Studies in Vitamin A

7. CAROTENE METABOLISM IN HERBIVORES

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(Received 30 March 1948)

It is generally agreed that the vitamin A found in the livers of herbivorous animals has its origin in the biologically active carotenoids present in the diet, for the intake of preformed vitamin A is nil. It has been widely assumed that the site of the conversion of the active carotenoids into vitamin A is the liver itself, although the results of *in vitro* experiments designed to test this view are equivocal (see, e.g., Woolf & Moore, 1932). In the cow, carotenoids are present in the systemic blood plasma in amounts which reflect the dietary intake of carotenoids (Mitchell & Wise, 1944), and the plasma levels presumably represent a balance between absorption from the intestine and removal by the liver, the pigments being merely *en route* to the liver from the gut. In other herbivorous animals (sheep, goat, rabbit), however, the systemic plasma contains only very small traces of carotenoids (c. 2 $\mu\text{g.}/100\text{ ml.}$), although considerable amounts of vitamin A are found in the liver. In all these animals the administration of vitamin A causes an increase of vitamin A in the systemic blood but administration of carotene is without apparent effect on either the vitamin A or carotene levels.

This fundamental difference between cattle and other herbivora is also shown in other ways, viz. the body and milk fat of the former contains carotenoids and is yellow, whereas that of the latter is colourless. The explanation for this striking difference between the cow and the sheep, goat and rabbit, might well be found by considering the possible factors concerned in the mode of absorption and transport of carotene from the intestine, regarding which there is at present only fragmentary experimental evidence.

Assuming that in these animals carotene* is brought to the liver in the portal blood stream and converted there into vitamin A, it follows that its presence in or absence from the systemic blood will depend on the rates of intestinal absorption and uptake (and conversion) from the portal blood by the liver. Carotene may be present in cow plasma because the rate of absorption relative to that of

uptake by the liver is high, whereas in sheep, goats and rabbits it is low and thus no carotene passes from the liver into the systemic circulation. On the other hand, if carotene is transported by the lymphatic route to the systemic circulation, so bypassing the liver, its accumulation in the systemic blood would depend on (a) the rate of absorption from the gut, (b) the rate of lymphatic transport (concn. $\times$ rate of flow), and (c) the rate of uptake by the liver.

In attempting to decide which route is concerned an obvious preliminary is to ascertain whether carotene is present in the portal blood stream after its administration in large amounts. Two difficulties arise in planning such an experimental approach. One is that absorption processes in general, and particularly those concerning fats, are retarded by anaesthesia and trauma (e.g. opening of abdomen) of several hours duration. Consequently, samples of body fluids obtained under such conditions of an acute experiment on an anaesthetized animal are much less likely to give positive results and the duration of the experiment must be considerably shorter than if the animal were conscious and unharmed. The other difficulty is that, owing to the large size and complicated nature of the ruminant stomach, the passage of material through it takes days, so that orally administered solutions are likely to enter the small intestine greatly diluted and at a very slow rate.

Both difficulties can be overcome by providing the animals beforehand at an aseptic operation with (a) a London cannula (London, 1928) on the portal vein, and (b) fistulae of either the abomasum or duodenum, so that serial portal (and systemic) samples can be withdrawn and solutions introduced directly into the intestine without operative interference.

Using these techniques, a study has been made of the carotene and vitamin A levels of portal and systemic venous blood plasma in conscious goats and sheep after the administration of large amounts of vitamin A or carotene. Consistent failure to detect carotene in transport in these experiments, and the results of concurrent investigations carried out in this laboratory (Glover, Goodwin & Morton, 1947*a, b*;

* Throughout this report the term 'carotene' indicates a mixture of α - and β -carotenes, in which the β -isomer predominates.

1948*a, b*) compelled the conclusion that the older views on the transformation of carotene into vitamin A must be abandoned in favour of the view that the conversion takes place in the intestinal wall. Further experiments on unanaesthetized goats provided with fistulae of the thoracic duct proved this conversion to occur in the conscious animal and showed that part, at least, of the vitamin A formed is transported from the gut via the lymphatic system.

A preliminary report of the first part of this work has already appeared (Goodwin, Dewar & Gregory, 1946).

EXPERIMENTAL

Determination of vitamin A and carotenoids in blood plasma and lymph. The method for blood plasma, which was found applicable to lymph, was that normally used in this laboratory and described in detail by Glover, Goodwin & Morton (1947*c*). All optical measurements, except those in a few preliminary experiments, were carried out using the Beckman quartz photoelectric spectrophotometer. Vitamin A levels were measured by the intensity of the blue colour produced in chloroform solution at 617 m μ . with the SbCl₃ reagent, and carotenoid levels by the intensity of direct light absorption at 449 m μ . of the blood lipid extract dissolved in light petroleum (b.p. 40–60°). The standard error of a vitamin A determination is about 4%, and of a carotenoid determination 1%.

Determination of faecal carotenoids. The carotenoids were extracted according to the method of Goodwin & Morton (1948) and the carotene fraction separated by chromatography on defatted bonemeal (Goodwin & Morton, 1946).

Carotene and vitamin A supplements. The vitamin A concentrates used were potent shark-liver oils. Carotene was used in the form of crystalline β -carotene, red palm oil concentrate, or homogenized spinach (Nestlé). These various preparations were dispersed in a suitable volume of water by means of a Waring Blendor, using, in various experiments, lecithin, bile salts and bile to aid emulsification.

Animals. Rabbits, sheep and goats were used as experimental animals. The rabbits were maintained on a diet of Lever's cubes and fresh cabbage, the sheep and goats on hay and Bibby's cattle food (No. 1 Cakelets).

The goats and sheep were provided with London cannulae (London, 1928; Gregory, 1946), abomasal or duodenal cannulae, and fistulae of the thoracic duct, at aseptic operations performed under general anaesthesia (Gregory, 1947).

Collection of thoracic lymph in the conscious animal. The thoracic duct was exposed and drained either by tying all other branches of the jugular vein, into which the duct usually opened, and then introducing a cannula into the vein, or by passing directly into the duct a thin-walled plastic tube in which the lymph did not clot for many hours. The cannula or tubing was brought out through a stab-wound in the skin adjacent to the incision, and lymph collected in a small rubber bag containing a small amount of heparin. In some experiments in which cannulation of the jugular was performed, clotting was successfully avoided by subcutaneous injection of 'chlorazol sky blue' in the vicinity of the incision. The dye promptly appeared in the lymph and this was stained blue and rendered incoagulable

for many hours. In other experiments, however, this method was not successful, and as supplies of suitable plastic tubing became available to us, this was used in later experiments.

The duration of collection of lymph in experiments which were successful in that clotting was avoided, was in some cases limited by the death of the animals from an unknown cause. The usual sequence of events was that, following recovery from the anaesthetic (about 15 min. after completion of the operation), the animal remained in an apparently normal state, walking about and eating, for a period of 10–20 hr. Rapid deterioration in its general condition then became apparent, indicated chiefly by stertorous and rapid breathing, and increasing weakness of the limbs. Death from respiratory failure occurred within 1–2 hr. from the first appearance of these signs and post-mortem examination failed to reveal any condition likely to have been responsible for death. The rate of flow of lymph was constant at about 30–50 ml./hr. and tended to decrease during the period of introduction of the carotene solution into the intestine, particularly if this was rapid. The flow continued up to the moment of death. In other experiments, which were failures owing to clotting of the lymph, the animals recovered completely and regained a normal healthy state; and as in these cases the same procedures had been carried out, it seems unlikely that any of these was itself a factor in the death of the other animals.

Since deterioration in the animal's condition would be expected, if anything, to depress absorptive processes, and in the experiments reported here absorption of the solution introduced (as indicated by the presence of vitamin A and fat in the lymph samples) was, in fact, well advanced before the death of the animal, we feel that the fact that these experiments were semi-acute, in that they were not followed by permanent recovery of the animal, does not detract from their value.

RESULTS

Carotene and vitamin A in portal and systemic venous blood

A. Rabbits. Experiments were first carried out to ascertain whether oral administration of massive doses of carotene was followed by the appearance in the systemic blood of appreciable amounts of carotene. Crystalline β -carotene dissolved in refined arachis oil or dispersed in water was administered by stomach tube to groups of 5 rabbits in doses of 4800 μ g. each. In two experiments the blood samples were drawn at intervals of 3, 6, 27, 48, 72 and 96 hr. after dosing, and in a third at hourly intervals for the first 4 hr. after dosing. These experiments were carried out before the photoelectric spectrophotometer was available, and in view of the large quantities of plasma required for a determination it was considered preferable to pool the samples from the group rather than to withdraw excessive amounts of blood from any one animal. In no instance was carotene observed in the plasma. The possibility that the absorption was defective

was investigated by determination of the faecal carotene content; it was found that whereas absorption from arachis oil was poor (almost 100 % excretion) that of the colloidal solution was apparently good, only 13.6 % of the administered carotene being accounted for in the faeces. The bulk of the carotene given in this form had either been absorbed or destroyed in the intestine; experiments in which colloidal carotene solutions were incubated at 37° with rabbit intestinal contents provided no evidence for such destruction. It seemed reasonable to conclude therefore that the carotene had been absorbed without gaining access to the systemic circulation.

Examination of the portal blood for vitamin A and carotene under similar conditions was obviously indicated; and in the hope of avoiding more complicated experimental procedures, a number of acute experiments were performed on rabbits in which blood samples were taken simultaneously from the portal vein and the inferior vena cava under light nembutal or cyclopropane/O₂ anaesthesia at various times after the oral administration of large doses of carotene. No detectable amounts of carotene were found in any of the blood samples.

B. *Sheep and goats.* In the experiments just described, failure to detect carotene in the portal blood was not unexpected in view of the notorious sensitivity of absorption processes to the conditions of such experiments. Further experiments were therefore carried out on goats and sheep provided with portal and abomasal cannulae.

In these experiments, the results of three of which are summarized in Table 1, the carotene preparation (dispersed unsaponifiable matter from red palm oil prepared by the method of Goodwin & Morton, 1946) was introduced slowly into the abomasum and 20 ml. samples of portal and systemic venous

blood were drawn simultaneously at frequent intervals for periods up to 24 hr. after dosing. The results were consistently negative; the carotene values of the bloods were so low as to be indistinguishable from zero for they correspond to a reading on the spectrophotometer of $E=c \cdot 0.01$. The vitamin A levels were, on the whole, reasonably constant, and certainly showed no significant rise after administration of the carotene.

Two similar experiments were performed on sheep, in one of which the carotene was administered in the form of spinach purée. Both gave results identical with those obtained on goats, and are therefore not reported in detail.

Extent of absorption of carotene from the alimentary tract

A possible explanation of the negative results so far recorded was that little or none of the carotene given in the manner described had been absorbed. An experiment carried out on a sheep, in which vitamin A was administered under similar conditions instead of carotene, showed that this is well absorbed, a normal blood tolerance curve being obtained. The vitamin A levels in the portal and systemic blood samples were never significantly different, and the time required to reach maximum blood levels was 4–6 hr., approximately the same as that needed in man (Table 2).

However, as the dose of vitamin A used was considerably higher than that of carotene and as it is well known that vitamin A is more readily absorbed than carotene (e.g. Wald, Carroll & Sciarra, 1941), further experiments were made to check more directly the efficiency of carotene absorption. In goat no. 2 (Table 1) carotene was introduced in the

Table 1. *Carotene and vitamin A levels of portal and systemic blood*

(Samples of plasma of goats drawn at varying intervals after administration via an abomasal cannula of 31,000 µg. carotene as red palm oil unsap. dispersed in 2 l. of water with potassium stearate.)

Goat no.	Time after dosing (hr.)	Plasma carotene and vitamin A levels			
		Systemic		Portal	
		Carotene (µg./100 ml.)	Vitamin A (i.u./100 ml.)	Carotene (µg./100 ml.)	Vitamin A (i.u./100 ml.)
2	0	3.9	44	3.0	43
	3½	3.1	39	2.8	29
	7	2.3	—	7.2	—
	24	6.1	36	1.5	23
3	0	5.6	43	7.1	47
	4	4.2	39	6.6	66
	8	3.4	60	4.2	76
	24	1.8	50	2.4	56
4	0	4.2	49	2.4	55
	1	—	56	3.0	47
	3	1.8	30	3.0	47
	6	1.2	—	1.2	35
	24	1.8	—	1.2	32

Table 2. *Vitamin A levels of the portal and systemic blood*

(Samples from a sheep after receiving 3×10^6 i.u. of vitamin A dispersed in 2 l. of water with 0.2% (w/v) of bile salts and 0.1% (w/v) sodium stearate.)

Time after dosing (hr.)	Vitamin A levels (i.u./100 ml. plasma)	
	Portal	Systemic
0	20	28
1½	68	73
4½	131	129
7	121	116
24	99	118
72	48	—

Table 3. *Excretion of carotene*

(Estimation of carotene in fluid draining from an ileal fistula of a sheep fed 32,850 µg. of carotene as homogenized spinach via an abomasal cannula.)

Time of draining after administration of carotene (hr.)	Volume of liquid (ml.)	Carotene content (µg.)	Hourly excretion (µg.)
3	50	Nil	Nil
5	20	Trace	Trace
7	Nil	Nil	Nil
11	160	427	107
14	250	58	194
16	Trace	Trace	Trace
21	250	300	60
23	50	252	126
25	50	400	200
28	50*	106*	c. 70
32	50	252	63
34	150	163	82
37	200	840	280
49	100	1250	104

Total 4048

* Incomplete collection about 75% of total.

usual way into the abomasum and this was emptied 5 hr. afterwards. Only 6% of the carotene introduced was still present, indicating that there was no appreciable retention in the abomasum. The excretion in these animals was ascertained in two ways: (a) by the usual balance experiment in which the amount of carotene excreted for several days before and after administration of a known dose was followed; and (b) by providing a sheep with a cannula in the terminal ileum in addition to that in the abomasum so that after administering carotene all material which passed through the small intestine could be collected and examined. This sheep received into the abomasum homogenized spinach containing 32,850 µg. of carotene, and the ileal contents were collected for the next 48 hr. (Table 3). From the results it was estimated that 87.7% of the carotene had failed to reach the terminal ileum.

The balance experiments were carried out on two goats each provided with (a) a portal cannula, and (b) an abomasal or duodenal fistula. As it was not feasible to remove hay from the diet either before or during the experiment, a base-line of carotene excretion had to be determined. This was obtained by taking a weighted mean value for the daily carotene excretion over 5–7 days previous to the experiment and for a similar period after the experiment, when the levels had returned to, or dropped below, the original levels. This tended to eliminate any error due to variation in the amount of hay consumed by the animals during the experiment. Table 4 records the results of two such experiments, which indicate that carotene is well absorbed under these conditions. Only 18.9 and 7.1% respectively of the carotene given appeared in the faeces.

It seemed probable from the results of the experiments in the previous series that the carotene was largely absorbed, but possibly at a rate which was slow and dependent on the rate of passage from the abomasum, so that detectable amounts of carotene

Table 4. *Faecal excretion of carotenoids*

(Goats fed via a duodenal cannula, red palm oil unsap. dispersed in water with lecithin and bile salts.)

Goat no. 5 (abomasal tube)			Goat no. 6 (duodenal tube)		
Duration of collection	Total daily excretion (µg.)	Excess excretion over basal (µg.)	Duration of collection	Total daily excretion (µg.)	Excess excretion over basal (µg.)
5 days prior to dosing	2,205	—	7 days prior to dosing	2,615	—
2 days after dosing	13,460	9,390	1 day after dosing	5,245	2,700
1 day later	12,970	10,935	2 days later	16,360	11,270
1 day later	3,080	1,045	5 days later	2,250	—
1 day later	1,190	—			
Weighted mean of basal excretion (µg.)	2,035		Weighted mean of basal excretion (µg.)	2,545	
Total excess excretion (µg.)	21,370		Total excess excretion (µg.)	13,970	
Dose (µg.)	113,000		Dose (µg.)	197,400	
Percentage excretion	$\frac{21,370}{113,000} \times 100 = 18.9$		Percentage excretion	$\frac{13,970}{197,400} \times 100 = 7.1$	

in the portal venous blood were not observed. Goats were therefore provided with portal cannulae and duodenal cannulae in the hope that direct introduction of carotene solutions into the upper parts of the small intestine would result in more rapid absorption. Larger amounts of carotene were also

Table 5. *Vitamin A plasma levels of portal and systemic blood*

(Samples drawn from goats at varying intervals after administration of 100,000 µg. of carotene, via a duodenal cannula, as red palm oil unsap. dispersed in 2 l. of water with lecithin and bile salts.)

Animal no.	Dose level (µg.)	Time after dosing (hr.)	Plasma vitamin A levels (i.u./100 ml.)	
			Systemic	Portal
6	197,400	0	65	72
		4	67	75
		10	67	67
		24	64	62
7	39,600	0	90	104
		4*	99	105
8	197,400	0	86	90
		4	85	106
		8	84	105
		24	82	88

* Goat died.

used. In three experiments of this type, in which samples of portal and systemic venous blood were drawn at intervals after the duodenal introduction of carotene, no detectable amounts of carotene could be observed (Table 5). The results on goat no. 6 were obtained during the balance experiments previously described.

The implications of our inability to demonstrate carotene in the blood of goats and sheep were becoming increasingly clear; the carotene was being converted into vitamin A before it reached the blood, i.e. in the intestinal wall. Concurrent experiments which were being carried out in this laboratory on rats and which are reported fully in an accompanying paper (Glover *et al.* 1948*b*) made it clear that this was the case. A review of all the vitamin A plasma levels obtained in our previous experiments did not reveal the expected rise following carotene administration. It was possible that the rise was so slight that it could not be detected by the analytical methods available.

It is reasonably certain that vitamin A itself travels to the liver via the lymphatic pathway (Drummond, Bell & Palmer, 1935; Popper & Volk, 1944; Eden & Sellers, 1948). To complete this investigation, therefore, it was decided to provide goats with thoracic cannulae as well as duodenal tubes so that thoracic lymph could be collected continually during the experiment. In two preliminary experiments in which the unsaponifiable fraction of red palm oil, dispersed in 1–2 l. water, was administered quickly, no carotene appeared in the lymph but there were small increases in the vitamin A levels. The clearly positive results on three animals recorded in Table 6 were obtained after administering the carotene dispersed in a much smaller volume of water (250 ml.) slowly over 6–8 hr.

The concentration of the lymph lipids extracted by light petroleum (b.p. 40–60°) was also determined in these experiments and the variations in it follow closely those for vitamin A. The lipid and vitamin A

Table 6. *Vitamin A levels in the thoracic lymph*

(Samples drawn at intervals after administration of 112,000 µg. of carotene, as red palm oil dispersed in 250–500 ml. of water with either bile or bile salts.)

Time after dosing (hr.)	Vitamin A lipids in lymph					
	Goat no. 12		Goat no. 13		Goat no. 14	
	Vitamin A (i.u./100 ml.)	Lipids (mg./100 ml.)	Vitamin A (i.u./100 ml.)	Lipids (mg./100 ml.)	Vitamin A (i.u./100 ml.)	Lipids (mg./100 ml.)
0 Control	43	34	69	173	29	256
1	—	—	—	—	20	394
2	64	107	64	167	30	351
3	—	—	—	—	31	429
4	79	131	63	226	33	455
5	—	—	—	—	45	419
6	80	165	79	310	55	1390
7	—	—	—	—	54	1830
8	72	232	82	669	48	1342
9	—	—	—	—	45	1270
10	84	498	147	1053	42	1209
11	—	—	—	—	52	1428
12	77	597	97	957	45	1128
13	—	—	—	—	—	—
14	101	262	—	—	—	—
15	—	—	—	—	—	—
16	—	195	—	—	—	—
17	—	—	—	—	—	—
18	67	67	—	—	—	—

values obtained on goat no. 14 are presented graphically in Fig. 1. The parallelism is very striking and the drop in absorption after 6 hr. when the carotene addition was discontinued and the increase after 9 hr. when it was resumed, is well reflected in both curves. The increase in the lymph lipids was, however, much greater than that in the vitamin A levels. The maximum increase in vitamin A obtained in each experiment was c. 100 %, whereas that of the lipids was of the order 500–800 %. The reason for this is not immediately apparent, but the well-known inhibition of the antimony trichloride test by excess fat probably makes our vitamin A values determined around maximum absorption rather low.

Fig. 1. Variation in lipid and vitamin A levels of lymph of a goat dosed with carotene (112,000 μ g. as red palm oil) via a duodenal cannula. ○○○○ vitamin A; ●●●● lipids.

Two other interesting but limited observations were made on the lymph: (a) The vitamin A levels of two samples of goat lymph were much less than the levels in the corresponding plasma. The two lymph samples contained 29 and 37 i.u./100 ml., whilst the corresponding plasma levels were 135 and 67 i.u./100 ml. respectively. One experiment indicated that at least 85 % of the lymphatic vitamin A is esterified. A recent preliminary note (Eden & Sellers, 1948) states that vitamin A in the lymph of bullocks is also preponderantly ester, but that the lymph levels are higher than the plasma levels. (b) Light petroleum (b.p. 40–60°) extracts of lymph obtained from goats recently removed from summer pasture were quite yellow, although similar extracts from the corresponding blood plasma were colourless; extracts from lymph of goats kept on hay and cattle food were also colourless. Spectrophotometric examination of the pigment indicates that it is not a carotenoid; the

absorption curve (Fig. 2) shows only one maximum at 433–434 $m\mu$. and a very small inflexion at about 590 $m\mu$. (not recorded in the curve). The pigment is destroyed by treatment with alkali, which further indicates that it is not a carotenoid. From these data the possibility that it is derived from chlorophyll must be considered, but further investigations are necessary finally to determine this.

Fig. 2. Absorption spectrum of a pigment extracted by light petroleum from lymph of a goat grazing on fresh pasture.

Carotenoids in other tissues

The apparent selective accumulation of carotenoids often reported in certain mammalian tissue, viz. adrenals, corpora lutea, yellow bone marrow, has led to a certain amount of speculation as to the function of carotene *per se*, as distinct from its activity as a vitamin A precursor. It was obviously important to determine whether, in those animals in which carotene appears to get into the blood stream only in traces if at all, carotene accumulates in these organs. The adrenals, ovaries, liver and bone marrow (from the long bones) of a number of goats killed after carotene administration were examined. In no case was carotene detected in any of the organs. The bone marrow was perfectly white.

DISCUSSION

The failure to obtain positive results in our first series of experiments, carried out to determine the route whereby carotenoids are transported in sheep and goats, is explicable, in the light of our later work. We have now shown that in the unanaesthetized animal the carotene is converted into vitamin A in the gut wall and transported via the lymph to the liver where it is stored. It is difficult in experiments of this type to rule out completely the lumen of the intestine as the site of conversion, but the

failure to destroy carotene by incubation with rabbits' intestinal contents (*vide infra*) indicates that this is so, and the experiments on rats (Glover *et al.* 1948b) supply additional proof. The transport of the vitamin A via the portal system is not ruled out but is highly unlikely to occur to any great extent. This confirms the work of Drummond *et al.* (1935), Popper & Volk (1944), and Eden & Sellers (1948). It is suggested that the failure to find vitamin A in the plasma of sheep and goats after feeding carotene is due to the dynamics of the situation. The vitamin A is taken up by the lymph in quantities which are sufficiently great to be experimentally demonstrable; however, in the delivery of the lymph into the systemic blood supply the excess vitamin A becomes so quickly dispersed and removed that the concentration increment is too small to be detected.

Support for this reasoning has been provided by the following experiment: 10⁶ i.u. of vitamin A were administered in similar dispersions (250 ml. water, 1% bile salts) via duodenal cannulae into two goats, one of which was provided with a thoracic cannula, and blood and lymph samples were collected from both animals at varying times afterwards. It is clear (Table 7) that the increase in vitamin A is much greater in lymph than in blood and obviously there is also a dilution aspect when lymph is compared with blood. Complete confirmation of this observation has been provided by the recently reported work of Eden & Sellers (1948).

Table 7. *Comparison of vitamin A levels in plasma and lymph of goats*

(The goats received identical doses of vitamin A (10⁶ i.u.) via duodenal cannulae.)

Time after dosing (hr.)	Goat no. 16 vitamin A (i.u./100 ml. plasma)	Goat no. 17 vitamin A (i.u./100 ml. lymph)
0	55	33
2	—	40
3	87	—
4	—	83
6	167	189
8	181	232.5
10	—	452
12	—	517
14	—	496
16	—	293
18	—	232
24	78	—

The fact that in goats the normal levels of vitamin A in lymph are lower than the blood levels is not in opposition to this point of view. Normally, the vitamin A in blood is in the alcohol form and its concentration is controlled at an optimum functional level by the liver (Glover *et al.* 1947c). The vitamin A in normal lymph is that which has been formed from

carotene and is now in transport to the blood; in other words, in a normal animal, blood and lymph vitamin A levels need not and do not bear any relationship to each other.

It is now possible to sum up the position regarding the conversion of carotene into vitamin A in mammals. Although the experimental basis for the hypothesis has been severely criticized (Rea & Drummond, 1932; Woolf & Moore, 1932), it was generally assumed until recently that the conversion takes place in the liver. Sexton, Mehl & Deuel (1946) first suggested that the liver was not the site of conversion, and demonstrated that injected carotene accumulated in the liver but did not appear to be converted into vitamin A. The first results reported here (see Goodwin *et al.* 1946) also pointed in this direction, and when Glover *et al.* (1947b, 1948a) found that retinene, a possible intermediate in the carotene-vitamin A transformation, was converted into vitamin A in the gut, it appeared even more probable that conversion of carotene takes place in this organ. The accompanying paper (Glover *et al.* 1948b) proves this for the rat by experiments carried out *in vitro* and *in vivo*; and Wiese, Mehl & Deuel (1947) and Mattson, Mehl & Deuel (1947) have completely confirmed these results. Meanwhile, Thompson, Ganguly & Kon (1947), approaching the problem from a slightly different angle, have come to the same conclusion, using the pig. The work reported here on goats confirms these findings, and proves that the vitamin A thus formed is transported via the lymphatics, a fact previously suggested in an acute experiment by Thompson *et al.* (1947).

SUMMARY

1. Carotene could not be demonstrated in the portal or systemic blood of rabbits, goats or sheep after administration of massive doses of carotene in various forms by the oral, abomasal or duodenal route.

2. Carotene, either as dispersed red palm oil or homogenized spinach, is well absorbed by goats and sheep irrespective of its route of administration.

3. In goats provided with thoracic cannulae, increases in the vitamin A levels of the lymph were obtained after feeding carotene, proving that vitamin A is formed from carotene in the gut wall and transported to the liver via the lymph.

4. Increases in vitamin A plasma levels, to be expected if carotene is converted into vitamin A in the gut wall, could not be demonstrated. The probable reason for this is discussed.

5. After a dose of vitamin A the concentration of vitamin A was much higher in lymph than in plasma.

6. A restricted number of experiments indicate that the normal vitamin A level in the lymph in

goats is less than that in the plasma, and that the lymph vitamin A is mostly in the ester form.

7. A non-carotenoid yellow pigment, which may be a chlorophyll derivative, is present in the lymph of goats feeding on fresh grass.

8. No carotene could be detected in the adrenals, liver, ovaries, or bone marrow of goats.

We are grateful to a number of people who helped to make this investigation possible: to Mr A. D. Dewar, Department

of Physiology, for help at the beginning of the investigation; to Mr H. V. Hughes, Department of Veterinary Anatomy, for much help in locating the thoracic duct; to Lever Bros. and Unilever Ltd., for gifts of red palm oil; to Nestlé Ltd. for a gift of homogenized spinach; to Dr C. F. Code, The Mayo Clinic, U.S.A., for special plastic tubing for the thoracic cannulae; to Prof. R. A. Morton, not only for financing most of the investigation by grants from his Ministry of Food Research Fund, but also for his constant help and encouragement; and to Prof. W. H. Newton for his advice.

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Studies in Vitamin A

8. CONVERSION OF β -CAROTENE INTO VITAMIN A IN THE INTESTINE OF THE RAT

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(Received 30 March 1948)

Since the work of Moore (1930), which showed that carotene administered orally to the rat was converted into vitamin A, which was stored in the liver, many investigators have attempted to determine the locus of the transformation. The problem has been approached in different ways and attention has been focused on two organs, the intestine and the liver, in either of which 'carotene'* given by the oral route could conceivably be transformed into vitamin A.

The results of the earlier *in vitro* work, where minced liver tissue or extracts were incubated with

* 'Carotene' is used here to denote mixtures of the α - and β -isomerides.

colloidal suspensions of 'carotene', were conflicting. Ahmad (1931), Olcott & McCann (1931), Parienti & Ralli (1932) and von Euler & Klusmann (1932) obtained evidence for the formation of vitamin A as indicated by the production of a lipid with an ultra-violet absorption band in the region of 328 m μ . (the maximum for vitamin A) or by the detection of an increase in colour intensity with the antimony trichloride reaction. On the other hand, Rea & Drummond (1932), von Euler (1932) and Ahmad (1934), were unable to prepare vitamin A under similar conditions. Furthermore, Drummond & MacWalter (1933) could not obtain vitamin A from carotene which was actually taken up by the liver cells prior