phase certainly occupies a longer period in the calcium experiments than it did in the phosphate ones. If Neuman & Weikel's explanation, postulated for PO_4 exchange, is accepted it can be concluded that the exchange of calcium in this particular bone is mainly limited to the surface of the crystal, and the exchange of surface and sub-surface ions plays a very small part.

The exchangeable calcium fraction of nearly 33 % is higher than that found by Falkenheim *et al.* (1951) in ashed rabbit bone (25 %). A pointer towards an explanation is that Robinson (1951), from electron microscope studies, found that the average bone crystal size was greater in cattle than in man, dog or cat (decreasing in this order). Whether there is sufficient difference in the crystal size, and, therefore, of the surface area available for exchange, of cattle and rabbits to explain the differences in calcium exchange is not known. If this is so, the amount of exchangeable calcium will depend on the species, age and type of bone. It may also depend on the technique used in measuring it; as Neuman & Neuman (1953) pointed out, any manipulative procedure, even washing with water, can introduce marked alterations in the state of an apatite crystal.

SUMMARY

1. The percentage of exchangeable bone Ca in unashed compact ox bone has been determined as 33 % by the loss of ^{45}Ca ions both from a radioactive solution, and from bone previously equilibrated with ^{45}Ca . The results were similar.

2. The time taken for the exchange process to reach equilibrium depends on the size to which the bone has been ground. For particles passing through a 200/in. mesh the initial rapid exchange is completed in 2–4 hr.

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The Effects of Deprivation of Carotene and Anterior Pituitary Hormone on the Partition of Vitamin A in the Blood and Milk of the Cow

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Chanda, Clapham & Owen (1954) showed that the partition of vitamin A between its free and esterified forms in cow's milk is different from its partition in blood serum. Both forms of the vitamin were found together in cow's milk (Chanda & Owen, 1952) irrespective of whether or not the diet contained carotene, though in both circumstances the vitamin A ester formed the major portion. If the diet contained no greenstuff or other source of vitamin A, vitamin A alcohol was present by itself in the blood serum, but if there was greenstuff in the diet the vitamin A alcohol, which still greatly preponderated, was accompanied by some vitamin A ester. The fact that several hundred volumes of blood pass through the blood system of the mammary gland for every volume of milk secreted raises the question whether the vitamin A ester in the milk arises directly from the vitamin A ester of the blood or

whether it comes from esterification by the gland of the vitamin A alcohol which preponderates in the blood. The results of the present experiments in which the effects of a pituitary-hormone preparation (Ambinon) on the metabolism of carotene have been studied, are consistent with the supposition that vitamin A ester in milk comes from both these sources and thus has a dual origin.

In the experiments certain similarities between the action of the hormone preparation and the action of thyroxine (Chanda & Owen, 1952) were observed and are now recorded. In these same experiments also, concentrations of carotene and xanthophylls in blood and milk were compared in order to see whether the vitamin A precursor, β -carotene, behaved differently from the biologically inactive xanthophylls once it had reached the blood stream.

EXPERIMENTAL

Hormone used. This was a proprietary preparation of Organon Laboratories Ltd., called Ambinon. It is sold in 1 ml. sterile sealed glass ampoules each of which is stated to contain the thyrotrophic and gonadotrophic factors from 2.5 g. of fresh anterior pituitary gland (and 0.3% cresol as a bacteriostatic agent).

but xanthophylls from the straw continued to be defaecated.

The partition of vitamin A between alcohol and ester and the content of carotene and xanthophylls in the milk were determined in representative samples of Sunday's and Wednesday's milk throughout the experiment. On the last day of each period these same determinations were made in the blood of each cow. During periods 2, 4 and 5

Fig. 1. Effect of anterior pituitary hormone treatment and deprivation of carotene on the output of vitamin A alcohol in cow's milk. Cow no. 3, ▲, received hormone injections during period 4, when the diet contained carotene and also during period 6 when the diet was devoid of carotene. Cow no. 2, ●, received at the same times as cow no. 3 control injections of isotonic saline.

Plan of experiment. The experiment which lasted 118 days was divided into seven periods of duration shown in Fig. 1. In periods 1, 3, 6 and 7 each of four Ayrshire cows received a diet which was free from carotene and contained (in parts by weight): oats 6, bran 1, field beans 2 and decorticated earthnut meal 1. During period 2 the diet contained carotene and consisted of oats 18, beans 12, dried grass 9 and lucerne meal 4 parts by weight. In periods 4 and 5 a diet richer in carotene was fed. It contained oats 18, beans 12, dried grass 7 and lucerne meal 5 parts by weight. The dried grass meal and lucerne meal being green were the source of β -carotene. The diet for each period was mixed in a single batch. In addition to this diet, roughage, an essential component of ruminant diets, was provided by oat straw and beet pulp, neither of which contains any carotene. On the carotene-free ration, faecal carotene became negligible in 7 or 8 days

samples of the concentrate ration were analysed every 4 days for carotene and the carotene intake calculated. These intakes are recorded in Table 1.

Administration of hormone. Each of the cows nos. 3 and 4 received 1 ml. of Ambinon by subcutaneous injection, every second day during period 4 when carotene was present in the diet, and during period 6 when carotene was absent from the diet. At the same time cows nos. 1 and 2 each received subcutaneously, every second day, an injection of sterile isotonic saline (0.9% NaCl) as a control on the injection procedure.

Methods of analysis. These were as described in earlier papers (Chanda, Clapham, McNaught & Owen, 1951; Chanda, Owen & Cramond, 1951; Chanda, Clapham, McNaught & Owen, 1952; Chanda & Owen, 1952; Chanda *et al.* 1954).

RESULTS

Milk yield and percentage of fat in milk

Table 2 shows that neither the rate of decline of the milk yield nor the percentage of fat in the milk was affected by either the hormonal treatment or the carotene deprivation. The decline in the milk yield was accompanied by a steady decline in the daily fat yield in all four cows.

Vitamin A alcohol in the milk and in the blood serum

Table 3 records the average figures for all the carotene-free periods for the vitamin A alcohol and vitamin A ester in the milk. The numbers of samples of milk included in each average can be seen from Fig. 1, in which the outputs of vitamin A alcohol throughout the experiment by cow no. 3, which received hormonal treatment, and cow no. 2, which did not, are graphically represented. The differences in period 6 between the graphs of the vitamin A alcohol outputs of cow no. 4, which was hormonally treated, and of cow no. 1, which was not, were very similar to those shown between cows nos. 2 and 3 in Fig. 1. The graphs for cows nos. 1 and 4 have therefore been omitted. Fig. 1 clearly shows an inverse relationship between vitamin A alcohol in the milk and carotene in the diet, for when greenstuff was fed (periods 2, 4 and 5) the output of vitamin A alcohol decreased steadily but when none was fed (periods 1, 3, 6 and 7) the output of vitamin A alcohol in the milk steadily increased (Chanda & Owen, 1951, 1952; Chanda *et al.* 1954). In Fig. 1 the vitamin A alcohol has been represented in terms of output per cow to emphasize that during the hormonal treatment (period 6) the vitamin A alcohol in the milk increased in amount as well as in concentration.

A particularly noteworthy feature of Fig. 1 is the contrast between the large increase of the output of vitamin A alcohol in period 6, during which Ambinon was given in the absence of dietary carotene, and the apparent lack of any change during period 4 when the hormonal treatment accompanied a diet containing greenstuff.

In Table 3 the average vitamin A alcohol figures for period 6, during which the hormonal treatment was given to cows nos. 3 and 4, have been compared with those for the preceding and succeeding carotene-free periods. This has been done by calculating the difference between the figure for period 6 when treatment was given and the average of the preceding and succeeding carotene-free periods (periods 3 and 7). Table 3 shows that this difference was positive (+ 700 and + 270 i.u. per day) for the two treated cows nos. 3 and 4, and negative (− 220 and − 340) for the two control cows nos. 1 and 2. A similar comparison (Table 4) between the vitamin A

Table 1. Vitamin A alcohol and ester in the blood serum during periods 2, 4 and 5 when the diet contained carotene, hormone being administered in period 4 to cows nos. 3 and 4

Cow no.	Treatment in period 4	Intake of carotene* (mg./day)					β-Carotene in blood (μg./100 ml. serum)					Xanthophylls (non-carotene carotenoids) (μg./100 ml. serum)					Vitamin A alcohol (i.u./100 ml. serum)					Vitamin A ester (i.u./100 ml. serum)				
		Period					Period					Period					Period					Period				
		2	4	5			2	4	5			2	4	5			2	4	5			2	4	5		
1	Control	268	552	659			136	411	555			34.1	44.7	54.3			86.6	95.8	117.7			11.4	21.2	27.8		
2		268	552	659			150	446	614			36.6	48.0	56.3			107.9	114.0	120.1			12.5	26.5	29.6		
3	Hormone	256	473	561			150	386	481			47.6	40.0	46.5			91.2	104.1	96.5			11.6	32.6	26.7		
4		267	552	659			152	367	503			41.2	39.8	49.1			102.6	121.6	115.5			9.3	33.3	26.0		

* Average intake corrected for refusals of food.

alcohol output in the carotene period 4, during which cows nos. 3 and 4 were hormonally treated, and the preceding and succeeding carotene periods 2 and 5 shows that, in spite of the decrease of output of vitamin A alcohol caused by the greenstuff in period 4 (Fig. 1), there was a simultaneous but opposite effect resulting from the hormonal treat-

ment, for the differences calculated in this way are still much larger (+360 and +470) for the treated animals than for the controls (-30 and +160).

These effects of the hormonal treatment on the composition of the milk were paralleled by simultaneous changes in the blood (Tables 1 and 5). In Table 5 the analyses of the blood of each cow on the

Table 2. *Milk yield, percentage of fat in milk and β -carotene content in milk of cows*

Cow no.	Treatment in periods 4 and 6	Milk yield (kg./day)						
		Period						
		1 No carotene	2 Carotene	3 No carotene	4 Carotene +hormone	5 Carotene	6 No carotene +hormone	7 No carotene
1	Control	14.07	11.28	9.26	8.34	7.85	6.85	5.66
2		15.46	14.18	13.76	14.12	13.65	12.20	11.38
3	Hormone	14.74	15.43	14.06	15.34	14.03	13.45	11.56
4		16.94	14.95	15.58	14.22	12.78	13.03	12.28

Cow no.	Treatment in periods 4 and 6	Percentage of fat in milk						
		Period						
		1	2	3	4	5	6	7
1	Control	3.41	3.53	3.22	3.24	2.88	3.10	3.20
2		3.69	3.90	3.72	3.66	3.53	3.65	3.88
3	Hormone	3.60	3.53	3.40	3.16	2.90	2.88	3.00
4		3.91	3.95	3.88	3.68	3.85	3.53	3.44

Cow no.	Treatment in periods 4 and 6	β -Carotene in milk (μ g./100 ml.)						
		Period						
		1	2	3	4	5	6	7
1	Control	8.4	15.6	10.6	18.3	25.3	15.8	6.7
2		8.2	16.2	10.7	18.6	25.6	15.1	6.6
3	Hormone	8.3	16.4	11.4	20.0	25.1	13.5	5.6
4		8.2	16.9	11.0	20.8	24.4	15.4	6.5

Table 3. *Average daily outputs of vitamin A alcohol and vitamin A ester in the milk during periods 1, 3, 6 and 7 when there was no carotene in the diet, hormone being given in period 6 to cows nos. 3 and 4*

Cow no.	Treatment in period 6	Vitamin A alcohol (i.u./day)				$(b - \frac{a+c}{2})^*$	Vitamin A ester (i.u./day)				$(e - \frac{d+f}{2})^*$
		Period 1	Period 3	Period 6	Period 7		Period 1	Period 3	Period 6	Period 7	
		(a)	(b)	(c)	(d)		(d)	(e)	(f)	(g)	
1	Control	1 310	940	610	710	-220	9 060	5 470	5 050	2 520	+1060
2		1 630	1 630	1 310	1 670	-340	9 820	8 900	9 120	5 330	+2010
3	Hormone	1 300	1 600	2 330	1 670	+700	8 680	7 440	10 600	5 910	+3930
4		1 960	2 040	2 280	1 980	+270	10 630	8 400	10 600	6 490	+3160

* Corrected to the nearest 10.

Table 4. *Average daily outputs of vitamin A alcohol and vitamin A ester in the milk during periods 2, 4 and 5 when the diet contained carotene, hormone being given in period 4 to cows nos. 3 and 4*

Cow no.	Treatment in period 4	Vitamin A alcohol (i.u./day)				$(b - \frac{a+c}{2})^*$	Vitamin A ester (i.u./day)				$(e - \frac{d+f}{2})^*$
		Period 2	Period 4	Period 5	(c)		Period 2	Period 4	Period 5	(f)	
		(a)	(b)	(c)	(d)		(d)	(e)	(f)	(g)	
1	Control	800	610	480	-30	8 710	6 130	7 600	-2030		
2		1 150	1 190	910	+160	8 830	11 190	13 050	+250		
3	Hormone	1 250	1 460	950	+360	11 010	12 520	14 220	-100		
4		1 210	1 520	900	+470	10 660	16 380	14 020	+4040		

* Corrected to the nearest 10.

Table 5. Vitamin A alcohol*, β -carotene and non-carotene carotenoids in the blood serum when the diet was free from carotene, hormone being given to cows nos. 3 and 4 during period 6

Cow no.	Treatment in period 6	Vitamin A alcohol (i.u./100 ml. serum)					$\left(b - \frac{a+c}{2}\right)$	β -Carotene ($\mu\text{g.}/100$ ml. serum)					Xanthophylls (non-carotene carotenoids) ($\mu\text{g.}/100$ ml. serum)				
		Period 1	Period 3	Period 6	Period 7			Period 1	Period 3	Period 6	Period 7	Period 1	Period 3	Period 6	Period 7		
1	Control	87.8	82.1	107.9	100.3	+16.7	48.3	70.1	93.2	38.4	8.0	11.0	16.4	8.8			
2		103.7	98.4	117.0	113.2	+11.2	64.0	80.0	119.1	47.2	7.0	15.2	20.9	10.8			
3	Hormone	81.7	92.3	128.4	88.2	+38.2	49.0	72.3	112.4	44.4	10.5	9.9	19.2	11.2			
4		101.5	97.3	132.2	103.4	+31.9	67.6	78.7	95.2	38.0	8.6	8.7	17.2	8.4			

* The blood serum contained no detectable vitamin A ester when the diet was free from carotene.

last day of each of the carotene-free periods, 1, 3, 6 and 7 are recorded. Vitamin A ester was not detectable either spectrophotometrically or by means of SbCl_3 in the blood when carotene was missing from the diet. The difference between the concentration of vitamin A alcohol in the blood during hormonal treatment (period 6) and the average of the concentrations for the preceding and succeeding carotene-free periods (periods 3 and 7) is given in Table 5. As in the milk so in the blood the values of this difference were bigger in the treated (+38.2 and +31.9) than in the control cows (+16.7 and +11.2). There was no evidence of subnormal vitamin A alcohol concentrations in the serum. That was because, in cattle which have been reared on grass, more than 200 days of continuous deprivation are necessary to deplete the liver (Guilbert & Hart, 1934) of its stored vitamin A.

Table 1 shows that similar differences (+10.3 and +12.6 for the treated as against -3.4 and 0.0 for the controls) of vitamin A alcohol in the blood were produced by the hormone treatment on the green diet.

Fig. 2 shows the relation between the β -carotene in the blood and the vitamin A alcohol in the milk on the last day of each period. It shows that the vitamin A alcohol in the milk decreased as carotene intake (and therefore blood carotene) increased and that the vitamin A alcohol became steady at a minimum value of 5-6 i.u./100 ml. of milk, when the blood carotene increased from 400 to 600 $\mu\text{g.}/100$ ml. of blood serum. Two straight lines have been fitted (Snedecor, 1946) to the array of data in Fig. 2. One of these lines depicts the minimum values of the vitamin A alcohol corresponding to the larger titres of blood carotene. The marked points which were recorded when hormone was being administered were on the curve when there was carotene in the diet and widely aberrant when the diet contained no carotene.

Thus whether the diet contained greenstuff (Tables 1 and 4) or not (Tables 3 and 5) the pituitary preparation increased both the output of vitamin A alcohol in the milk and the concentration of vitamin A alcohol in the blood serum.

Vitamin A ester in the milk and blood serum

As reported by Chanda & Owen (1952) greenstuff caused vitamin A ester to appear in the blood (Tables 1 and 5). Comparisons of the data for the last milk samples of periods 2, 4 and 5, which showed as would be expected the maximum effect of re-alimentation with greenstuff, are given in Table 6. The differences between the figure for the treatment period when there was carotene in the diet (period 4) and the average of the figures for the carotene periods 2 and 5 were +7.8 and +9.1 i.u./100 ml. of milk for the control cows and +27.7 and

+62.3 for the hormonally treated ones. The blood analysed at the same time (Table 1) showed the same effect, i.e. +1.6 and +5.5 i.u./100 ml. for the vitamin A ester in the serum of the controls and +13.5 and +15.7 i.u./100 ml. for the treated ones.

When there was no carotene in the diet there was as already stated no vitamin A ester in the blood, but Table 3 shows what effect the hormone had

extent to which it was esterified. There being no vitamin A ester in the blood, the extra vitamin A ester secreted by cows nos. 3 and 4 in period 6 probably came from the vitamin A alcohol in the blood which was itself increased (Table 5).

Fig. 2. Relation of vitamin A alcohol, in the last milk sample of each period for each cow, and β -carotene in the blood serum. No distinction has been made between the data from different cows. The two straight lines fitted as recommended by Snedecor (1946) represent a degenerate hyperbola. The points marked differently are at the times when cow no. 3 (▲) and cow no. 4 (▼) were receiving hormone treatment; they are aberrant only at the time when the diet was devoid of carotene. These points were, of course, omitted from the computation of the fitted lines. The equation of the two lines is:

$$y - 11.73 = -0.16506(x - 83.24)$$

$$\text{and } (y - 6.589) = -0.0022832(x - 384.67),$$

where y = i.u. vitamin A alcohol per 100 ml. milk and x = μ g. β -carotene per 100 ml. blood serum. The independent variable was x .

Fig. 3. Relation of vitamin A ester, in the last milk sample of each period for each cow, and the β -carotene in blood serum. No distinction has been made between the data from different cows. The points marked differently are at the times when cow no. 3 (▲) and cow no. 4 (▼) were receiving hormone treatment. These points are aberrant only at the time when the diet contained carotene. They were, of course, omitted when the hyperbola was being computed. The curve shown through the points is a portion of a hyperbola with an asymptote parallel to the carotene axis. Its equation, fitted by the method of least squares using Taylor's theorem (Running, 1917) is

$$(y - 97.7)(y - 0.97174x) = 697.61,$$

where y = i.u. vitamin A ester per 100 ml. milk and x = μ g. β -carotene per 100 ml. blood serum. The independent variable was x .

under these circumstances on the output of vitamin A ester in the milk. Calculation of the difference between vitamin A ester output in period 6 when hormonal treatment was given with vitamin A ester output in periods 3 and 7 (the preceding and succeeding carotene-free periods) showed +1060 and +2010 for the controls and +3930 and +3160 for the treated cows (Table 3). Thus when greenstuff was absent from the diet the hormone preparation stimulated the output of both the ester and alcohol forms of vitamin A in the milk. Under these conditions therefore not only was the output of total vitamin A in the milk stimulated, but also the

The hormonal treatment did not affect blood carotene or blood xanthophylls (Table 1) but there was an interesting relationship between blood carotene and the vitamin A ester in the milk of the last collection of each period (Fig. 3). As blood carotene increased due to increased intake of greenstuff, the concentration of vitamin A ester in the milk increased up to a limiting value of about 100 i.u./100 ml. of milk. In Fig. 3 the plateau value of vitamin A ester has been emphasized by fitting to the data the hyperbola shown in the figure. The departure from the curve of the two points representing hormonal treatment during the eating of

greenstuff is obvious, but it should be noted that in Fig. 3 it is during the green diet that this departure occurs. In Fig. 2 in which vitamin A alcohol in milk was plotted against blood carotene it was the carotene-free diet which produced the two aberrant points.

Carotene in the milk

Carotene in the milk increased steadily from the beginning to the end of each of the periods 2 and 4 during which carotene was present in the diet. In period 5, which followed period 4 without a break, the carotene in the milk was relatively constant. In Table 2 the average level of β -carotene during all the periods is shown and the average in period 5 is seen to be noticeably larger than that in period 4. This is because the average in period 5 during which carotene was fed was near the maximum shown at the end of period 4, which was also a carotene period. The carotene in the milk in periods 1, 3, 6 and 7, during which the diet was free from carotene, declined steadily from the beginning to the end of each of these periods so that much lower averages resulted in all these periods except period 6. This higher average in period 6 was expected because that period followed period 5 in which the carotene intake (see Table 1) was maximal. Table 2 shows that the variations of carotene in the milk of each cow were controlled by the intake of greenstuff (see Table 1) and that the variations in the milk yield itself were much smaller. In Table 6 the carotene concentrations in the last milk sample collected from each cow in each of the carotene periods, 2, 4 and 5 are shown. The difference between the figure for period 4 (when hormonal treatment was given) and the average of the two figures for periods 2 and 5 was positive in all cases but it was larger for the treated (+4.5 and +6.7 $\mu\text{g./100 ml.}$ of milk) than for the control cows (+2.9 and +1.9). This effect of the hormonal treatment on the milk carotene was the same as the simultaneous effect on the content of vitamin A ester in the milk (Table 6) and the simultaneous effects on vitamin A alcohol and ester in the blood (Table 1). The carotene and xanthophylls (non-carotene carotenoids) in the blood (Tables 1 and 5) appeared to be determined by dietary effects, and as expected there was no evidence of a hormonal effect on the xanthophylls. The blood did not become free of carotene or of xanthophylls when greenstuff was withheld from the diet and even at the end of the carotene-free periods carotene was readily measurable in the milk as shown earlier (Chanda *et al.* 1954).

By calculation from the milk yields in Table 2, ranging from 16.94 to 5.66 kg./day, from the estimated blood volume of 36 l. for a 450 kg. cow (Dukes, 1943) and from the blood vitamin A and milk vitamin A it can be shown that the output of

Table 6. Concentration of vitamin A ester, vitamin A alcohol, β -carotene, and non-carotene carotenoids (xanthophylls) in the last day's milk sample of the carotene periods 2, 4 and 5, hormone being given to cows nos. 3 and 4 during period 4

Cow no.	Treatment in period 4	Yield of milk (kg./day)			Percentage of fat in milk			Vitamin A alcohol (i.u./100 ml. milk)			Vitamin A ester (i.u./100 ml. milk)			β -Carotene ($\mu\text{g./100 ml. milk}$)			Xanthophylls ($\mu\text{g./100 ml. milk}$)		
		2	4	5	2	4	5	2	4	5	2	4	5	2	4	5	2	4	5
1	Control	10.4	8.4	7.7	3.4	3.0	2.9	5.4	5.0	6.6	81.3	96.9	96.9	19.0	25.0	25.3	5.4	2.9	2.6
		13.8	14.4	13.6	3.9	3.7	3.6	7.9	6.8	6.4	80.2	98.8	99.2	19.3	24.4	25.8	5.4	2.4	2.2
3	Hormone	14.6	14.9	13.8	3.7	3.1	3.1	6.6	7.4	6.0	80.2	115.5	95.4	20.1	27.4	25.8	5.5	2.8	2.5
		13.4	12.8	13.0	3.2	3.9	3.9	7.4	6.3	6.6	83.6	153.5	98.8	20.4	29.4	25.0	5.4	3.2	2.7

* Note. If this column were calculated from yields of vitamin A ester/day instead of from concentrations these figures would be 0.2, 2.0, 4.8 and 7.7 for cows nos. 1, 2, 3 and 4 respectively.

total vitamin A in the milk each day ranged from about 31 % (period 1, cow no. 4) to about 8½ % (period 7, cow no. 1) of the total vitamin A circulating in the blood at the time. With the smaller fraction the blood reservoir would empty of vitamin A in 12 days, with the larger emptying would take only 3 days. Evidently an efficient mechanism keeps the blood reservoir filled with vitamin A even when there is no carotene in the diet.

In the carotene-free periods, 1, 3, 6 and 7, the average rate at which the milk was depleting the blood of carotene was such that the milk took 3.20 ± 1.04 (S.D.) % of circulating carotene on the last day of each period. The corresponding figure for xanthophylls was 4.68 ± 1.99 (S.D.) %. These are much smaller than the 8½–31 % shown by the vitamin A. Thus in this respect the blood carotene behaved in much the same way as the xanthophylls.

The ratios of total vitamin A in the blood to total vitamin A in the milk were calculated for all the samples of milk and blood collected on the last day of each period. The average of the 28 ratios so obtained was 1.42 ± 0.26 (S.D.). For exactly the same set of samples of blood and milk the average calculated in the same way was 12.31 ± 4.74 (S.D.) for β -carotene and 10.60 ± 5.68 (S.D.) for xanthophylls. The much larger ratios for carotene and xanthophylls than for vitamin A are indicative of the lesser permeability of the mammary epithelium to carotenoids than to vitamin A. The fact that β -carotene, a precursor of vitamin A, gave the same ratio as xanthophylls is in keeping with the assumption of the relative quantitative unimportance of carotene as a precursor of vitamin A after it has passed into the body through the intestinal mucosa, this latter tissue being the chief site of transformation of carotene to vitamin A (for literature citations see Owen, 1941, 1947, 1951, 1954).

DISCUSSION

Ambinon is a preparation which contains the thyrotrophic, luteinizing and follicle-stimulating activities of the anterior hypophysis. What part any or all of these fractions played in producing the effects in the present experiments cannot be assessed from the present experiments alone. There are many similarities between the present effects on carotene and vitamin A metabolism produced by Ambinon and the effects of thyroxine reported earlier by Chanda & Owen (1952), and such similarities might suggest that the thyrotrophic activity was chiefly involved. However, in the absence of any evidence as to how thyroxine itself may affect the output of anterior hypophyseal secretions even these similarities cannot be used as evidence in favour of attributing the action of Ambinon solely or even chiefly to its thyrotrophin content. It can nevertheless be asserted that two effects of thyroxine on

the cow can be simulated by a pituitary extract. These are the increase of carotene and vitamin A ester in the milk when the diet contains carotene and the increase of vitamin A alcohol when it does not (Chanda & Owen, 1952).

Dual origin of vitamin A ester in milk

These experiments have shown that the power of the mammary gland to esterify vitamin A is limited; for more vitamin A alcohol reached the gland when it was the only form circulating in the blood (Table 3). They have also shown that Ambinon increased the vitamin A alcohol in both the blood and the milk when greenstuff was withheld but that it at the same time increased the output of vitamin A ester in the milk. When greenstuff was present in the diet, vitamin A ester appeared in the blood in amounts which were increased by the simultaneous injection of Ambinon and at the same time a similar effect was seen in the milk. These observations are consistent with the hypothesis that vitamin A ester in the milk arises both from dietary carotene and from vitamin A alcohol set free from the liver into the blood (Glover, Goodwin & Morton, 1947). It may be esterified in the intestinal mucosa (Kon & Thompson, 1951) and it may be esterified in the mammary glands.

The present experiments confirm the observations on human subjects of Hoch & Hoch (1946) and Hoch (1946) that vitamin A of internal origin circulates in the blood as the alcohol but that alimentary vitamin A circulates as the ester.

The contrast shown in Figs. 1–3 between the effects of the hormonal treatment on a carotene-free diet and the effects when greenstuff was given suggest a possible antagonistic effect of greenstuff on the action of the hormone in releasing vitamin A alcohol from the liver. There is much previous evidence (none of it on lactating animals) in support of such an antagonism in the observations of Fasold & Peters (1933), Abelin (1935), Rappai & Rosenfeld (1935), Fellinger & Hochstädt (1936), Fleischmann & Kann (1936), Rokhlina (1936), Lindquist (1938), Logaras & Drummond (1938), Török & Neufeld (1938), and Sadhu & Brody (1947).

The authors wish to draw attention to the fact that each of the criteria used in the present experiments to show the effects of the hormone treatment is independent, and that the effects on the milk were simultaneous with those on the blood.

SUMMARY

1. Injections of an anterior pituitary preparation 'Ambinon' (Organon Laboratories Ltd.) which contains thyrotrophin and gonadotrophins, were given to Ayrshire cows, both in the presence and in the absence of dietary carotene in the form of greenstuff, and the figures for carotene, vitamin A ester and vitamin A alcohol in the blood and milk were

compared with the figures from two control cows receiving exactly the same dietary treatment.

2. The pituitary preparation increased the vitamin A alcohol output in the milk whether greenstuff was present in the diet or not.

3. When there was no greenstuff in the diet the output of vitamin A alcohol in the milk increased and this increase was enhanced in the hormonally treated cows.

4. In the absence of green food there was no vitamin A ester in the blood and yet the output of vitamin A ester in the milk was also increased by the hormone preparation so that the hormonal treatment increased the rate of esterification of vitamin A by the mammary gland.

5. When greenstuff was present in the diet the blood contained vitamin A ester in amounts which were increased by the hormonal treatment.

6. The hormonal treatment increased the vitamin A alcohol in the blood serum whether or not there was greenstuff in the diet.

7. The results lend weight to the hypothesis that, like thyroxine, the hormone preparation accelerated the mobilization of hepatic reserves as vitamin A alcohol into the blood and hence into the milk as alcohol and ester. There was also some evidence that, like thyroxine, the hormone preparation accelerated the transformation of dietary carotene into vitamin A in the gut.

8. The results provided evidence for the hypothesis that vitamin A ester in the milk could, when the diet contained carotene, have either a hepatic or a dietary origin according as it came from the alcohol or the ester of vitamin A circulating in the blood.

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Transglucosidation in *Penicillium chrysogenum* Q-176

ISOLATION AND IDENTIFICATION OF THE OLIGOSACCHARIDES

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The non-phosphorylating oligo- and poly-saccharide-synthesizing enzymes have recently been studied in great detail. Amylomaltase (Doudoroff, Hassid, Putman, Potter & Lederberg, 1949; Torriani & Monod, 1949), amylsucrase (Hehre, 1949), cyclo-dextrinase (Norberg & French, 1950), dextran sucrose and levan sucrose (Hassid & Doudoroff, 1950), dextran dextrinase (Hehre, 1951), *Q*-enzyme

(Nussenbaum & Hassid, 1951) and transfructosidase (Edelman & Bacon, 1951) have all been shown to fall in with the general class of 'transglycosidases', the name being suggested by Doudoroff, Barker & Hassid (1947). Yet another transglucosidase, capable of synthesizing branched-chain oligosaccharides from maltose has been reported in *Aspergillus niger* (Pan, Andreasen & Kolachov, 1950),