows nos. 19 and 20 which received thiouracil in period 4. That these increases in carotene output were probably of dietary rather than hepatic origin is shown by comparing periods 3 and 6 when no carotene was fed. In these circumstances thyroxine failed to cause any change in the output of milk carotene.

In this discussion it is, of course, assumed throughout that carotene, once it has passed into the lymph from the intestine, is of no further use to the cow as a source of vitamin A. It has long been known (Drummond & McWalter, 1933) that injected carotene is not converted to vitamin A by the rat and, indeed, Sexton, Mehl & Deuel (1946) have had rats dying of vitamin A deficiency while their livers contained much β -carotene resulting from injection of it. In the goat only traces of carotene could have been absorbed in these experiments (Tables 3, 4 and 7), so that the effect of thyroxine in increasing the apparent digestibility of carotene in these animals (Chanda *et al.* 1951*b*) may reasonably be attributed to an increase in the rate of conversion of carotene to vitamin A.

A considerable contribution to the vitamin A in the milk of the goats which were treated with thyroxine was probably made by their liver reserves after the overlarge dose of thyroxine had, by adversely affecting their appetites (Table 7), caused their carotene intakes to be reduced. The magnitude of this hepatic contribution can be judged by comparing, in Table 4, the liver reserves of the treated and untreated goats.

Tables 2 and 3 show that the goat's milk was

richer in vitamin A than the cow's milk and that the goat's milk unlike the cow's milk did not contain either carotene or vitamin A alcohol. The greater amount of vitamin A in goat's milk can perhaps be related to the more active thyroid gland of the goat as shown in Table 8. In that table data on both goats and cows from the present experiments and from those of Chanda *et al.* (1951*a*) are presented together with the data of Schultze & Turner (1945) on the rates of secretion of thyroxine by cows and goats. The table suggests that the greater apparent digestibility of carotene by goats is associated with both a higher vitamin A content of the milk and with a greater thyroid activity. The greater vitamin A content of the goat's milk could not have been a dietary effect because, as Table 8 shows, the

Table 8. *The rate of secretion of thyroxine, the digestibility of carotene and the secretion of vitamin A in the milk of cows and goats*

	Cows	Goats
Average body weight (kg.)	450	35
Average carotene intake (mg./day)	496	42
Carotene intake (mg./kg. body weight)	1.1	1.2
Apparent digestibility of carotene (%)*	56	63
Rate of secretion of thyroxine (mg./kg. body weight)†	22	34
Vitamin A in milk (i.u./g. fat)	21‡	39

* Data of Chanda *et al.* (1951*a*).

† Data from Schultze & Turner (1945).

‡ This value does not include the carotene.

carotene intakes of the cows and goats before hormone treatment were practically equal when calculated in terms of body weight. The larger concentration of carotene in the milk from cows treated with thyroxine leaves the observation of Fellenberg & Grüter (1932), that thyroidectomized goats secreted yellow milk, unexplained. Indeed, in the present experiments, goats treated with either thyroxine or thiouracil showed no evidence of the presence of carotenoids in the milk. Nevertheless, β -carotene was demonstrated in goat's colostrum (Table 4). It therefore seems that there is some hormonal influence on the goat's mammary gland at parturition which causes β -carotene regularly to appear together with other carotenoids in the colostrum. The appearance of carotene in goat's colostrum could hardly have been due to the thyroid which does not attain maximum activity until the peak of lactation at which time the goat's milk is free from carotenoids. Neither do oestrogens appear to be responsible for the occurrence of carotene in goat's colostrum. Thus goat no. 7 (Fig. 8), which resumed lactation after having been 'dried off' by stilboestrol, produced milk free from

carotenoids in the resumed lactation. Similarly, another goat which was giving some carotene-free milk 2 days before parturition gave β -carotene along with other carotenoids in the post-partum mammary secretion. If, therefore, the occurrence of β -carotene in goat's colostrum is under hormonal control it does not seem from the present experiments that the hormones concerned are either thyroxine or oestrogens.

SUMMARY

The metabolism of carotene in six lactating cows and in eight lactating goats was investigated with the following results:

1. Goat's milk contained 142 i.u. vitamin A/100 ml., all of it in the ester form. Cow's milk contained 88 i.u. vitamin A, 6% of which was in the alcohol form. Unlike the cow's milk, that of goat did not contain carotenoids, but in the colostrum and in the liver of goats β -carotene was demonstrated.

2. When the cows were deprived of carotene for a fortnight their milk yields were not affected, but the concentrations of vitamin A and carotene decreased. At the same time the proportion of vitamin A present in the alcohol form increased.

Superimposing thyroxine treatment upon carotene deprivation caused even greater increases of vitamin A alcohol in the milk.

3. On re-instatement of the carotene-containing diet the concentrations of both carotene and vitamin A increased at rates which were enhanced by thyroxine and diminished by thiouracil. The effects on vitamin A and carotene of discontinuing thyroxine while the cows were still ingesting carotene were similar to those produced by injecting thiouracil. Conversely, the effects of discontinuing thiouracil were similar to those of giving thyroxine.

4. Thyroxine and thiouracil produced changes in the composition of goat's milk which were similar to those found in cow's milk.

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REFERENCES

- Cama, H. R. & Goodwin, T. W. (1949). *Biochem. J.* **45**, 236.
 Chanda, R. (1951). Unpublished observations.
 Chanda, R., Clapham, H. M., McNaught, M. L. & Owen, E. C. (1951a). *J. agric. Sci.* **41**, 179.
 Chanda, R., Clapham, H. M., McNaught, M. L. & Owen, E. C. (1951b). *Biochem. J.* **50**, 95.
 Chanda, R. & Owen, E. C. (1949). *Biochem. J.* **44**, xxix.
 Chanda, R., Owen, E. C. & Cramond, B. (1951). *Brit. J. Nutrit.* **5**, 228.
 Davies, A. W. (1933). *Biochem. J.* **27**, 1770.
 Drill, V. A. (1943). *Physiol. Rev.* **23**, 355.
 Drummond, J. C. & McWalter, R. J. (1933). *Biochem. J.* **27**, 1342.
 Fasold, H. & Heidemann, E. R. (1933). *Z. ges. exp. Med.* **92**, 53.
 Fellenberg, T. von & Grüter, F. (1932). *Biochem. Z.* **42**, 253.
 Ganguly, J. & Deuel, H. J. (1951). *Fed. Proc.* **10**, 383.
 Ganguly, J., Kon, S. K. & Thompson, S. Y. (1947). *Brit. J. Nutrit.* **1**, iii.
 Glover, J., Goodwin, T. W. & Morton, R. A. (1947). *Biochem. J.* **41**, 97.
 Glover, J., Goodwin, T. W. & Morton, R. A. (1948). *Biochem. J.* **43**, 512.
 Goodwin, T. W. & Gregory, R. A. (1948). *Biochem. J.* **43**, 505.
 Greeves, J. D. & Schmidt, C. L. A. (1936). *Amer. J. Physiol.* **116**, 456.
 Hoch, H. & Hoch, R. (1946). *Brit. J. exp. Path.* **27**, 316.
 Johnson, R. M. & Baumann, C. A. (1947). *J. biol. Chem.* **171**, 513.
 Karrer, P. & Jucker, E. (1950). *Carotenoids*. Translation by E. A. Braude. Amsterdam: Elsevier.
 Kelley, B. & Day, H. G. (1948). *J. biol. Chem.* **175**, 863.
 Kon, S. K. & co-workers (1944-6). *Ann. Report. Nat. Inst. Res. Dairying*, Reading, England.
 Kunde, M. M. (1926). *Proc. Soc. exp. Biol., N.Y.*, **23**, 812.
 Lindquist, T. (1938). *Studien über das Vitamin A beim Menschen*. Uppsala: Appelberg.
 Malpress, F. H. & Owen, E. C. (1947). *J. Endocrinol.* **5**, lxii.
 Meites, J. & Turner, C. W. (1948). *Res. Bull. Mo. agric. Exp. Sta.* no. 416.
 Moore, T. & Payne, J. E. (1942). *Biochem. J.* **36**, 34.
 Morton, R. A. (1942). *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes*, 2nd ed. London: Adam Hilger.
 Morton, R. A. & Stubbs, A. L. (1946). *Analyst*, **71**, 348.
 Olson, F. R., Hegsted, D. M. & Peterson, W. H. (1939). *J. Dairy Sci.* **22**, 63.
 Owen, E. C. (1948). *Biochem. J.* **43**, 235.
 Schultze, A. B. & Turner, C. W. (1945). *Res. Bull. Mo. agric. Exp. Sta.* no. 392.
 Sexton, E. L., Mehl, J. W. & Deuel, H. J. (1946). *J. Nutrit.* **31**, 299.
 Smith, J. A. B. & Dastur, N. N. (1940). *Biochem. J.* **34**, 1093.
 Smith, V. R., Niedermeier, R. P. & Schultz, L. H. (1948). *J. Anim. Sci.* **7**, 544.
 Thompson, S. Y., Braude, R., Coates, M. E., Cowie, A. T., Ganguly, J. & Kon, S. K. (1950). *Brit. J. Nutrit.* **4**, 398.
 Thompson, S. Y., Ganguly, J. & Kon, S. K. (1949). *Brit. J. Nutrit.* **3**, 50.
 Thomson, A. M., Griffith, J. R., Mutch, J. R., Lubbock, D. M., Owen, E. C. & Logaras, G. (1939). *Brit. J. Ophthalm.* **23**, 698.