THE CONVERSION OF CAROTENE TO VITAMIN A IN SHEEP AND CATTLE*

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Summary

It has been established that the conversion of carotene to vitamin A in the sheep occurs in the wall of the intestine. This conclusion is based on surviving tissue experiments in which sections of intestine have been incubated with carotene and the product conclusively identified as vitamin A by colorimetric and spectrophotometric methods. It is further supported by the high vitamin A levels in intestinal as compared with non-intestinal lymph, and similar observations with cattle suggest the intestine as the site of conversion in this species also.

I. Introduction

The role of the carotenes as precursors of vitamin A was established about 20 years ago, principally by the work of Moore (1929, 1930), and his assumption (Moore 1931) that the liver was the main site of conversion in the animal body has been generally accepted until quite recently. No direct evidence of a satisfactory nature has, however, been advanced in support of this assumption and the observation that the parenteral introduction of carotene into rats failed to relieve symptoms of vitamin A deficiency, the pigment merely accumulating unchanged in the liver, led Sexton, Mehl, and Deuel (1946) to suggest an alternative site of conversion, possibly the wall of the intestine. Subsequent workers (Glover, Goodwin, and Morton 1947, 1948; Mattson, Mehl, and Deuel 1947; Wiese, Mehl, and Deuel 1947; Mattson 1948; Thompson, Ganguly, and Kon 1947; Krause and Pierce 1948), using a number of different techniques, have demonstrated conclusively that in the rat the conversion of carotene to vitamin A occurs in the wall of the intestine.

At this stage other aspects of the carotene metabolism of ruminants were under investigation in this laboratory and, as previous observations had been confined to rats, it seemed of interest, in view of possible species differences, particularly with herbivorous animals, to determine whether the wall of the intestine was also the site of conversion in sheep and cattle. Experiments were undertaken therefore, (1) to attempt, using sections of the small intestine of sheep, a repetition of the surviving tissue experiments by which Wiese, Mehl,

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and Deuel (1947) had so elegantly established the site of conversion in the rat, and, if positive results were obtained, (2) to investigate the mode of transport of the vitamin A from the intestine to the liver, and (3) to determine whether conversion occurred throughout the length of the small intestine or was restricted to certain regions. Since this work was commenced, Thompson, Ganguly, and Kon (1949) have extended previous observations with rats and have shown the site of conversion to be the wall of the intestine in pigs also, while similar findings have been reported for sheep, goats, and rabbits (Goodwin and Gregory 1948), and recently for calves (Stallcup and Herman 1950). Using somewhat different methods, the present investigation has, however, provided further evidence in support of the findings of these later workers and the results are submitted in confirmation of their conclusions regarding the site of conversion in cattle and sheep.

II. Experimental

(*a*) In Vitro *Conversion Experiments*

Intestines were removed from sheep as rapidly as possible following slaughter and placed in a bath containing Ringer-Locke solution* maintained at about 37°C. Sections (approx. 2-3 ft. in length) were removed from the jejunum and the contents flushed out with Ringer-Locke solution. Colloidal carotene (about 20 ml.), prepared as described by Wiese, Mehl, and Deuel (1947), was introduced into one section from each animal and after ligation at the ends, the tissue was incubated anaerobically in Ringer-Locke solution for 2-3 hours at 37°C. As controls, sections of equivalent length were taken immediately above and below that section into which carotene was introduced. After incubation the colloidal carotene was flushed out with 0.9 per cent. saline. The sections were comminuted in the Waring Blendor under nitrogen with ethanol containing 5 per cent. potassium hydroxide. The suspension was then refluxed until a clear solution was obtained (10 to 15 minutes) and the vitamin A extracted into light petroleum using a method similar to that described by Gallup and Hoeffer (1946) for liver samples. Vitamin A was estimated spectrophotometrically in the light petroleum solutions (concentrated where necessary by evaporation at room temperature under vacuum in a stream of nitrogen) using a Beckman Model D U photoelectric spectrophotometer and applying a three point correction procedure to allow for extraneous absorption (McGillivray 1950). The control sections were assayed in the same way.

The identity of the vitamin A formed in a number of these experiments was confirmed colorimetrically and by a measurement of its absorption spectrum after purification. The extracts containing the crude vitamin A from a number of incubated sections were combined, washed with water to remove traces of ethanol, dried over anhydrous sodium sulphate, and evaporated to

* The Ringer-Locke solution employed had the following percentage composition: sodium chloride 0.9; potassium chloride 0.042; calcium chloride 0.024; sodium bicarbonate 0.05; magnesium chloride 0.02; glucose 0.05.

about 20 ml. under reduced pressure. The absorption curve of this solution was measured and it was then chromatographed on a column of 1 : 1 magnesium oxide-"Hyflo Super-Cel" as described by Mattson (1948). The chromatogram was developed with light petroleum containing 5 per cent. benzene, and the vitamin A band, detected by its fluorescence under ultraviolet light, separated mechanically and eluted with light petroleum saturated with ethanol. After its absorption curve had been measured the solution was evaporated to dryness and the residue taken up in chloroform. The vitamin A content of this solution was estimated colorimetrically using activated glycerol dichlorohydrin. In addition the absorption curve of the coloured compound formed with glycerol dichlorohydrin was also measured. Glycerol dichlorohydrin was used in preference to antimony trichloride solution since the transitory nature of the colour produced by the latter reagent renders measurement of its absorption curve difficult in spectrophotometers of the Beckman type.

(*b*) *Transport of Vitamin A from the Intestines to the Liver*

If conversion of carotene to vitamin A occurs in the wall of the intestine, the vitamin A formed must be transported from there to the liver by either the portal or lymphatic routes or both, and carotene will not appear in the blood or lymph unless the rate of absorption exceeds the rate of conversion to the vitamin. This excess carotene may be transformed to vitamin A at a secondary site of conversion or it may be treated merely as a waste product and decomposed in various tissues. It was apparent therefore that a comparison of the carotene and vitamin A levels in portal and systemic blood and in intestinal and non-intestinal lymph would supply information regarding the site of conversion and the mode of transport of the vitamin or provitamin from the intestine.

Intestinal and non-intestinal lymph glands were removed from a number of sheep immediately following slaughter. Three groups of similar pasture-fed animals were used and sufficient were included in each group to provide about 5 ml. of both types of lymph. In each group the intestinal glands, which included duodenal, jejunal, and ileal, were combined and as much lymph as possible collected from them. In the same way, samples of non-intestinal lymph were collected from various other glands, mainly submaxillary and pharyngeal, from the same animals. Preliminary estimations of carotene and vitamin A were carried out using the method described by Kimble (1939) for blood plasma. Poor recoveries of vitamin A added as internal standard indicated the presence of colour inhibitors in amounts greater than encountered in blood plasma, and for the lymph samples the Kimble method was modified to include saponification as described by Parrish, Wise, and Hughes (1948).

Similarly, samples of intestinal and non-intestinal lymph were obtained from a pasture-fed bullock immediately following slaughter. These were assayed for vitamin A using the modified Kimble method. Sufficient lymph was obtained to carry out the assays in duplicate.

Attempts were made to compare the carotene and vitamin A levels in portal and systemic blood using an anaesthetized sheep following injection into the intestine of an excess of readily absorbable carotene. Although, in a preliminary experiment, the vitamin A level in the portal blood was found to be markedly higher than in the systemic blood, it is doubtful whether reliance can be placed on this observation, since, as pointed out by Goodwin and Gregory (1948), absorption processes in general are retarded by anaesthesia and trauma, and these experiments were not proceeded with.

(*c*) *Region of Conversion*

In an attempt to determine whether the conversion could occur at any point along the intestine or was limited to a particular region, whole intestines were incubated with colloidal carotene, using a method similar to that already described for the short sections. After incubation the intestines were cut into short sections, which were assayed separately for vitamin A. These experiments gave variable results owing possibly to injury to portions of the tissue caused during handling. It was possible, however, to obtain an indication of where carotene absorption and conversion occurred by estimating vitamin A in the various intestinal lymph glands. Duodenal, jejunal, and ileal lymph glands were removed from four pasture-fed sheep immediately following slaughter. Insufficient lymph could be obtained from each gland for assay so the assumption was made that the ratio of lymph to gland tissue was relatively constant, and whole glands, after grinding with sand, were assayed separately for vitamin A, using the method already described for the sections of intestine. For plasma and lymph samples it was found that, provided the extracts had been saponified, the three point correction procedure gave results agreeing to within about ± 8 per cent. with the Carr-Price figures.

III. Results and Discussion

For the surviving tissue experiments it was not possible to deplete the sheep of vitamin A before slaughter or even to maintain them for a short time on a carotene-free diet. In most cases therefore, carotene absorption was proceeding at the time of slaughter and the sections contained appreciable quantities of carotene and vitamin A before incubation. A large number of experiments were carried out on sections of this type and, omitting preliminary experiments, the incubated tissues showed statistically significant increases in vitamin A content of up to 15 per cent. Although this was considered evidence of conversion in the wall of the intestine, the increases were small and the results less convincing than those reported by Wiese, Mehl, and Deuel (1947) for vitamin A-depleted rats. It was possible, however, to carry out a few experiments on intestines from sheep that had been fed a poor quality hay of low carotene content for some time prior to slaughter. The vitamin A content of control and incubated sections from six of these animals is shown in Table 1. Analysis of variance carried out on these figures showed the increase on incubation with colloidal carotene to be highly significant.

The absorption curve for the combined light petroleum extracts from these six incubation experiments is shown in Figure 1, curve 1. Curve 2 gives the absorption of the purified solution obtained after chromatography. This curve resembles that of vitamin A, and application of the three point correction procedure gave an apparent total vitamin A content of 88 μg. (78 per cent. recovery) as illustrated by curve 3. Curves 4 and 5 represent the impurity apparently present in the original and chromatographed solutions respectively. The total vitamin A content estimated colorimetrically with glycerol dichlorohydrin was 98 μg. (86 per cent. recovery) and the absorption curve of the glycerol dichlorohydrin addition product agreed closely with that reported for pure vitamin A (Sobel and Werbin 1946).

TABLE 1
VITAMIN A IN SECTIONS OF THE INTESTINAL WALL OF SHEEP AFTER INCUBATION WITH CAROTENE AT 37°C.

Animal	Vitamin A (μg.)		
	Control	Incubated	Increase on Incubation
1	8.2	25.2	17.0
2	8.4	20.2	11.8
3	4.8	8.5	3.7
4	9.8	27.3	17.5
5	6.9	19.3	12.4
6	5.2	12.3	7.1
Mean	7.2	18.8	11.6

The vitamin A levels in the intestinal lymph samples collected from the three groups of sheep were 118, 84, and 102 μg./100 ml. while the corresponding non-intestinal lymph samples contained respectively, 38, 35, and 34 μg./100 ml. As might be expected from its virtual absence from the blood plasma of sheep, no carotene could be detected in any of the lymph samples. From these results it may be concluded that in sheep the wall of the intestine is a site of conversion of carotene to vitamin A. Eden and Sellers (1948) found the lymphatic route to be the main one by which the vitamin A absorbed was transported to the liver following oral administration to bullocks of emulsified halibut liver oil. From the vitamin A levels found in the intestinal and non-intestinal lymph of sheep in the present investigation, it is apparent that part at least of the vitamin A derived from the conversion of carotene in the wall of the intestine is also transported via the lymphatic route. Similar conclusions, that vitamin A is formed in the intestine and transported partly via the lymphatic system, may be drawn for the pasture-fed bullock. These intestinal lymph samples, taken from two positions along the small intestine, contained 142 and 159 μg. vitamin A per 100 ml. and two non-intestinal samples 48 and 60 μg. per 100 ml. Carotene was also present in the intestinal lymph, the two samples assayed averaging 345 μg. per 100 ml.

The vitamin A levels in the individual intestinal lymph glands of the four pasture-fed sheep are shown in Figure 2. Since only relative figures were required, the average vitamin A content of the glands from each sheep, in μg./g., was calculated and a comparison between sheep made by expressing the vitamin A concentration (μg./g.) in each gland as a percentage of the mean for the animal. These percentages are plotted against the relative position of the gland along the intestine also expressed as a percentage of the total length.

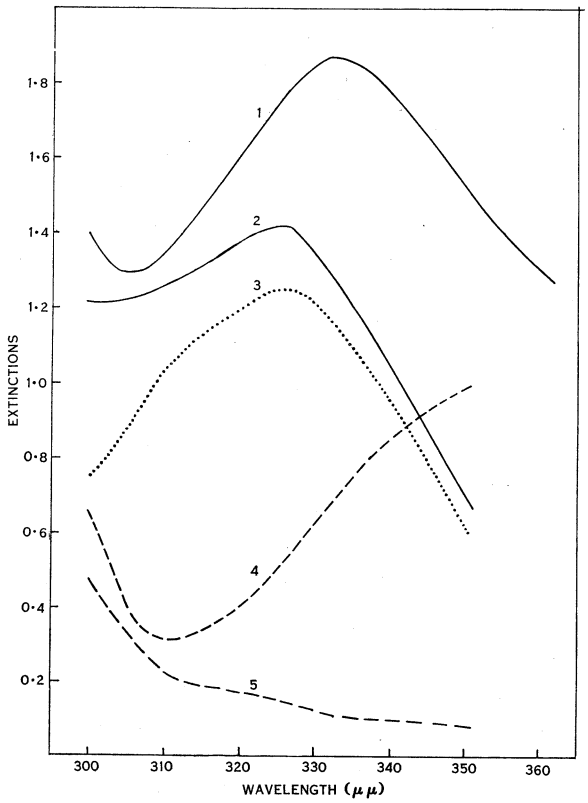

Fig. 1.—Vitamin A formed on incubating intestines at 37°C. with colloidal carotene.

Owing to the variations in size and number of the glands their relative positions are plotted as lines, the lengths of which represent the relative sizes of the glands and give an indication of the length of intestine from which lymph is drained by each gland. The low level of vitamin A and the small size of a number of the glands limits the reliance that can be placed on individual assays but the results obtained from the four sheep indicate that, although there is some carotene absorption and conversion over the whole intestine, maximum vitamin A formation occurs in the upper and lower portions.

IV. Acknowledgments

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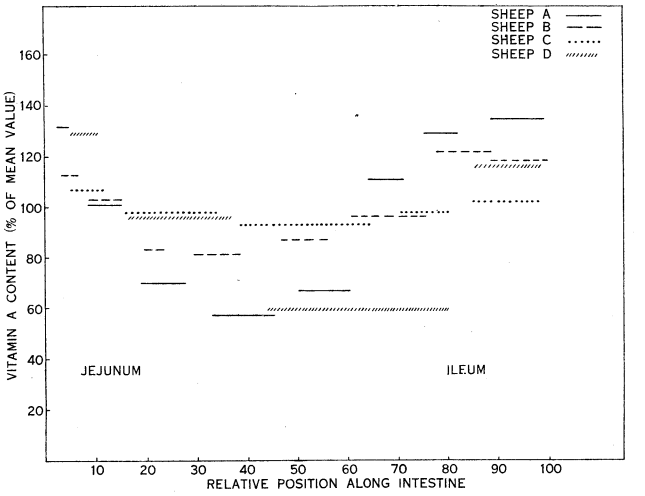

Fig. 2.—Relative vitamin A levels in intestinal lymph glands. Relative position along intestine expressed as percentage of total length.

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