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Studies in Vitamin A

15. THE VARIATIONS IN THE SERUM β -CAROTENE AND VITAMIN A LEVELS OF COWS NEAR PARTURITION

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Kuhlmann & Gallup (1944), Braun (1945*a*) and Sutton, Kaeser & Soldner (1945), working in the United States, have reported that at or immediately after parturition the serum carotenoid and vitamin A levels of cows drop sharply, to recover slowly during the next 14–21 days. It was considered important to find out whether such a situation obtained with cows kept under normal English farming conditions, and to this end the work reported here was carried out during 1948.

EXPERIMENTAL

Animals. The cows used in this investigation belonged to the Veterinary Science Division of the Research Department of Boots Pure Drug Co. They formed a small mixed herd used for a long-term experiment on the spread of *Streptococcus agalactiae* infection, but this experiment cannot be considered to affect in any way the vitamin A and carotenoid metabolism of the cows. The general details of the experimental animals are given in Table 1. During the period of the investigation (May–November, 1948) all the cows except no. 7 were out at grass day and night until

17 October, having been turned out on 11 April. Cow no. 7 was kept in after early October because of an illness; after 17 October cows nos. 2, 3 and 7–9 were out at grass only during the day (approx. 5 hr.) and received a daily supplement of third-year seeds hay, up to 20 lb. (9.1 kg.) of kale and concentrates according to milk yield. Cows nos. 4–6 remained out day and night until the investigation ended. On calving the cows received a supplement of 8 lb. (3.6 kg.) per day of a production ration consisting of 1 part bran, 1 part oats, 1 part flaked maize (Kosistos) and 1 part dairy nuts. Throughout the period under investigation there was always plenty of grass available, for, owing to the wet summer, the pasture never dried up.

Determination of serum β -carotene and vitamin A. Blood samples were drawn from the jugular vein into McCartney bottles and immediately posted to Liverpool, where they arrived in good condition, showing little or no haemolysis. The serum was separated from the clot by centrifugation and stored at 0° until it was assayed. Determinations were always carried out as soon as possible after arrival and never more than 48 hr. later. It was assumed that no loss of carotenoids and vitamin A had occurred in transit. The carotenoids and vitamin A were extracted from the serum by the method in general use in this laboratory (Glover, Goodwin & Morton, 1947*a*). Owing to the very high concentration of β -carotene in the extracts, direct determinations of vitamin A were impracticable, because any correction applied

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Table 1. *Details of the cows used during the present investigation*

Cow no.	Date of birth	Breed	No. of days dry before calving	Date of calving
1	Jan. 1944	Ayrshire ×	57	30 Apr. 1948
2	Jan. 1944	Ayrshire ×	113	30 May 1948
3	Dec. 1944	Ayrshire	1st calf	6 Sept. 1948
4	Mar. 1945	Ayrshire	1st calf	8 Sept. 1948
5	Dec. 1944	Ayrshire	1st calf	21 Sept. 1948
6	Jan. 1945	Shorthorn (Pedigree)	1st calf	15 Nov. 1948
7	Jan. 1945	Ayrshire	1st calf	21 Nov. 1948
8	Feb. 1944	Ayrshire (Pedigree)	1st calf	Feb. 1948
9	Oct. 1944	Ayrshire	1st calf	Feb. 1948

for the presence of β -carotene would have been much too great; consequently the extracts were chromatographed on defatted bone meal (Mann, 1943; Goodwin & Morton, 1946; Glover, Goodwin & Morton, 1947b) before determinations were made. The blood extract, dissolved in a little light petroleum (b.p. 40–60°), was poured on to a bone-meal column; elution with light petroleum removed the β -carotene together with any small amounts of vitamin A esters which were present. The xanthophylls and free vitamin A which remained in the column were then eluted with acetone.

The light petroleum fraction was made up to a convenient volume and the amount of β -carotene present determined by measuring the $E_{450}^{1\text{cm}}$ value of the solution in a Beckman spectrophotometer. The vitamin A esters present in this fraction were ignored because they form only a minor fraction of the total serum vitamin A (Glover *et al.* 1947a). The fraction containing the xanthophylls (lutein is by far the major pigment present) and vitamin A was then examined for vitamin A. No attempt was made to separate the lutein from the vitamin A because, being only a minor component of the total serum carotenoids (about 5%), it did not interfere seriously with the SbCl_3 test for vitamin A, and this slight interference could be allowed for by applying a correction, which never amounted to more than 5% of the uncorrected value. The correction applied was based on our observation that the ratio $E_{450}^{1\text{cm}}/E_{446}^{1\text{cm}}$ is 6.35 for a chromatographically homogeneous sample of lutein isolated from grass.

The xanthophyll-vitamin A fraction was made up in a known volume of acetone, the $E_{446}^{1\text{cm}}$ value measured in the Beckman instrument, and the $E_{450}^{1\text{cm}}$ for the serum calculated in the usual way. The acetone was then removed under a stream of N_2 , the residue dissolved in 1 ml. of CHCl_3 and the SbCl_3 test carried out at 617 m μ . again using the Beckman instrument (Glover *et al.* 1947a; Goodwin & Gregory, 1948). The observed $E_{450}^{1\text{cm}}$ value was then corrected by subtracting from it the $E_{450}^{1\text{cm}}$ value divided by 6.35.

RESULTS

Changes in the serum β -carotene and vitamin A levels around parturition. The variations in the concentrations of both these constituents were followed in six cows (nos. 2–7) and all showed a gradual decline during the last week of pregnancy until at, or just after, parturition they were, on the average, between 60 and 70% of their normal values. The levels of all the cows except no. 7 then gradually improved until at 1 month post-partum they were approaching the normal levels. Following parturition, cow no. 7 behaved quite abnormally, for neither the β -carotene nor the vitamin A level recovered from the drop at parturition, but continued to fall until the end of the experiment. No obvious clinical condition could account for this at the time (end of November), but the animal had been ill early in October.

The results obtained for the serum vitamin A and β -carotene values for cows nos. 2–6 around parturition are recorded in Figs. 1 and 2 as scatter diagrams. The basic value for each cow (except

Fig. 1. The variations in the serum vitamin A levels of cows near parturition. The mean values for the 6th, 7th and 8th months of pregnancy are taken as 100 and the levels around parturition adjusted accordingly. $\circ$, cow no. 2; $\bullet$, cow no. 3; $\square$, cow no. 4; $\blacksquare$, cow no. 5; $\triangle$, cow no. 6; $\blacktriangle$ — $\blacktriangle$, mean values.

Fig. 2. The variations in the serum β -carotene levels of cows near parturition. The mean values for the 6th, 7th and 8th months of pregnancy are taken as 100 and the levels around parturition adjusted accordingly. $\circ$, cow no. 2; $\bullet$, cow no. 3; $\square$, cow no. 4; $\blacksquare$, cow no. 5; $\triangle$, cow no. 6; $\blacktriangle$ — $\blacktriangle$, mean values.

no. 2) has been taken as the means of the routine monthly values obtained during the 6th, 7th and 8th months of pregnancy and has been given the value 100. The values near parturition were then adjusted accordingly. The values obtained 14 days before calving for cow no. 2 were taken as her base line for no previous values were available; this is justified because reference to the diagrams will show that no significant drop had occurred in the remaining cows when examined between 7 and 14 days ante-partum. The first serum value obtained for cow no. 1 was for the day of parturition, but the values obtained post-partum all showed an increase on these values. Cows nos. 8 and 9 were not followed through parturition.

Seasonal changes in serum β -carotene and vitamin A levels. The monthly serum levels for vitamin A and β -carotene obtained between May and October 1948 are recorded in Tables 2 and 3. An analysis of

variance of these results revealed no significant month to month variations for either component.

The vitamin A and β -carotene content of colostrum. The values obtained on a number of samples of colostrum from cows nos. 1, 2 and 9 are recorded in Table 4. Very high values were obtained for early colostrum, but these fell off rapidly until 14 days after parturition they were generally less than 10% of their original values. This confirms previous observations (see e.g. Sutton, Warner & Kaeser, 1947; Ganguly, Kon & Thompson, 1947). Colostrum obtained 2 and 6 days before delivery was as high as that obtained on the day of calving. Samples of colostrum from cow no. 8 were examined and the results calculated per ml. of colostrum (the lipid content was not measured); in this case the values of vitamin A and β -carotene fell from 1.73 to 2.44 $\mu\text{g./ml.}$, respectively, at parturition to 0.41 and 0.75 $\mu\text{g./ml.}$ 7 days later.

Table 2. *The serum vitamin A levels ($\mu\text{g./100 ml.}$) of cows during May–October 1948*

Cow no.	Month					
	May	June	July	Aug.	Sept.	Oct.
2	Calved	22	27	31	30	29
3	33	32	43	34	Calved	29
4	46	38	34	34	Calved	35
5	33	29	20*	28	Calved	31
6	30	32	33	29	30	30
7	30	46	38	43	38	—
8	33	31	31	35	30	29
9	34	31	30	36	—	30

* Doubtful value.

Table 3. *Serum β -carotene values ($\mu\text{g./ml.}$) of cows during May–October 1948*

Cow no.	Month					
	May	June	July	Aug.	Sept.	Oct.
1	Calved	8.9	6.5	9.9	9.6	10.2
2	Calved	15.3	16.8	19.0	16.4	12.6
3	18.2	14.0	13.6	—	Calved	14.4
4	9.1	10.6	8.6	8.6	Calved	11.2
5	8.8	8.2	9.9	9.8	Calved	7.3*
6	6.3	5.1	3.9	4.0	5.4	5.4
8	30.0	28.0	—	22.0	15.0	16.0
9	12.0	11.0	11.2	12.2	13.0	—

* Only 5 hr./day on grass.

Table 4. *The vitamin A and β -carotene content of colostrum fat*

(All values expressed as $\mu\text{g./g.}$)

Cow no.	Time pre-partum (days)				Time post-partum (days)							
	6		2		Day of parturition		1–2		7		14	
	Vit. A	β -Carotene	Vit. A	β -Carotene	Vit. A	β -Carotene	Vit. A	β -Carotene	Vit. A	β -Carotene	Vit. A	β -Carotene
1	—	—	—	—	31	98	12	13	1.8	5.5	—	—
2	—	—	—	—	55	139	37	62	4.0	10.1	—	—
9	37	112	35	131	—	—	15	37	—	—	1.2	9.5

DISCUSSION

In considering the significance of the fall in the serum β -carotene and vitamin A levels at parturition, it must be taken into account that the serum vitamin A is functional whilst the serum β -carotene is, in all probability, of no physiological value, merely being that which has escaped conversion into vitamin A in its passage across the intestinal wall. When the same variations are noted for two blood components, one physiologically active, the other inert, it is reasonable to assume that the changes have no significance for either, but merely indicate a general variation in the concentration of blood constituents at parturition. This conclusion is supported by the recent observations that similar changes associated with parturition of cows have been reported for plasma lipids (Niedermeiler, Smith & Whitehair, 1949) and for calcium and phosphorus (Niedermeiler *et al.* 1949; Wilson, 1951) and vitamin E (Parrish, 1949). (There is, however, one report (Kaay, Teunissen, Emmerie & Eekelen, 1949) which states that plasma vitamin E levels do not alter at parturition.)

During the 6 months covered by this investigation there were no statistically significant variations in the serum vitamin A or β -carotene levels except at parturition. Because of the 'buffering' action of the liver vitamin A (Glover *et al.* 1947*a*), there is no reason to expect major changes in the vitamin A levels, but changes in the β -carotene levels might have been expected, for it has been noted on many occasions that there is a sudden increase in the β -carotene levels during the 'spring flush' (in late March and April in Britain). These high levels are maintained until about midsummer when usually a fall is observed owing to the drying up of the pasture during the hot months. The values then increase to a second (lower) maximum in early autumn, when a second crop of pasture appears (e.g. Lord, 1945). In the case of animals in the present experiment, the cows had already been out to grass a month before the first blood samples were taken and thus the spring rise had already occurred. Further, the summer of 1948 had rainfall above the average and during May–November there was always a good 'bite' of grass. At no period did the pasture dry up.

The significance of the serum vitamin A/ β -carotene (A/C) ratio in cows. Braun (1945*a, b*) first attempted to attribute significance to the A/C ratio in cattle. He found the ratio to decrease with increasing carotene concentration until the latter reached about

9 $\mu\text{g./ml.}$; above this level the ratio remained constant. This was taken to indicate a decreasing efficiency of conversion of β -carotene into vitamin A with increasing β -carotene intake, until, at and above the level quoted, a constant proportion is converted.

The A/C ratios have been calculated for the results recorded in Tables 2 and 3 and analysed statistically. The regression lines connecting β -carotene levels with the A/C ratio indicate a highly significant correlation between these two parameters over the whole range of β -carotene levels examined. Similar analyses indicate no significant correlations either between the A/C ratio and the vitamin A levels or between the vitamin A and β -carotene levels themselves. This statistical demonstration, that the A/C ratio is solely dependent on the β -carotene levels of the blood, confirms the experimental observations that serum vitamin A levels are relatively stable, owing to the 'buffering' of the liver vitamin A, whilst the plasma β -carotene levels are extremely labile, quickly reflecting variations in intake. No greater significance than this can be ascribed to the A/C ratio for normal cattle.

SUMMARY

1. The tendency of the serum β -carotene and vitamin A levels of cows to drop at, or just after, parturition has been confirmed. These changes are considered not significant for vitamin A and β -carotene *per se*, but are probably only a reflexion of general changes in the concentration of blood constituents at parturition.

2. No seasonal variations in serum vitamin A or β -carotene levels of cows at grass occurred during May–October 1948.

3. The high values for the vitamin A and β -carotene content of early colostrum and their rapid post-partum decline has been confirmed. Colostrum drawn up to 6 days ante-partum is also very rich in vitamin A and β -carotene; 94% of the colostrum vitamin A is esterified.

4. The significance in cows of the ratio serum vitamin A/ β -carotene is discussed.

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The Constitution of Hexadecatrienoic Acid from the Glycerides of Rape (*Brassica napus* L.) Leaf

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Apart from hexadec-9-enoic acid, the C₁₆ unsaturated acids are of somewhat rare occurrence. From sardine oil there has been isolated hexadeca-6:10:14-trienoic acid (Toyama & Tsuchiya, 1935) and a hexadecatetranoic acid (Tutiya, 1940, 1941). More recently Shorland (1945) reported the presence of hexadecatrienoic acid in the glycerides of rape leaf.

The purpose of the present work is to determine more precisely the constitution of the hexadecatrienoic acid found in rape-leaf glycerides. In addition, the method of Armstrong & Hilditch (1925), commonly used to locate the position of the double bonds by disruptive oxidation, has been examined using linolenic acid, the constitution of which has already been determined by previous workers (cf. Erdmann, Bedford & Raspe, 1909). This step has been deemed advisable in view of difficulties which have occasionally been experienced by other workers in the isolation of the oxidation products from polyethenoid acids (cf. Shinowara & Brown, 1940).

EXPERIMENTAL

Oxidation of ethyl linolenate

Ethyl linolenate used for this work was prepared as described by McCutcheon (1938, 1940). Spectroscopic analysis by the method of Hilditch, Morton & Riley (1945) showed the presence of 95.5% (0.2% conjugated) triene acids and 2.7% (1.7% conjugated) diene acids, while the iodine value 245.8 (theoretical for ethyl linolenate 248.9) also confirmed the purity of the preparation.

The ester (8.1 g.) was dissolved in 140 ml. of acetone, and

finely divided KMnO₄ (75 g.) was added (cf. Armstrong & Hilditch, 1925) gradually under reflux until vigorous reaction ceased. The mixture was then boiled for a further 4 hr.

After removal of the acetone on a water bath, the residue was extracted with 600 ml. of hot 5% (w/v) KOH and the extract concentrated to 150 ml. (cf. Haworth, 1929). The extract was acidified with dil. H₂SO₄ to congo red, and the volatile acids separated by steam distillation. By comparing the amount of 0.1 N-KOH required for neutralization with the dried weight (3.41 g.) of the K salts (cf. Dolby, Nunn & Smedley-MacLean, 1940) the equivalent of the volatile acids was found to be 71.3 as compared with 74.0 required for propionic acid. To test the validity of the method a sample of propionic acid was steam distilled as above and gave an equivalent of 74.9.

After extraction of the non-volatile residue with ether, the extract was boiled with water and filtered to remove any oily insoluble matter. Recrystallization from hot water yielded 1.53 g. crystals, equiv. 93.1, m.p. 104.5–106.5°. From the mother liquors a further crop of crystals (1.42 g., equiv. 91.0) was obtained. By applying counter-current distribution methods (Craig, 1944) to 0.117 g. of this latter fraction, using a mixture of 4:3 by volume ether-light petroleum and 20% aqueous ethanol, there was obtained, in addition to azelaic acid, 0.016 g. of suberic acid, equiv. 87.8, m.p. 131–137° (not depressed by admixture with authentic suberic acid).

Other similar oxidation experiments with ethyl linolenate as well as with methyl oleate also indicated that in addition to azelaic acid there were produced small but variable amounts of suberic acid.

Oxalic acid was estimated as described by Haworth (1929), the yield amounting to 60% of the theoretical. Malonic acid was not detected, indicating presumably, as was found by Haworth (1929), that under these conditions this acid is further oxidized to yield oxalic acid.