The Apparent Intestinal Synthesis of Carotene by Sheep*

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During an investigation into certain aspects of the carotene metabolism of ruminants, it was desired to obtain an indication of the effect of the composition of the feed on the availability of carotene to the animals. The most convenient method of measuring approximately the carotene absorption appeared to be by determining its apparent digestibility using the ordinary digestibility-trial technique. However, if any appreciable decomposition occurred in the digestive tract, the calculated digestibility might bear no relationship to the actual absorption. It seemed desirable, therefore, to verify whether there is any oxidative decomposition in regions where absorption of carotene does not occur by determining carotene content, relative to an inert reference substance, at various points through the digestive tract. In addition, it was anticipated that the same experiment would afford some explanation of an apparently anomalous excretion of carotene observed in preliminary digestibility trials, where some of the sheep were found to be excreting more carotene than they were consuming, excretion in some cases reaching 160 % of the ingested provitamin.

EXPERIMENTAL

Lignin as reference substance. Lignin was selected as a suitable reference substance for these experiments, and a digestibility trial with pasture-fed sheep provided an opportunity for checking the recovery of lignin from grass. At the same time, the excretion of carotene was further investigated by estimating carotene:lignin ratios in samples of faeces collected at intervals from these animals.

Estimation of carotene and lignin. Carotene was estimated in feed, ingesta and faeces by a modification (McGillivray, 1950) of the cold-extraction method using a 'foaming mixture' of light petroleum and ethanol as described by Moore & Ely (1941). The method of Ellis, Matrone & Maynard (1946) was used for the lignin determinations. All assays were carried out in duplicate and, in order to reduce sampling errors, the residue from the carotene determinations was dried at room temperature, ground, and used for the lignin estimations. Carotene:lignin ratios were calculated in all instances in mg. carotene/g. lignin. The reproducibility of these ratios was investigated in a number of instances, a typical result, where six determinations were made on a well-mixed sample of dried grass being, carotene 355 ($\pm$ 15) mg./kg. and lignin 48 ($\pm$ 2·0)g./kg. giving a carotene:lignin ratio of 7·4 with a standard deviation of $\pm$0·4 or 5·5 %.

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Differences of the same order were found between determinations on samples of ingesta and faeces.

Carotene ratios through the digestive tract. Samples of ingesta, each equivalent to about 5 g. of dry matter, were collected from the four stomachs, from various points along the small intestine and from the caecum, colon and rectum of a pasture-fed sheep immediately after slaughter. These, together with a sample of freshly voided faeces and a sample, as representative as possible, of the pasture on which the animal had been grazing, were assayed for carotene and lignin. Similar determinations were made on three other sheep, samples on one occasion being taken at more frequent intervals through the intestine.

Carotene excretion and lignin recoveries. The level and uniformity of carotene excretion by sheep was investigated by determining carotene:lignin ratios in samples of faeces collected twice daily from four pasture-fed animals which formed part of a group used for a digestibility trial. For the trial, the faeces were collected by the conventional bag method and, when these bags were emptied, samples (about 25 g. each) of the most recently voided faeces were collected. These, together with representative pasture samples, were held at 0° until the end of the trial, when they were assayed in duplicate for carotene and lignin. The digestibility of lignin was calculated in the usual way from the trial figures. As a further check on the constancy of its composition, the nitrogen contents of samples of lignin extracted from pasture, caecal contents and faeces were also determined.

RESULTS

The variations in carotene:lignin ratios found in the first sheep investigated are shown in Table 1. These figures show a decrease in the carotene ratios to a minimum in the jejunum, and then an increase reaching a maximum in the caecum followed by a small decrease through the colon and rectum. Table 2 shows the ratios found in the second animal, where particular attention was paid to the changes through the intestine. The trends, which were similar to those observed in the first animal, were also apparent in the other two sheep examined, the carotene:lignin ratios decreasing from 16·7 in the pasture to a minimum of 13·7 in one case, and 15·6 in the other, in the mid-jejunum and increasing to maxima of 17·3 and 20·0, respectively, in the caecum. With the first

Table 1. *Carotene:lignin ratios in grass and through the digestive tract of a pasture-fed sheep*

Origin of sample	Ratio (mg. carotene/g. lignin)	Origin of sample	Ratio (mg. carotene/g. lignin)
Grass	21·2	Ileum:	
Rumen	12·3	mid 2 ft.	23·4
Reticulum	16·7	last 2 ft.	25·5
Omasum	14·0	Caecum	28·1
Abomasum	22·0	Colon	27·0
Duodenum	22·8	Rectum	21·4
Jejunum:		Faeces	22·5
first 6 ft.	23·3		
mid 6 ft.	15·2		
last 6 ft.	15·9		

animal the ratios varied considerably through the four stomachs owing, possibly, to the retention of more fibrous material of low carotene content in the rumen and omasum. In all instances, however, good agreement was found between the ratios in the pasture and in the abomasum, indicating little loss of carotene during passage through the stomachs.

Table 2. *Carotene:lignin ratios through the digestive tract of another sheep*

Origin of sample	Ratio (mg. carotene/ g. lignin)	Origin of sample	Ratio (mg. carotene/ g. lignin)
Grass	16·7	Small intestine:	
Abomasum	14·9	tenth 6 ft.	22·2
Duodenum	15·5	eleventh 6 ft.	18·8
Small intestine:		twelfth 6 ft.	17·6
first 6 ft.	11·7	Caecum	22·2
second 6 ft.	9·0	Colon:	
third 6 ft.	9·9	first 2 ft.	21·8
fourth 6 ft.	12·0	second 2 ft.	21·0
fifth 6 ft.	13·9	third 2 ft.	20·1
sixth 6 ft.	13·4	fourth 2 ft.	20·4
seventh 6 ft.	16·0	fifth 2 ft.	18·7
eighth 6 ft.	13·8	sixth 2 ft.	18·9
ninth 6 ft.	16·6	Faeces	17·5

The excretion of carotene by the four pasture-fed sheep was fairly uniform for each animal over the 3-day collection period but varied somewhat between animals. The average carotene:lignin ratio in the pasture was 18·5 and the ratios found in the faeces are shown in Table 3. The percentage carotene excreted was high in all cases, animal no. 2 showing a marked negative balance. The average lignin recovery was 96·0 %, indicating negligible digestibility. No significant differences were found in the nitrogen content of the lignin isolated from pasture, caecal contents and faeces, all samples containing 1·8–2·5 % nitrogen.

Table 3. *Carotene:lignin ratios in faeces of four sheep*

	Sheep no.			
Day	1	2	3	4
1st: a.m.	15·7	22·4	17·0	16·0
p.m.	13·4	19·8	18·6	18·8
2nd: a.m.	12·2	21·8	18·2	21·1
p.m.	15·4	21·5	17·9	17·9
3rd: a.m.	15·4	20·3	16·5	16·3
Average	14·4	21·2	17·6	18·0
Carotene excreted (as percentage of that ingested)	78·0	114·5	95·2	97·4

DISCUSSION

It seems reasonable to assume that carotene absorption would, at least in part, account for the decrease in carotene:lignin ratios through the upper portions of the small intestine, and that the decrease through the colon and rectum might be attributed to oxidative decomposition of the pigment. The increase in the ileum and caecum is more

difficult to explain, but could result from (*a*) a partial digestion of the lignin, (*b*) the formation in the lower intestine of a pigment not separated from carotene on the Hyflo Super-Cel (Fisher Scientific Company and Eimer and Amend, New York) chromatographic column, or (*c*) a synthesis of carotene in the intestine. Ellis *et al.* (1946) have shown that the lignin estimated by their method represents an almost completely indigestible fraction of herbage. This, supported by the 96 % recovery of ingested lignin from faeces in this investigation, excludes the possibility of the increase in carotene:lignin ratios in the caecum being due to the use of lignin as an inert reference substance. This conclusion is also supported by the constancy of the nitrogen content of the lignin samples isolated from various sources. Although the lignin molecule is believed not to contain nitrogen, it has not been possible to isolate nitrogen-free lignin from succulent plant tissues. If, as is generally considered (MacDougall & DeLong, 1948*a–c*), this nitrogen is due to the condensation of protein molecules with the lignin, possibly during extraction, even small changes in the nitrogen content would have markedly affected the carotene ratios.

An extensive rechecking and investigation of the accuracy of the method used for the estimation of carotene (McGillivray, 1950) eliminated the possibility of appreciable errors in the carotene assays. Although the possible presence in faeces and other materials of a yellow pigment or artifact that cannot be separated from carotene by the normal phasic methods is well recognized (Booth, 1945), it should be possible to effect a separation by chromatographic methods. However, repeated chromatographing on columns of Hyflo Super-Cel or of magnesium oxide and Hyflo Super-Cel failed to reveal the presence of pigments other than the carotenes. The identity of the pigment obtained from caecal contents was further confirmed by a comparison of its absorption spectrum with that of a sample of carotene extracted from fresh pasture. As shown in Fig. 1 the pigment from the intestine appears to be identical with the carotene in the pasture, allowing for some isomerization in the digestive tract.

It appears, therefore, that some synthesis of carotene must have occurred in the digestive tracts of the sheep. Negative balances of carotene have been reported previously (e.g. by Whitnah, Peterson, Atkeson & Cave, 1939) but in all such instances phasic separation methods had been used for separating the carotenes from other pigments. Since these methods are not specific for the carotenes, subsequent workers have apparently attributed the findings entirely to the presence in the carotene fraction of other epiphasic pigments. The presence of these pigments, which form a yellow band immediately above the carotenes on the chromatographic columns, has been noted in this investigation. The quantities have, however, been small, representing less than 15 % of the total epiphasic pigments present, and it seems possible that carotene synthesis might also have contributed to the negative digestibilities previously reported.

Several micro-organisms, e.g. *Staphylococcus aureus*, are capable of synthesizing carotenes (Zechmeister & Cholnoky, 1943, pp. 126, 137), and the possibility of intestinal synthesis by these organisms has apparently been considered previously, but in a recent report it was concluded that formation of carotene did not occur in the digestive tract of man (Hume & Krebs, 1949). If micro-organisms are responsible for the synthesis in sheep it should be possible to show an increase in carotene content on incubating

caecal or ileal contents. Although this aspect of the work is still under investigation, it has not so far been possible conclusively to demonstrate carotene formation under these conditions, but synthesis has been shown to occur on a solid medium containing agar 2 %, tryptose 2 %, dextrose 0·1 %, and sodium chloride 0·5 %, inoculated with caecal contents collected under aseptic conditions. Carotene, identified by its absorption spectrum, was formed in quantities equivalent to 1·2–1·8 μg./ml. medium. No attempt has been made to identify the micro-organisms responsible.

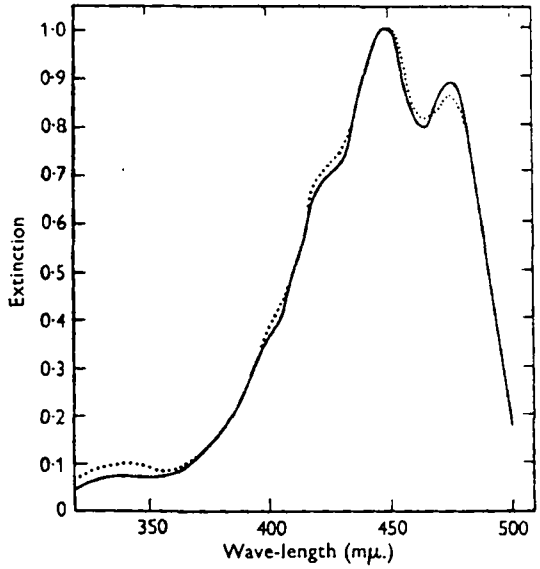

Fig. 1. Absorption spectrum of carotene. Isolated from: ———, grass; . . . . ., caecal contents of a sheep.

Apart from this synthesis of carotene, marked decomposition of the provitamin appears to occur in the colon and rectum, so that the apparent digestibility, as calculated from the difference between the amounts ingested and excreted, gives no indication of actual absorption. A point of immediate interest is whether the synthesized carotene can be utilized by the animal. No absorption of carotene or vitamin A occurs in the caecum or colon (Barrick, Andrews & Bullard, 1948) but it is possible that some absorption occurs in the lower portions of the ileum.

SUMMARY

1. Carotene:lignin ratios were determined at different points through the digestive tracts of four sheep. The ratios decreased through the upper portions of the small intestine, increased through the ileum reaching a maximum in the caecum, and decreased slightly through the colon and rectum.

2. The increase in carotene:lignin ratio was not due to a partial digestibility of the lignin fraction of the herbage or to the erroneous estimation as carotene of some non-carotene pigment formed in the intestine.

3. It is suggested that carotene is synthesized by the micro-organisms of the ileum and caecum. Such synthesis of carotene by intestinal micro-organisms has been demonstrated on an agar medium inoculated with caecal contents.

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The Composition of Human Milk with Special Reference to the Relation between Phosphorus Partition and Phosphatase and to the Partition of Certain Vitamins

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Two of the present authors are making a detailed study of the composition of the milk and colostrum of cows and goats, as affected by hormonal and nutritional influences. The results, which will be published shortly, showed that ester phosphorus was negatively correlated with phosphatase, the coefficient of correlation being $-0{\cdot}95$. In cows treated with thyroxine or thiouracil the same correlation obtained (Chanda & Owen, 1949). Lipid phosphorus and phosphatase were similarly correlated. During the change from colostrum to milk the correlations were still found. There was likewise (Chanda, McNaught & Owen, 1949) a negative correlation between phosphatase and phosphorylated vitamin B_1 in milk, as previously reported by Houston, Kon & Thompson (1940). The present work was undertaken to see whether the changes in human milk at the beginning of lactation were at all comparable with those in cow's