I. VITAMIN A AND CAROTENE.
IX. NOTES ON THE CONVERSION OF CAROTENE
TO VITAMIN A IN THE COW.

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In a previous paper [Moore, 1930] evidence has been advanced to show that carotene, or some part of it, suffers conversion in the rat to the colourless vitamin A. It is well known, however, that the degree of yellow pigmentation of the body-fat of animals varies considerably between different species. Thus the rat resembles the sheep and pig in possessing colourless body-fat, while man, the ox and the fowl have more or less pigmented fats. Before reaching any conclusion as to the general applicability of results obtained with the rat, therefore, it seemed desirable to examine the fate of carotene in animals of the latter group, in case any fundamental difference should be revealed.

In regard to the fowl, Capper, McKibbin and Prentice [1931] have already shown that the administration of carotene leads to the appearance of vitamin A in the liver oil, the fate of the pigment being in marked contrast with that of xanthophyll, which appears neither to suffer obvious change nor to be selectively absorbed in the liver, being found fairly evenly distributed throughout the body- and egg-yolk-fats, and endowing them with their characteristic colour [Palmer and Kempster, 1919]. The present experiments deal with the equally familiar case of the ox, which differs from the fowl in having carotene as the predominant pigment of the body-fat [Palmer and Eccles, 1914].

Since the experiments of Stephenson [1920] upon the vitamin A activity of decolorised butter-fat it has been assumed that the colourless vitamin A plays its part in bovine metabolism, and this finding has been amply confirmed by the recent work of Morton and Heilbron [1930], who have shown by spectroscopic means that both carotene and vitamin A are present in the unsaponifiable fraction of New Zealand butter-fat. It has also been found that whereas the feeding of carrots or green stuff to cows leads to increases both in the carotene content and vitamin A activity of the butter-fat, the feeding of cod-liver oil results only in increased vitamin A activity, the degree of yellow pigmentation remaining unchanged [Drummond *et al.*, 1924; Golding, Soames and Zilva, 1926]. These observations, which first seemed to support the view that no relation could exist between carotene and the vitamin, might now seem to

point to an incomplete conversion of carotene in the cow, or even to an interchangeability of the functions of the pigment and vitamin in this animal. The present work was carried out in the hope of shedding light on this question.

EXPERIMENTAL.

Since the large size of the cow makes it an unsuitable subject for such rigid experimental procedure as can be adopted in the case of the rat it is unavoidable that the evidence as to the conversion of carotene should be largely obtained by indirect means. Two main lines of investigation have been taken up. In the first place specimens of liver, butter, *etc.* obtained from different cows have been examined by the colorimetric method for carotene and vitamin A. In these experiments special attention has been directed to a comparison of specimens obtained from Jersey cows with those from other breeds, since the Jersey cow is reputed to show unusually deep pigmentation in its body- and milk-fat. Secondly an experiment has been carried out on the effects of feeding carotene, as carrots or red palm oil, on the degree of pigmentation and vitamin A content of butter-fat obtained from a cow at the end of the winter stall-feeding period.

The estimation of carotene and vitamin A.

Ox-liver. Specimens of liver from cows of known breed and history were kindly provided by Dr F. Carr, of British Drug Houses, Ltd. The extraction of the oil and colorimetric examination were carried out in the manner previously described [Moore, 1930; Dann and Moore, 1931]. The results obtained are shown in Table I. Vitamin A, as characterised both by a low yellow/blue ratio and by absorption at $m\mu$ 620 in the $SbCl_3$ test, was demonstrated in all cases, the amounts present varying considerably between different animals. The extracts were all somewhat yellow in colour, but the amount of carotene present, even in the most deeply pigmented samples, could have represented only a small fraction of the total chromogenic value.

Table I.

Sex and Age	Breed	Feeding	Yellow units per g. liver	Blue units per g. liver
Ox, 3 yrs.	Hereford	Grass	2	25
Ox, 3 yrs.	Black Angus	Grass	12	300
Ox, 4 yrs.	Devon	Grass	2	30
Bull, 7 yrs.	Jersey	Store	6	230
Cow, 7 yrs.	Jersey	Grass	18	30
Cow	Jersey	Unknown	9	350

Butter-fat. In view of the occasional commercial practice of increasing the yellow shade of butter-fat by the use of foreign colouring matter, it was essential that butter-fat of known history should be used. At the suggestion of Mr J. S. Penicud, milk recorder for the Cambridge district, samples of milk were collected from cows of the University Shorthorn herd through the

courtesy of Mr W. Mansfield, and from cows of Lady Walston's pedigree Jersey herd through the courtesy of Mr H. B. Hart. Collections were made on the same date at a time when both herds had been at grass for many weeks. On superficial examination it was obvious that the samples from the Jersey cows were in each case of a much more pronounced yellow shade than those given by the Shorthorns. At first an attempt was made to examine the samples individually, but the technique necessary to ensure the complete extraction of the fat was found to be too laborious to permit of this course. The samples from each herd were therefore combined, the cream was removed by skimming and converted into butter in a shaking machine. The butter-fats, after rendering, were examined colorimetrically both before and after saponification. The results obtained are shown in Table II.

Table II.

	Rendered fat		Unsaponifiable matter		
	Yellow units per g.	Blue units per g.	% unsaponifiable matter	Blue units calculated per g. original fat	Position of absorption bands in SbCl_3 reaction
Jersey cows	25	7.5	0.3	20	Dense vitamin A band $620\text{ m}\mu$, hazier band $575\text{ m}\mu$
Shorthorn cows	11	5.0	0.26	25	Vitamin A band present

The presence of vitamin A in both specimens was made apparent by the appearance of the $620\text{ m}\mu$ band in the SbCl_3 test given by the unsaponifiable matter, although with the Jersey butter a fainter band at $\text{m}\mu\ 575$ was also observed¹. The yellow/blue ratios were consistent with this finding, indicating, on the unproven assumption that the yellow pigmentation was due entirely to unchanged carotene, that about 10 % and 5 % of the total blue value was attributable to the pigment in the Jersey and Shorthorn butter respectively. The decidedly greater yellow value given by the Jersey sample was in good accordance with the deeper colour of the original milk.

It will be noticed that the blue value calculated per unit of original fat was much increased by the process of saponification. The author's attention was directed to this point by Dr H. N. Green, who had previously observed the same phenomenon. The increase would seem to be caused by the removal of substances which interfere with the SbCl_3 reaction, similar increases being usually observed on saponifying cod-liver oils.

Blood-fat and body-fat. A few experiments were carried out upon specimens of body-fat and blood-fat obtained from animals of unknown history. In all cases only small amounts of chromogenic material were present, suggesting that in the cow, just as in the rat, the main reserves of vitamin A are concen-

¹ Heilbron and Morton described the position of the second band shown in New Zealand butter-fat (derived mainly from Jersey cows) as $592\text{ m}\mu$, characteristic of carotene. The band at $575\text{ m}\mu$ observed in the present experiments on the other hand, was in the same position as the band later detected by these workers in certain cod-liver oils.

trated in the liver. In general the colorations obtainable in the SbCl_3 test were too transient to permit of spectroscopic examination or even accurate matching in the tintometer. From a rough comparison of the yellow and blue values, however, it was obvious that the relative proportion of yellow pigment present was always much greater than in liver-fat.

Corpus luteum. A single specimen of dried cow's corpus luteum of unknown source was kindly made available by Mr C. W. Bellerby. The material was extracted and examined in the usual way. A yellow/blue ratio of 4 was given, while the main absorption band in the SbCl_3 test was at $m\mu$ 590, the position characteristic of carotene. While it would be unjustifiable on the evidence available to exclude the possibility that traces of vitamin A were present, it is obvious that the main fraction of the chromogenic value was in this case represented by a carotenoid.

Weight, powdered corpus luteum g.	Weight fat mg.	Total yellow units	Total blue units	SbCl_3 ab- sorption band $m\mu$
1.9	80	600	150	590

*The effect of carotene-feeding upon the carotene and
vitamin A contents of butter-fat*

In this experiment cows belonging to the National Institute for Research in Dairying were used, through the kindness of Mr James Mackintosh, who arranged for the feeding of the animals and the dispatch of samples of butter to Cambridge. The work was so timed that the feeding of the supplements containing carotene commenced in the early spring at a point when the carotene and vitamin A contents of the milk would have been expected to have stood at their lowest levels, as the result of maintenance through the winter months upon a diet relatively low in carotene. This diet consisted of two parts, (1) a "basal ration" of mangels 25 lbs., potatoes 5 lbs., oatstraw chaff 4 lbs., treacle 1 lb., hay 3-4 lbs., oats 2 lbs.; (2) an allowance of a mixture of oats 3 parts, dried grains 1 part, rice meal 2 parts, maize gluten feed 2 parts, decorticated ground-nut cake 1 part, soya meal 1 part, adjusted to the milk yield of the individual cow. Although no actual examination of these food materials was carried out several of the components, notably the mangels, maize gluten feed and soya meal may be suspected to have contained small amounts of carotenoids. At the same time the pale colour of the butter-fats at the commencement of the experiment made it plain that the carotene content of the diet as a whole was inadequate to permit pigmentation at the normal summer level.

At suitable intervals milk was collected from each cow and samples of butter were prepared, this part of the work being supervised by Miss Dearden. For this purpose the milk from two or three days had to be used, the intervals of collection have therefore usually been calculated from the first day on which samples were taken. After three batches of butter had been examined, in order to determine the preliminary levels of the vitamin A and carotene, the cows

were treated individually; (a) Winnie 3rd served as control receiving no supplement, (b) Campion 2nd received 30 lbs. of carrots daily in place of mangels, (c) Woodruff 2nd received 8 oz. red palm oil daily. The butter samples were placed in cold storage when received, and were examined later as opportunity arose.

Table III. *Yellow and blue values given by rendered butter-fats.*

Sample	Winnie 3rd		Campion 2nd		Woodruff 2nd	
	Yellow units per g.	Blue units per g.	Yellow units per g.	Blue units per g.	Yellow units per g.	Blue units per g.
0	10	12.5	4	7.5	8	7.5
6	7	12.5	3.5	5	9	7.5
13	4	10	2.5	5	6.5	7.5
	Negative control		Carrots 30 lbs. daily		Red palm oil 8 oz. daily	
20	3	10	2.5	10	4	7.5
28	3	10	6	10	5.5	7.5
30	3	10	7	12.5	6	8.5
36	2	7.5	7	12.5	5	8.5
43	7	10	9	12.5	8	7.5

The results obtained are given in Table III.

(a) The samples from the control animal, Winnie 3rd, showed a gradual decrease in yellow value, with the exception of the last sample taken, which, for reasons quite obscure, showed a partial recovery towards the original value. This decline in yellow coloration could easily be appreciated by the naked eye in the butter even before rendering, the four samples preceding the final being remarkably faintly coloured. The blue values given by the rendered fat showed a much less marked decline.

(b) The preliminary samples of butter-fat from Campion 2nd decreased both in yellow value and blue value. On feeding carrots, however, increases in both these values to levels considerably above the initial values were observed. Although eventually both values were increased in about the same proportion it is noteworthy that the increase in blue value was shown in sample 4, and thus definitely preceded the increase in yellow value, which was first shown in sample 5.

The significance of these results may be best appreciated by reference to Fig. 1. The broken curve has been drawn to represent the fraction of the blue values theoretically attributable to carotene, being deduced from the yellow curve on the assumption that carotene gives a yellow/blue ratio of about 11. It will readily be seen that the increase in blue value as the result of carrot feeding was much too great to be related to the increase in unchanged carotene. It is therefore obvious that the feeding of carotene led to the appearance of vitamin A in the butter-fat. Confirmation for this view was afforded by the predominance of the $620\text{ m}\mu$ absorption band in the SbCl_3 reaction applied to unsaponifiable matter obtained from the various samples.

(c) The samples given by Woodruff showed declining yellow values during

the preliminary period. The feeding of red palm oil seemed to result in a restoration of the yellow colour towards its original value, although the results were irregular. The blue value remained practically constant throughout the whole experiment. The supplement was therefore less marked in its effect than the carrots, either on account of the relatively small amount given or through poor absorption.

Fig. 1. Campion 2nd. The effect of carrot-feeding on the vitamin A and carotene content of the butter-fat.

DISCUSSION.

A general consideration of the above evidence supports the view that the cow resembles the rat in its ability to convert carotene into vitamin A. Since the cow is exclusively herbivorous under natural conditions the invariable presence of vitamin A in the liver oil in itself would place this conclusion almost beyond doubt. At the same time the presence of carotene in the milk-fat and body-fat would seem to result from some genuine modification in its metabolism, and not merely from the large amounts which are normally included in the diet. Thus, in contrast with the cow, the sheep maintains colourless body-fat under identical conditions of nutrition, while the pig, in the experience of the writer, may consume large amounts of red palm oil without showing the least degree of pigmentation in its fat. The finding that the butter-fats of Jersey and Short-

horn cows show different degrees of pigmentation under similar conditions of nutrition indicates that the factors determining the presence of carotene in the fat may vary even between different breeds of the same animal.

Although in the liver of the cow vitamin A represents the main fraction of the chromogenic value it seems probable that in the rest of the body carotene is present in much higher proportion. The experiments of Euler, Euler and Hellström [1928] have indeed suggested that the blue colour given by ox blood-fat corresponds exactly with the carotene content. Of particular interest in this connection is the presence of carotene in the corpus luteum. Since Escher [1913] succeeded in isolating the crystalline pigment from mixed cow and sheep corpora lutea there can be no doubt as to its identity. The presence of such considerable amounts of carotene, apparently unaccompanied by vitamin A, must present an interesting problem in the mechanism of reproduction.

The economy of vitamin A and carotene in the cow during lactation.

The results obtained in the above experiments possess secondary interest in permitting a rough visualisation of the vitamin A economy of the cow. Considerations may be based on the following approximate data for an average cow.

(1) When having access to pasture, the cow may eat up to 40 kg. of grass daily. If the grass be taken to contain 10–100 mg. of carotene per kg. (fresh wt.) this intake must represent 0.4–4 g. of carotene, equivalent to 80,000–800,000 B.U. of the pigment, which should presumably be capable of yielding about 800,000–8,000,000 B.U. of vitamin A on complete conversion.

(2) The weight of the liver may be taken as roughly 5 kg. If the vitamin A content be taken as 300 B.U. per g., typical of the highest values obtained in the present experiments, the total reserve may therefore be computed as about 1,500,000 B.U.

(3) A cow may secrete about 1 kg. of milk-fat daily. This should involve the loss of 20,000 B.U., together with 10,000–20,000 Y.U., according to breed.

In the case of the cow at pasture, therefore, the daily output of vitamin A and carotene in the milk-fat is very small in comparison with the amount of carotene available in the diet. Storage of vitamin A in the liver takes place, while a large excess of pigment must undoubtedly be excreted unchanged. It is of interest to note that just as in the rat the vitamin A content of the body-fat remains within normal limits in the face of large dietary excess of carotene [Moore, 1931], so in the cow the vitamin A and carotene of the butter-fat are maintained at normal values under similar conditions. This point is most clearly illustrated by the experiment on Campion 2nd described above. During the period of experimental feeding the cow consumed about its own weight of carrots, an amount obviously sufficient, in the absence of selective absorption by the liver and rejection by the intestine, to render the body-fat and milk-fat deep orange in colour. Nevertheless the vitamin A and carotene content of the butter-fat did not rise above the normal summer level.

On the other hand if the cow is stall-fed upon a diet almost devoid of carotene the daily drain of vitamin A into the milk-fat can no longer be neglected. On the basis of the typical figures given above it is obvious that the reserves of vitamin A in the liver, assuming that they are available for transference to the milk-fat, would last for only 75 days. The reserves of carotene and vitamin A in the body-fat, from experiments on the rat, would be expected to represent a much smaller aggregate. Unless the liver has been unusually highly charged with vitamin A, therefore, the cow must be incapable of maintaining the concentration of the vitamin in its milk-fat at the normal level when exposed to long periods of carotene deficiency. In practical dairying a small allowance of carrots or other material rich in carotene should suffice to restore a positive balance.

SUMMARY.

(1) Although the body-fat of the cow is normally characterised by the presence of small amounts of carotene, vitamin A as such is present in preponderating amounts both in the liver- and milk-fats. Since the cow is purely herbivorous this finding suggests that carotene undergoes the conversion to vitamin A previously demonstrated in the rat.

(2) This conclusion is supported by the finding that the feeding of carotene, in the form of carrots, to a cow at the end of the winter stall-feeding period resulted in an increase not only in the carotene content but also in the vitamin A content of the butter-fat.

(3) The degree of pigmentation of butter-fat, when adequate carotene is available in the diet, is determined by breed. Butter-fat derived from Jersey cows gave a yellow value twice that obtained with butter-fat derived from Shorthorn cows under similar nutritive conditions.

(4) When the cow is at pasture the output of carotene and vitamin A in the butter-fat is very small in comparison with the amount of carotene available in the diet. On the other hand during stall-feeding upon a diet almost devoid of carotene the reserves of vitamin A available in the liver and elsewhere may be insufficient to meet the demands of prolonged lactation. In practical dairying a small allowance of carrots, or other material rich in carotene, should suffice to restore a positive balance.

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