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Further Studies of the Conversion of β-Carotene to Vitamin A in the Intestine*

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We have already reported in full some of our findings on the formation of vitamin A in the intestine (Thompson, Ganguly & Kon, 1949). The present paper deals with further evidence, in particular that derived from observations on the appearance of vitamin A in the portal and systemic circulations of pigs and in the mesenteric lymph of pigs and rats.

EXPERIMENTAL

The methods and materials used were in the main as described in the earlier paper (Thompson, Ganguly & Kon, 1949) with the following additions or modifications.

Colloidal carotene and vitamin A

These were prepared by the method of With (1939) and, except for the experiment described on p. 406, were mixed for feeding with the fat-free vitamin A-deficient diet (Thompson, Ganguly & Kon, 1949).

Portal cannula

One of London's (1935) cannulas was established in the portal vein of pigs, at first fairly close to the liver but later so as to tap only the mesenteric stream (Copher & Dick, 1928). For ease of insertion the cannula was modified by making it in two parts, the stainless steel circular knurled head being threaded on to the stainless steel shaft. In this way the size of the stab wound could be reduced to that of the diameter of the tube. In position, the cannula was protected by a Perspex dome, 3 in. in diameter and 1 in. high, with a central hole, 1 in. in diameter, which allowed the flange restricted movement (see Pl. 1).

Simultaneously with the withdrawal of samples of portal blood, samples of systemic blood were obtained from an ear vein.

* Read in part before the Biochemical Society (Thompson, Braude, Cowie, Ganguly & Kon, 1949; Coates, Thompson & Kon, 1950).

Lymph cannula

Rats. In rats the intestinal lymphatic that drains most of the small intestine was cannulated by the technique of Bollman, Cain, Grindlay & van Hook (1948), except that polythene, instead of Transflex (polyvinyl chloride), tubing was used and that the tube was pushed directly through the wall of the lymph vessel and not through a prepared hole. For operation normal rats were anaesthetized with Nembutal (Abbott Laboratories); those made vitamin A-deficient on the diet of Thompson, Ganguly & Kon (1949) were given ether. Rats weighing 200–300 g. were used and were accommodated in the cage described by Bollman & van Hook (1948). In sham operations the lymphatic was exposed but not cannulated.

Pigs. In the pig the operation was carried out under *cyclo*propane-oxygen anaesthesia, which proved very satisfactory. A well-fitting mask, as described for the dog by Wright (1947), was used, since it was found impossible to insert a tracheal tube owing to the narrowness of the rima glottidis in the pig. The abdomen was opened through a mid-line incision and a lymph vessel of suitable dimensions for cannulation was sought near the root of the mesentery. The end of the plastic tube was then pushed either directly through the wall of the lymph vessel or through a small slit as already described for the rat.

At first polythene tubing of 1 mm. internal bore was used; it proved, however, too brittle and broke off at the point of passage through the abdominal wall. Later a special food-grade polyvinyl-chloride tubing, internal diameter 1 mm., wall thickness 0·25 mm., containing no toxic plasticizers, was made for us by Tenaplas Ltd.; it proved entirely satisfactory. For collecting samples a small brown glass bottle of 5 ml. capacity was inserted into the hole of the Perspex protector used for London's cannula (see p. 398 and Pl. 1) where it was connected to the lymph cannula. Bottle and protector were then fixed in a suitable position by elastic surgical tape. As a rule lymph flowed at a rate of about 20 ml./hr. and was collected at 15 min. intervals. Clotting of lymph in the bottle was prevented by addition of one drop of saturated ammonium-oxalate solution. Samples were transferred from the bottle to small test-tubes, and fluorescence in ultraviolet light screened by a Woods glass filter was noted. For analysis the lymph was bulked into half-hourly samples. In the interval between operation and dosing the lymph dripped freely from the cannula and was not collected.

Measurement of carotene and vitamin A in lymph

Measurements were made as described for blood plasma by Thompson, Ganguly & Kon (1949). The fat from the pig lymph was separated by evaporation of the final light petroleum extract, and weighed before it was transferred to *n*-hexane.

Measurement of the ultraviolet absorption of extracts of pig lymph

For these measurements the vitamin A-ester fraction obtained by chromatography was evaporated under diminished pressure and taken up in *n*-hexane, boiling range 67–69°. The absorption curve was then determined in a model D.U. Beckman photoelectric spectrophotometer over the range 310–400 mμ.

Curves obtained even for rich lymph extract (see p. 413 and Fig. 5) indicated considerable irrelevant absorption on the short wave-length side of the maximum, the extinction at 313 mμ. being decidedly greater than that at 338·5 mμ. (see Morton & Stubbs, 1948). A correction was therefore necessary. In this particular instance we were in the fortunate position of having at our disposal a more direct method than that essential for most of the material examined by Morton & Stubbs (1948). The procedure was to apply the correction by subtracting extinction values for lymph before the appearance in it of vitamin A from those obtained when vitamin A was at its highest concentration, the two sets of values being corrected to an equal fat content.

Removal of intestinal contents in the rat

Fisher & Parsons (1949–50) have shown that when the blood supply to the intestine is cut off changes occur with great rapidity in its mucous membranes. For this reason the technique described by Thompson, Ganguly & Kon (1949) was modified and the contents of the intestine were washed out in the living animal. Under ether anaesthesia the intestine was exposed, a glass cannula attached to a length of rubber tubing was inserted into an incision in the duodenum about 0·5 cm. from the stomach, the ileum was severed at the ileo-caecal valve, the blood supply to the last 2 in. of the ileum was clamped off and the stump was inserted into a measuring cylinder. About 60 ml. of 0·9% sodium-chloride solution at 37° were then passed by gravity with a head of about 2 ft. through the cannula. Of these some 35 ml. ran freely into the cylinder and the remainder was blown out. Blood, if needed for analysis, was then obtained by heart puncture; the rat was killed and the intestine removed immediately and treated further as already described by Thompson, Ganguly & Kon (1949).

Vitamin A-deficient diets for pigs

Diet A, used for young pigs up to weaning and for pigs that had been operated on, had the following composition: barley meal 40, fine wheat feed (offals) 30, fish meal 10, dried skim milk as used by Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell (1949) 20. Analysis of the whole diet, and also of the dried skim milk alone, failed to demonstrate the presence of vitamin A and of more than traces of carotene.

Diet B, for sows, gilts and young pigs after weaning, consisted of barley meal 50, fine wheat feed (offals) 40 and fish meal 10.

RESULTS

Partition of vitamin A between wall and contents of the intestine after a meal of carotene

In our earlier work we found that some 75% of the vitamin A formed from carotene in the intestine of the rat was in the wall and the remainder in the contents. However, when the technique of Fisher & Parsons (1949–50) was used and the contents were washed out while the intestine was still connected with the blood supply, about 90% of the vitamin A was found in the wall. An experiment quoted in Table 1 illustrates

Table 1. *The influence of different methods of washing out the contents of the small intestine on the proportion of vitamin A ester and alcohol found in the wall and contents. Control rats received 1 g. diet and 400 mg. arachis oil only. Dosed rats received 1 g. diet and 400 mg. arachis oil containing 4 mg. β-carotene. The contents were removed in vivo or 1·5 hr. after killing*

(Values expressed per organ)

				Wall					Contents						
					Vitamin A					Vitamin A*					
					Alcohol		Ester			Alcohol		Ester		Total vitamin A in wall and contents	
Exp. no.	State of rat	Rat no.	Treatment	Carotene (μg.)	(i.u.)	Percentage of total in intestinal tract	(i.u.)	Percentage of total in intestinal tract	Carotene (μg.)	(i.u.)	Percentage of total in intestinal tract	(i.u.)	Percentage of total in intestinal tract	Alcohol (%)	Ester (%)
1	Undosed	1	Contents of	0	1·7	—	2·6	—	0	1·7	—	2·1	—	—	—
	Dosed	2	small intestine	59	24	29	51	62	415	5·0	6·0	2·6	3·2	35	65
		3	washed out	81	18	19	69	72	750	2·6	2·7	6·0	6·3	22	78
		4	in vivo	158	23	21	80	71	1290	5·5	4·9	4·3	3·8	25	75
		5		120	20	18	80	70	1240	7·1	6·2	6·9	6·0	24	76
2†	Undosed	1	Contents	0	1·7	—	1·7	—	0	1·0	—	0·9	—	—	—
	Dosed	2	washed out	39	5·2	8·6	47	79	910	6·3	10	1·3	2·1	19	81
		3	in vivo	43	7·9	7·6	86	83	1030	8·0	7·7	2·2	2·2	15	85
		4	Contents	39	5·6	8·3	27	40	810	12	17	24	35	25	75
		5	washed out 1·5 hr. after killing	37	6·5	7·7	46	54	1040	12	14	21	24	22	78
3	Undosed	1	Contents‡	0	0·6	—	1·0	—	0	0·7	—	0·9	—	—	—
	Dosed	2	washed out	36	5·6	8·6	48	74	450	9·6	14	2·4	3·7	22	78
		3	in vivo	64	7·2	9·4	56	73	870	12	16	1·6	2·0	25	75
		4	Contents‡	16	5·6	7·9	25	35	850	16	23	24	34	31	69
		5	washed out 1·5 hr. after killing	24	7·2	9·1	34	43	360	26	33	12	15	42	58

* See p. 402, for the way in which these values can be corrected to allow for the presence of the carotene artifact.

† In this experiment vitamin A alcohol was eluted from the chromatographic column with 20 % acetone (eluent (*b*), Thompson, Ganguly & Kon, 1949) instead of 8 % ethanol (eluent (*c*), Thompson, Ganguly & Kon, 1949). It was subsequently found that with the batch of alumina used the elution of vitamin A alcohol was not quite complete. Hence values obtained in this experiment for vitamin A alcohol should not be compared with those quoted in Exps. 1 and 3.

‡ After being washed out the small intestine was split longitudinally and rinsed in 0·9 % sodium chloride.

this and indicates that in the earlier experiments some of the vitamin must have leaked out of the wall in the brief interval between the death of the tissue and extraction. Our more recent experience indicates, moreover, that at least some of this 'vitamin A' may have been an artifact produced by the oxidation of carotene. Carotene dissolved in arachis oil, mixed with the diet as for feeding (cf. Thompson, Ganguly & Kon, 1949), and put through the chromatographic-separation process, yielded in the vitamin A fraction small quantities of a yellow-coloured substance contributing appreciably more to the vitamin A value than an amount of β-carotene of equivalent intensity of yellow colour (cf. Thompson, 1949). The error introduced by the artifact is quite small. Usually after a meal of 4 mg. carotene about 1 mg. was found in the contents of the small intestine of the rat within 2 hr. of dosing. This quantity would yield some 5–8 'i.u.' of apparent vitamin A, three-quarters of it usually appearing in the alcohol fraction. The proportions of the artifact measured as alcohol or ester varied somewhat with small changes in experimental conditions and with the adsorptive power of the batch of alumina used. For this reason we prefer to apply the correction to the total amount of vitamin A determined in the intestinal contents rather than to apportion it between alcohol and ester. It will be apparent from Table 1 that it is doubtful whether any true vitamin A is present in the intestinal contents washed out in vivo when the correction for the artifact has been made. It is difficult to decide whether the blank obtained with vitamin A-deficient control rats should be added to the value for the artifact before the latter is subtracted from the value for rats given carotene. It should be pointed out that some of the values quoted in the earlier paper for the amounts of vitamin A present in the contents of the small intestine require a similar correction but, though the proportion of vitamin A found in the lumen of the intestine would thereby be reduced, the conclusions reached in that paper would not be invalidated.

Table 1 shows how easily vitamin A is stripped off the dead intestine. Vitamin A-deficient rats received β-carotene in the usual way and 2 hr. later the intestines of some were washed out in vivo. Other rats were bled from the heart, killed and left for 1·5 hr.; their intestines were then washed out in situ (Table 1, Exps. 1 and 2). Of the total intestinal vitamin A some 10% was in the contents of the intestine of the animals washed out during life, but with the dead intestine the value was about 45%.

In a further experiment (Table 1, Exp. 3) the procedure was similar, but with both groups of animals the intestine after being washed out was split longitudinally and rinsed in 0·9% sodium-chloride solution. Again the difference between the two treatments was marked. In addition to leakage of vitamin A from the wall of the dead intestine some hydrolysis of the ester form had taken place also. Thus, in Exps. 1 and 3 of Table 1 the total vitamin A ester found by washing out the contents in vivo amounted to some 75% of the total vitamin A present, whereas in the dead intestine (Exp. 3) the proportion was about 60%. Even this value, however, is higher than those reported in the earlier paper (Thompson, Ganguly & Kon, 1949) for the proportion of vitamin A ester. It will be recalled that there the vitamin A alcohol and ester were reported to appear in roughly equal amounts after the administration of carotene or vitamin A alcohol or ester.

Table 2. *The appearance of the two forms of vitamin A in the stomach and in the wall and contents of the small intestine of vitamin A-deficient rats 2 hr. after a meal of β-carotene, vitamin A alcohol or vitamin A ester. The rats received 1 g. diet and 400 mg. arachis oil containing 4 mg. β-carotene or 500 i.u. vitamin A alcohol or 500 i.u. vitamin A ester, except the control rat which received only 1 g. diet and 400 mg. arachis oil*

(Values expressed per organ)

		Stomach					Small intestine							
							Wall			Contents				
		Vitamin A								Vitamin A				
				Total								Total		
Treatment	Rat no.	Alcohol (i.u.)	Ester (i.u.)	Uncorrected (i.u.)	Corrected* (i.u.)	Carotene (μg.)	Alcohol (i.u.)	Ester (i.u.)	Total (i.u.)	Alcohol (i.u.)	Ester (i.u.)	Uncorrected (i.u.)	Corrected* (i.u.)	Carotene (μg.)
Control	1	2·4	0·9	3·3	3·3	0	0·7	1·0	1·7	0·9	0·9	1·8	1·8	0
β-Carotene	2	5·3	4·7	10	0	1400	15	89	104	6·6	1·2	7·8	4·0	490
	3	6·4	4·7	11	0	1500	11	86	97	6·8	1·0	7·8	4·0	370
	4	8·6	6·0	15	0	2400	19	94	113	9·4	1·2	11	3·0	850
Vitamin A alcohol	5	77	14	91	91	0	20	47	67	5·1	0·7	5·8	5·8	0
	6	115	10	125	125	0	24	68	92	6·8	0·6	7·4	7·4	0
	7	86	7·7	94	94	0	26	63	89	9·4	0·7	10	10	0
Vitamin A ester	8	1·9	145	147	147	0	17	47	64	9·0	1·7	11	11	0
	9	2·0	124	126	126	0	20	53	73	9·4	3·4	13	13	0
	10	1·7	111	113	113	0	15	49	64	5·4	1·5	6·9	6·9	0

* See p. 402. In this particular instance the correction was 7·6 'i.u.' vitamin A for each mg. carotene accompanying the vitamin A.

The findings just described throw doubt on the validity of these earlier observations, and further experiments were made by the technique of Fisher & Parsons (1949–50).

Appearance of the two forms of vitamin A in the wall and contents of the small intestine of vitamin A-deficient rats after a meal of β-carotene, of vitamin A alcohol or of vitamin A ester

Rats depleted of vitamin A were given β-carotene or vitamin A alcohol or ester, and 2 hr. later the intestine was washed out while still attached to the blood supply. Vitamin A alcohol and ester were determined in the wall and contents. The results are shown in Table 2. Those for carotene confirm the observations recorded in Table 1 that over 90% of the vitamin A arising from carotene in the intestine is present in the wall. Naturally, the distribution between wall and contents is of much less significance when preformed vitamin A is given, as the amount present in the contents depends on the chance emptying of the stomach. It is of interest, however, that under the conditions of this experiment only some 10 i.u. vitamin A were found in the contents though at the same time roughly 100 i.u. were still present in the stomach.

The significant finding is that, whichever form of vitamin A was given, the vitamin A in the contents was almost exclusively in the form of alcohol. Clearly the vitamin A ester must have been hydrolysed in the lumen of the intestine. In the wall the alcohol form, regardless of the form of vitamin A given, amounted to only about 25%. It is of interest that after the carotene meal the proportion of the alcohol in the wall was even lower, about 15%. Further evidence for the rapid hydrolysis of the vitamin A ester in the intestine was obtained when four rats were given 1200 i.u. synthetic vitamin A acetate and were killed 5–15 min. later. The vitamin A found in the stomachs was almost exclusively in the ester form but roughly equal quantities of the alcohol and ester were detected in the contents of the intestine, though again the ester form predominated in the wall (Table 3).

Table 3. *The hydrolysis of vitamin A ester in the lumen of the intestine. Rats received 1 g. diet and 400 mg. arachis oil containing 1200 i.u. vitamin A acetate. They were allowed 5 min. to consume the dose and were then anaesthetized for 5–10 min. and killed. Rats nos. 1 and 2 were anaesthetized with* cyclo*propane, rats nos. 3 and 4 with Nembutal**

(Values expressed per organ)

		Vitamin A					
				Small intestine			
		Stomach		Wall		Contents	
Treatment	Rat no.	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u.)	Ester (i.u.)
Vitamin A acetate, 1200 i.u. in 400 mg. arachis oil and 1 g. diet	1	14	585	7·3	25	47	36
	2	11	690	15	45	8·2	5·6
	3	2·1	500	3·6	7·7	1·9	4·8
	4	5·6	448	2·2	9·4	6·8	7·7

* These results were obtained in the course of an experiment on the effect of anaesthetics on the conversion of carotene to vitamin A, not quoted in the present paper.

Efficiency and speed of conversion of carotene in different states of dispersion

So far only the conversion of oily solutions of carotene has been considered. Fig. 1 shows that, as would be expected, powdered crystalline carotene mixed with the fat-free diet was converted to only a very slight extent. The conversion was better when

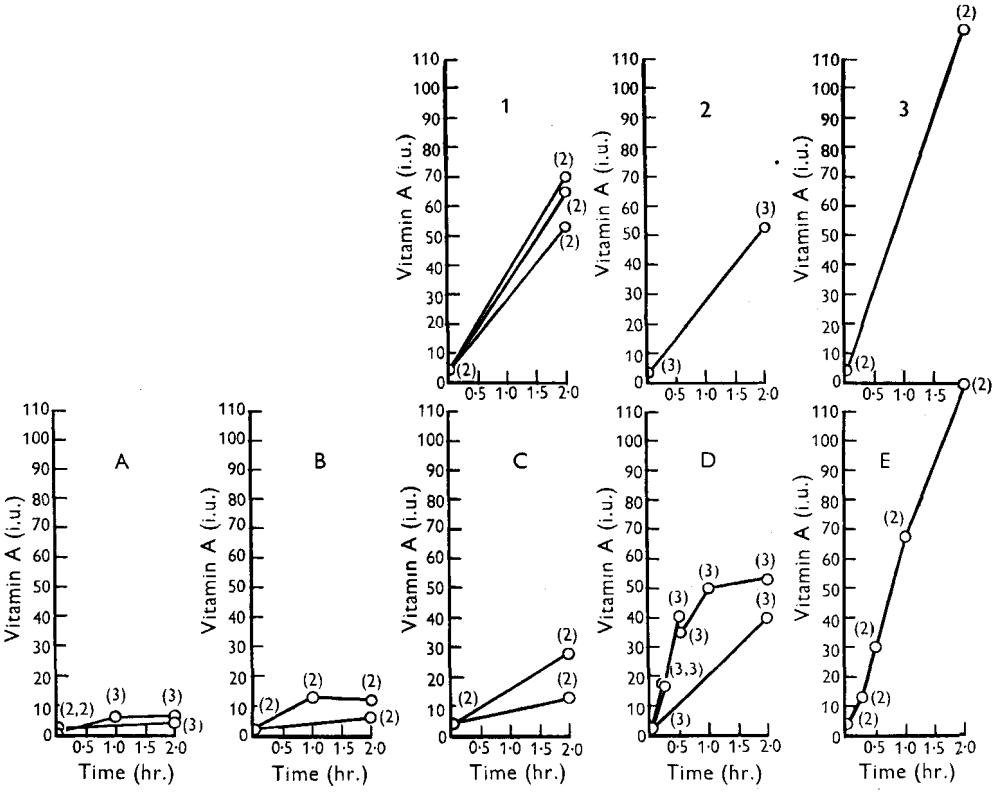

Fig. 1. The effect of the state of dispersion on the conversion of β-carotene to vitamin A and on the absorption of preformed vitamin A in the small intestine of vitamin A-deficient rats. Rate of increase in hr. in the concentration of total vitamin A in i.u. in the wall of the small intestine. A, 2·5 mg. powdered β-carotene on 1·5 g. fat-free vitamin A-deficient diet; B, 2·5 mg. powdered β-carotene on 1·5 g. vitamin A-deficient diet containing 10% fat; C, 2·6 mg. β-carotene, dissolved in ether, on 1 g. fat-free diet, ether removed by evaporation; D, 3·2–5 mg. β-carotene as 2–5 ml. colloidal solution mixed with 1 g. fat-free diet; E, 4 mg. β-carotene in 400 mg. arachis oil, or 400 mg. arachis oil alone, either mixed with 1 g. diet; 1, 600 i.u. vitamin A acetate dissolved in *n*-hexane on 1 g. fat-free diet, hexane removed by evaporation; 2, 1200 i.u. vitamin A as 2 ml. colloidal solution of vitamin A acetate, and 1 g. fat-free diet; 3, 600 i.u. vitamin A acetate in 100 mg. fat and 1 g. fat-free diet. Figures in parentheses indicate the number of rats on which values are based.

the diet contained 10% fat. A further improvement was obtained by dissolving the carotene in diethyl ether, pouring it over the fat-free diet and allowing the ether to evaporate. Finally, with colloidal solutions of carotene (see p. 398) the conversion was efficient, indicating that, if sufficiently dispersed, carotene is taken up by the intestine in the absence of fat. As fat is, however, present in the intestine itself, it is possible that carotene passes there into an oil phase before or after conversion to vitamin A.

Fig. 1 indicates that with vitamin A the uptake is less related to the state of dispersion than with carotene.

The conversion of carotene in colloidal solution is extremely rapid; in fact, in an isolated experiment, detectable quantities of vitamin A appeared in the intestine of a vitamin A-deficient rat 5–10 min. after a meal of fat-free diet mixed with 2 ml. of a colloidal solution containing 3 mg. β-carotene.

Table 4. *The appearance of vitamin A alcohol and ester in the wall and contents of the small intestine of vitamin A-deficient rats after a meal of 4 mg. β-carotene in 400 mg. arachis oil or of 4 mg. β-carotene as 2 ml. of colloidal solution, both doses being mixed with 1 g. vitamin A-deficient diet, 80 mg. α-tocopherol and 0·5 ml. 1% sodium-taurocholate solution*

(Values expressed per organ)

Dose	Time between dosing and killing (min.)	Rat no.	Wall			Contents		
			Carotene* (μg.)	Vitamin A		Carotene (μg.)	Vitamin A	
				Alcohol (i.u.)	Ester (i.u.)		Alcohol† (i.u.)	Ester† (i.u.)
None‡		1	—	0·2	1·5	19	0·5	0·4
Carotene in oil	5	2	10	1·0	2·6	198	1·3	0·7
		3	4·7	0·8	4·5	166	1·2	1·0
	10	4	9·3	1·3	4·5	208	1·5	1·0
		5	7·1	1·8	6·5	93	2·1	0·9
	20	6	7·0	2·5	15	205	2·1	1·4
		7	9·0	2·2	11	503	3·4	1·2
None §		8	—	0·6	0·3	19	0·5	0·2
Carotene in colloidal solution	5	9	13	2·1	4·9	212	2·7	1·3
		10	15	2·4	4·6	436	5·3	1·2
	10	11	12	2·6	4·7	156	2·9	1·0
		12	13	4·3	7·3	206	3·9	0·9
	20	13	6·4	6·9	19	137	1·7	0·9
		14	7·8	4·8	19	128	2·1	0·9

* Probably some adhering mechanically, see Thompson, Ganguly & Kon (1949, p. 54).

† The correction for the artifact (see p. 402) in this instance would be for the oily solution 1·5 'i.u.'/mg. carotene and for the colloidal solution 15 'i.u.'/mg. carotene.

‡ Arachis oil (400 mg.) with diet, tocopherol and sodium taurocholate.

§ Diet, tocopherol and sodium taurocholate.

The observation was confirmed by the experiment shown in Table 4, in which colloidal carotene and carotene dissolved in oil were given side by side. With both techniques vitamin A was already detected in the intestine 5 min. after the meal, but at all stages the conversion was more marked in those rats given the carotene in colloidal form. It will be noted that in this experiment the carotene was given with sodium taurocholate, since this improved the stability of the colloidal solution. As usual, most of the vitamin A was found in the wall. Owing to the presence of relatively large quantities of carotenoids, the values for vitamin A in the contents required correction for the carotene artifact.

The appearance of vitamin A in different segments of the small intestine of the rat after a meal of β-carotene, of vitamin A aldehyde, or of preformed vitamin A

It will be recalled (Thompson, Ganguly & Kon, 1949) that the conversion of β-carotene, as judged by the appearance of the vitamin A formed from it, is at its maximum in the middle of the small intestine. Further experiments, presented in Fig. 2, in which the small intestine was, as before (Thompson, Ganguly & Kon, 1949),

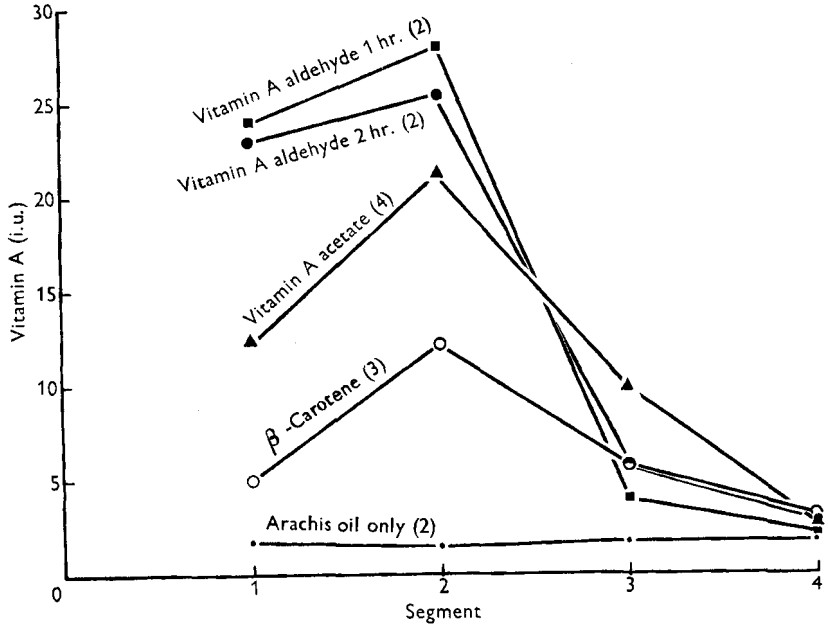

Fig. 2. The appearance of vitamin A in four consecutive segments of equal length of the small intestine of vitamin A-deficient rats 2 hr. after a meal of β-carotene or vitamin A acetate and 1 and 2 hr. after a meal of vitamin A aldehyde. The rats received 4 mg. β-carotene in 400 mg. arachis oil, or 600 i.u. vitamin A acetate in 400 mg. oil, or 500 μg. vitamin A aldehyde in 600 mg. oil, or 400 mg. oil only, all with 1 g. fat-free diet. Figures in parentheses indicate number the of rats on which values are based.

divided into four equal lengths, show that vitamin A aldehyde, which, according to Glover, Goodwin & Morton (1948*a*) is converted to vitamin A in the intestine, behaves in the same way as β-carotene. The curve of uptake of preformed vitamin A demonstrates that conversion of the vitamin A precursors and absorption of the vitamin A itself occur preferentially in the same site.

Division of the small intestine into sixteen segments

To define more exactly the site of maximum conversion, two experiments were made in each of which ten vitamin A-deficient rats were used; two served as undosed controls and eight received the usual dose of 4 mg. β-carotene in arachis oil. The rats were killed 2 hr. later and, after the contents had been washed out, as described on p. 400, the small intestine was divided into sixteen segments, the first from the pylorus

to, and including, the entrance to the common bile duct, and the remaining fifteen of equal length. The length of the first segment was about one-third that of the others. Corresponding segments obtained from the eight rats were combined and analysed. The small intestine of the two control rats was divided into four equal lengths and these also were combined. In Fig. 3 the results of the second of these experiments are shown. Those of the first, essentially similar, were not as complete.

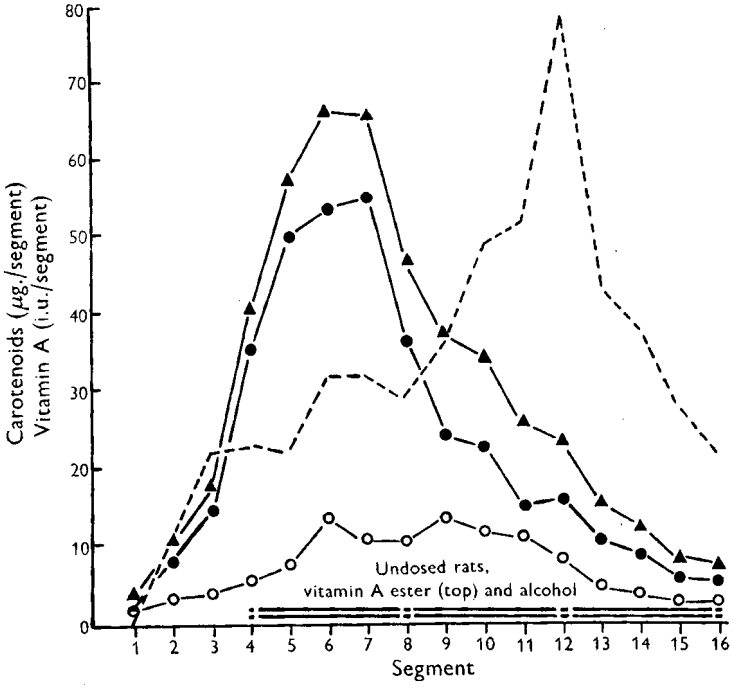

Fig. 3. Vitamin A and carotenoids in the wall of sixteen segments of the small intestine of vitamin A-deficient rats 2 hr. after a meal of 4 mg. β-carotene in 400 mg. arachis oil, and in the wall of four segments from similar, but undosed, rats. ○——○, vitamin A alcohol; ●——●, vitamin A ester; ▲——▲, total vitamin A; – – – –, carotenoids. Mean values for eight dosed and two undosed rats.

It will be seen that no vitamin A beyond the normal traces was detected in the first segment. This confirms our early observation based on measurement of fluorescence of vitamin A (Thompson, Ganguly & Kon, 1949, p. 73) and indicates that carotene is not converted in the rat in the absence of bile and pancreatic secretion. Beyond the first segment the content of vitamin A ester in the intestine rose sharply, with the peak of conversion at the sixth segment, that is just proximal to the middle point, and then the concentration curve sloped off more gradually towards the caecum. As expected, the alcohol form of vitamin A amounted only to one-third to one-fifth of the ester. Its increase was not as rapid, and peak values were found between the sixth and eleventh segments, i.e. farther down the intestine than the ester peak. A flatter peak for vitamin A alcohol was found in the first experiment also, but there it covered the fifth to the eighth segments. β-carotene, present in the wall in quantities relatively small in comparison with those usually found in the contents, was in greatest amount

towards the end of the small intestine, in the same position where the carotene present in wall and contents was found in earlier experiments (see Thompson, Ganguly & Kon, 1949, Fig. 2).

Vitamin A and β-carotene content of systemic blood and of blood obtained with a portal cannula from pigs given carotene or vitamin A

The evidence so far presented makes it virtually certain that the intestine is the main, if not the only, site of formation of vitamin A from carotene. We have shown that vitamin A appears in the intestine before it is detected in the liver. Moreover, we could never detect carotene in measurable quantities in the systemic blood of the rat or of the pig at any stage after these animals had had a substantial meal of carotene. Goodwin, Dewar & Gregory (1946) and Goodwin & Gregory (1948) showed conclusively that carotene is absent under such conditions from the portal circulation also of the rabbit, goat and sheep, thus excluding the possibility that in these animals carotene is carried to the liver for conversion without appearing in the systemic blood.

We made similar experiments with pigs and reached similar conclusions. Samples of systemic and portal blood were obtained before dosing and 1, 2, 4 and 6 hr. after dosing from three pigs. Pigs nos. 1 and 2 (Table 5) were from the litter of a sow (no. 2104) that had been on the vitamin A-deficient diet B (p. 400) for 6 months. They were 3 and 4 months old, respectively, when used and had received the vitamin A-deficient diets (p. 400) throughout. Pig no. 3 was a normal animal about 7 weeks old. All three, after insertion of the portal cannula, received the vitamin A-deficient diet A. The dose consisted of 300 mg. β-carotene in 30 g. arachis oil mixed with about 200 g. diet. In no sample of blood could we detect more than traces of β-carotene, of the order of 1 μg./100 ml. On the other hand, the level of vitamin A ester and vitamin A alcohol increased markedly in the blood of two of the pigs, and to a less extent in that of the third (Table 5). It will be seen from the table that in the three pigs the concentration of vitamin A ester in the blood increased earlier, at the 2 hr. sampling, than that of the alcohol, which did not increase till the next sampling at 4 hr.

A similar result was obtained when one of the pigs, no. 3, received, 1 week before it was given carotene, a dose of 40,000 i.u. vitamin A ester as 0·5 g. of a potent fish-liver oil dissolved in arachis oil and mixed with the food (Table 5). Here again, the increase in the ester form in the blood preceded that of the alcohol. Thus the changes in systemic and portal blood in these animals were much the same whether they received carotene or vitamin A. This is in agreement with our earlier findings with systemic blood (Thompson, Ganguly & Kon, 1949). We observed no obvious differences between the concentration in the portal and the systemic blood of either form of vitamin A.

Vitamin A and β-carotene content of the lymph obtained with a lymph cannula from pigs and rats given carotene or vitamin A

The negative results with the portal route were not unexpected, since the experience of Frazer (1946, 1948) with fat and of Drummond, Bell & Palmer (1935), Popper & Volk (1944), Eden & Sellers (1948), Goodwin & Gregory (1948) and our own

Table 5. *The appearance of vitamin A alcohol and ester in the portal and systemic blood of pigs at intervals after a meal of carotene or vitamin A in arachis oil*

Dose	Pig no.	Time interval between dosing and sampling (hr.)	Vitamin A: Alcohol — Systemic blood* (i.u./100 ml. plasma)	Vitamin A: Alcohol — Portal blood† (i.u./100 ml. plasma)	Vitamin A: Ester — Systemic blood* (i.u./100 ml. plasma)	Vitamin A: Ester — Portal blood† (i.u./100 ml. plasma)
β-carotene, 300 mg. in 30 g. arachis oil mixed with 200 g. vitamin A-deficient diet	1	0	36	40	10	11
		1	34	45	12	23
		2	37	49	102	118
		4	67	83	133	132
		6	124	105	114	117
	2	0	25	22	8	8
		1	24	22	8	8
		2	23	24	22	24
		4	51	70	38	37
		6	92	89	31	28
	3	0	25	30	9	9
		1	45	27	13	10
		1·5	26	30	15	15
		2	30	43	20	23
		4	39	39	35	27
		5	45	44	24	27
Vitamin A ester as fish-liver oil, 40,000 i.u. in 10 g. arachis oil mixed with 200 g. vitamin A-deficient diet	3	0	96	54	10	8
		1	75	70	25	16
		2	81	60	99	130
		4	73	87	40	50‡
		5	118	125	145	171

* From the ear. † From cannula in portal vein. ‡ Given food.

(Thompson, Ganguly & Kon, 1949) with vitamin A made it clear that the transport occurs largely through the lymphatics. It seemed, therefore, reasonable to look there for the vitamin formed from carotene in the intestine.

Pigs

The appearance of vitamin A in the mesenteric lymph nodes of the pig after a meal of carotene has already been described (Thompson, Ganguly & Kon, 1949). Further experiments were made with pigs having a plastic cannula inserted in a mesenteric lymph duct (see p. 399). Two pigs were used. One, a 10-week-old male pig, weighing 28 lb., was from a normal litter and received a normal ration after weaning at 8 weeks. For 2 days after operation the pig was given without stint the vitamin A-deficient diet A (p. 400) and fresh skim milk. It was fasted overnight and dosed with vitamin A, as set out in Table 6. The other pig, a 3-month-old female, was from a further litter of sow no. 2104 (see p. 409), this time after 1 year on diet B (p. 400), and had itself been on the vitamin A-deficient diet throughout. Its treatment after operation was similar to that of the first pig, but it received β-carotene instead of the preformed vitamin (Table 6).

It will be seen that the sequence of events with carotene was very similar to that with vitamin A. In both instances the concentration of vitamin A ester in the lymph increased very markedly, in the vitamin A-deficient pig some 200 times and in the normal one some fifty times, whereas the level of vitamin A alcohol was in comparison scarcely affected. The fluorescence in ultraviolet light was a useful and reliable indication of the appearance of increased quantities of vitamin A in the lymph. Values for the fat content of lymph (p. 399), also given in Table 6, indicate that the increase in it, though very slight compared with that of vitamin A ester, followed its trend. The lymph of the pig dosed with carotene contained, even before dosing, measurable quantities of a yellow pigment equivalent, at the absorption maximum for β-carotene in the appropriate solvent, to some 15 μg./100 ml. The colour increased after dosing to reach the equivalent of some 60 μg./100 ml. after 2 hr., at which time the concentration of vitamin A had risen markedly (see Table 6). The absorption curves in *n*-hexane of the pooled samples of lymph before and after the appearance of vitamin A (see below) are given in Fig. 4. It is evident that the absorption before dosing was not due to a carotenoid pigment. The flat maximum near 447 mμ. after dosing might be that of a carotenoid pigment, though the absence of a second peak near 480 mμ. makes it doubtful whether the pigment was β-carotene. Correction for irrelevant absorption carried out as for vitamin A (see below) yielded the third curve in Fig. 4; it bears more resemblance to that of β-carotene (fourth curve) than the uncorrected one. The pigment was not destroyed by saponification (cf. Goodwin & Gregory, 1948). It was possibly an oxidation product of β-carotene. From the corrected curve its concentration expressed as β-carotene can be calculated as about 6·5 μg./100 ml.

Vitamin A increased also in blood from the ear vein taken on three occasions during the carotene experiment, the ester form increasing first, simultaneously with the increase in the lymph, though to a much smaller extent. Vitamin A alcohol increased later, but the concentration was higher than that of the same form in the lymph.

Table 6. *Appearance of vitamin A in the lymph from a mesenteric lymphatic and in the blood of a 3-month-old vitamin A-deficient pig after a meal of 300 mg. β-carotene in 30 g. arachis oil dispersed in separated milk and mixed with 200 g. food, and in the lymph, obtained in the same way, from another, normal, 10-week-old pig after a similar meal of 1 g. fish-liver oil containing 106,000 i.u. vitamin A*

	Meal of β-carotene									Meal of vitamin A						
	Lymph							Blood plasma Vitamin A		Lymph						
				Vitamin A									Vitamin A			
				Yield		Concentration							Yield		Concentration	
Time after meal (hr.)	Fluorescence in ultra-violet light	Fat content (%)	Yield (ml.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u./100 ml.)	Ester (i.u./100 ml.)	Alcohol (i.u./100 ml.)	Ester (i.u./100 ml.)	Fluorescence in ultra-violet light	Fat content (%)	Yield (ml.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u./100 ml.)	Ester (i.u./100 ml.)
−0·5 −0	—	—	—	—	—	—	—	24	8	Bluish	1·74	6·5	2·0	2·2	31	33
0 −0·5	Bluish	1·74	7·9	1·3	1·1	16	14	—	—	Bluish	1·73	11·0	2·1	4·7	19	43
0·5 −1·0	Bluish	1·33	10·3	1·6	8·7	16	84	—	—	Bluish	1·59	9·5	1·8	3·1	19	33
1·0 −1·25	Yellow	1·56	7·7	1·1	76	14	960	18	27	—	—	—	—	—	—	—
1·25−1·5	Yellow	2·18	6·0	0·9	69	15	1150	—	—	Bluish	1·68	13·0	2·9	8·0	22	62
1·5 −1·75	Yellow	1·89	4·8	1·0	51	21	1060	—	—	—	—	—	—	—	—	—
1·75−2·0	Yellow	3·06	7·3	2·1	124	29	1700	—	—	Bluish	1·70	11·0	2·3	22	21	200
2·0 −2·5	Yellow	2·80	10·2	3·0	190	29	1860	—	—	Yellow	2·02	7·0	1·8	30	26	430
2·5 −3·0	Yellow	2·40	5·7	1·1	84	19	1470	—	—	Yellow	2·02	5·0	1·3	33	26	650
3·0 −3·5	Yellow	2·86	6·5	2·7	121	42	1860	70	60	Yellow	3·18	9·0	3·5	123	39	1370
3·5 −4·0	Yellow	4·66	7·0	3·9	224	56	3200	—	—	Yellow	2·49	4·5	1·4	65	31	1450
4·0 −4·5	Yellow	2·76	5·7	3·2	84	56	1470	—	—	Yellow	2·22	12·0	5·3	185	44	1540
4·5 −5·0	Yellow	3·25	10·5	5·8	171	55	1630	—	—	Yellow	1·75	4·0	2·2	54	55	1350
5·0 −5·5	Yellow	2·45	9·5	5·8	122	61	1280	—	—	Yellow	3·24	5·0	3·5	85	69	1700
5·5 −6·0	Yellow	2·16	8·9	5·0	111	56	1250	—	—	Yellow	2·22	7·0	3·9	84	55	1200

Characterization of the vitamin A appearing in the lymph. Fig. 5 shows absorption curves of extracts of mesenteric lymph from the two pigs.

The curves for the pig dosed with vitamin A are shown in Fig. 5 B. Curves 1 and 2 show the gross absorption in the pooled samples of lymph obtained between 0·5 and 1·5 hr. after dosing, that is, before the appearance of vitamin A (see Table 6), and of the pooled samples collected thereafter. The third curve, being the difference

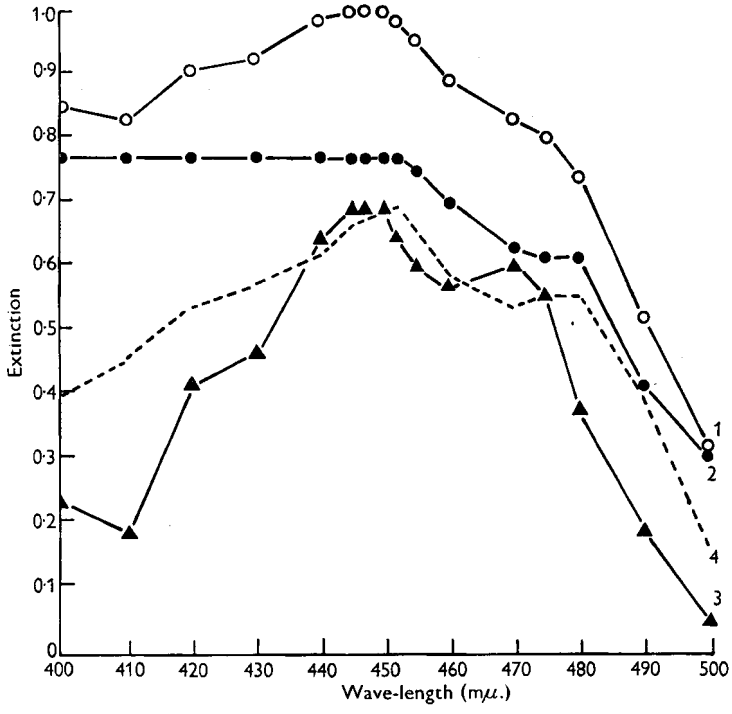

Fig. 4. Occurrence of a yellow pigment in the lymph of the pig. Spectral absorption curves in *n*-hexane of pooled samples of lymph from a mesenteric lymph duct of a vitamin A-deficient pig before and after the appearance of vitamin A in the lymph after a meal of 300 mg. β-carotene in 30 g. arachis oil. Curve 1, after meal; curve 2, before meal; curve 3, difference between curves 1 and 2 magnified three times; curve 4, curve for pure β-carotene, recalculated to have the same extinction at 451 mμ. as curve 3.

between the first two, shows the net absorption due to vitamin A. It will be seen that its shape is very similar to that given by pure vitamin A ester (curve 4), and that the two wave-lengths at which the E value is 6/7 of $E_{max.}$ are 314 and 337·5 mμ. instead of 313 and 338·5 mμ. found by Morton & Stubbs (1948) in *cyclo*hexane.

The curves for the pig dosed with β-carotene are shown in Fig. 5 A. Samples collected for 1 hr. after dosing were pooled for examination of the lymph before the appearance of vitamin A. Thereafter, samples collected from 1·25 hr. after dosing were again pooled. Curves 1 and 2 show again the gross absorption of both specimens of lymph, and curve 3 the net absorption due to vitamin A, obtained by difference.

In this instance the E values equal to 6/7 of $E_{max.}$ corresponded to wave-lengths

318·5 and 337 mμ. The agreement with the curve given for pure vitamin A ester (curve 4), though slightly less good than that given by the lymph of the pig dosed with vitamin A, is nevertheless striking.

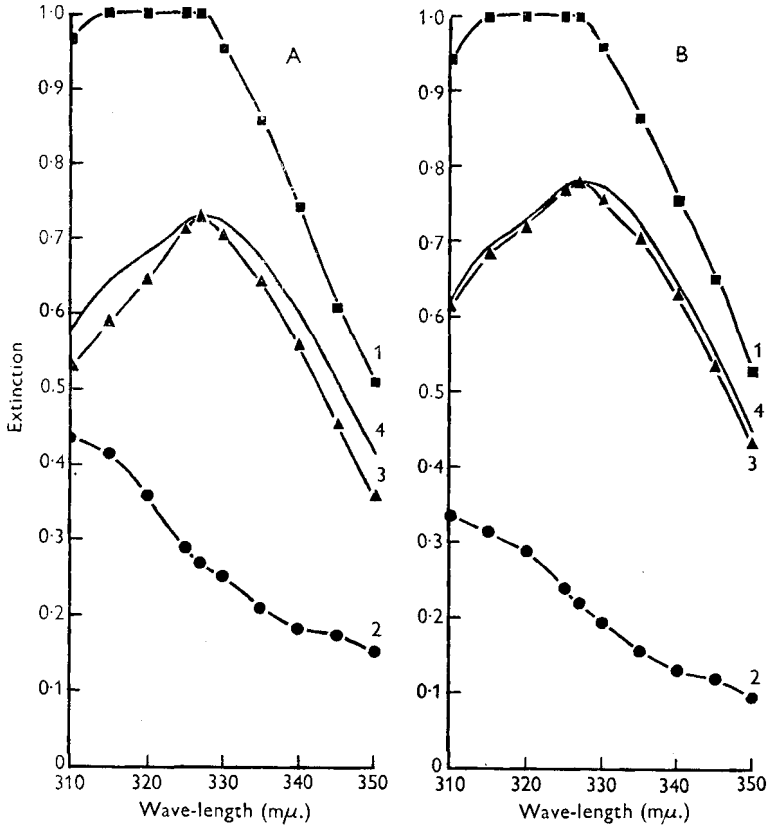

Fig. 5. Spectral absorption curves in *n*-hexane of pooled samples of lymph: A, from a mesenteric lymph duct of a vitamin A-deficient pig before and after the appearance of vitamin A in the lymph after a meal of 300 mg. β-carotene in 30 g. arachis oil; B, from similar samples from a normal pig after a meal of 106,000 i.u. vitamin A as 1 g. fish-liver oil in 30 g. arachis oil. Curve 1, after meal; curve 2, before meal; curve 3, difference between curves 1 and 2; curve 4, curve for pure vitamin A ester (Morton & Stubbs, 1948) recalculated to have the same extinction at 327 mμ. as curve 3.

Rats

Experiment with normal rat. Table 7 shows the results of an experiment, similar to those with pigs described on p. 411, in which a normal rat was fitted with a lymph cannula. The rat was allowed 18 hr. to recover from the operation. The flow of lymph was sluggish overnight but became more rapid the next morning. The rat was then given on a small piece of bread 4 mg. β-carotene dissolved in 400 mg. arachis oil and consumed the dose completely within 20 min. The lymph was thereafter collected at the intervals stated in Table 7 into small test-tubes containing a trace of heparin to prevent clotting. The procedure was repeated on the following day when the β-carotene meal was taken within 5 min. It will be seen that on both occasions the results were much the same as with the pig given β-carotene; the vitamin A content of the lymph

Table 7. *Appearance of vitamin A in the lymph from the lymphatic draining the small intestine of a stock-colony rat given on 2 successive days 4 mg. β-carotene in 400 mg. arachis oil on a piece of bread*

	Lymph											
	First day, 18 hr. after cannulation						Second day, 42 hr. after cannulation					
			Vitamin A						Vitamin A			
			Yield		Concentration				Yield		Concentration	
Time after meal (hr.)	Fluorescence in ultraviolet light	Yield (ml.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u./ml.)	Ester (i.u./ml.)	Fluorescence in ultraviolet light	Yield (ml.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u./ml.)	Ester (i.u./ml.)
−0·5– 0*	Bluish	1·0	0·8	0·9	0·8	0·9	Bluish	3·5	0·4	0·4	0·1	0·1
0 – 0·5	Bluish	0·5	0·4	0·5	0·7	0·9	Bluish	1·3	0·1	0·3	0·1	0·2
0·5– 1·0	Bluish	0·7	0·3	0·7	0·4	1·0	Bluish	1·4	0·3	0·3	0·2	0·2
1·0– 1·5	Bluish	2·0	0·4	1·0	0·2	0·5	Yellow	1·0	0·2	2·0	0·2	2·0
1·5– 2·0	Yellow	1·0	0·4	0·7	0·4	0·7	Yellow	1·6	0·3	3·0	0·2	2·0
2·0– 2·5	Yellow	1·0	0·7	0·7	0·7	0·7	Yellow	1·4	0·3	4·0	0·2	2·5
2·5– 3·0	Yellow	1·0	0·5	1·0	0·5	1·0	Yellow	1·4	0·3	4·0	0·2	2·9
3·0– 3·5	—	—	—	—	—	—	Yellow	0·6	0·1	1·3	0·2	2·1
3·5– 4·0	Yellow	1·5	0·8	7·7	0·5	5·1	Yellow	1·2	0·5	1·4	0·4	1·2
4·0– 5·0	Yellow	1·0	0·5	2·6	0·5	2·6	Yellow	1·7	0·3	1·0	0·2	0·6
5·0– 6·0†	Yellow	1·0	0·6	5·2	0·6	5·2	Yellow	1·1	0·3	2·2	0·3	2·0
6·0– 7·0	Yellow	1·3	0·7	4·0	0·5	3·1	Yellow	1·7	0·3	2·0	0·2	1·2
7·0– 8·0	Yellow	2·0	0·8	2·0	0·4	1·0	Yellow	1·9	0·4	4·9	0·2	2·6
8·0–22·0	Yellow	52·0‡	10·4	10·4	0·2	0·2	—	—	—	—	—	—
8·0–23·0	—	—	—	—	—	—	Yellow	10·0	1·0	7·0	0·1	0·7

* On second day −1·75–0.
† Given a piece of bread at this time on both occasions.
‡ Drank Ringer-Locke solution containing 5 % glucose.

rose after the carotene meal and the increase was only in the ester form. A change from bluish to yellow fluorescence in ultraviolet light accompanied the rise, though on the first occasion it preceded the first noticeable chemical change. On both days of the experiment the rat was given a piece of bread some 5 hr. after the carotene meal when, after the peak at 3–4 hr., the concentration of vitamin A ester in the lymph was diminishing. This was followed by an increase in concentration of vitamin A, possibly because the bread caused a further release of β-carotene solution from the stomach into the intestine, and then a renewal of the conversion to vitamin A, this becoming noticeable in the lymph within about 1 hr. After completion of the 1st day's run Ringer-Locke solution containing 5% glucose was offered to the rat to compensate for the loss of lymph. The rat drank it avidly and as a result the flow of lymph increased about fourfold and 52 ml. were collected between 6.40 p.m. and 8.40 a.m.

Experiments with vitamin A-deficient rats. Results of experiments in which the cannulated rats receiving β-carotene were vitamin A-deficient, and in which other deficient but not cannulated rats were given carotene or arachis oil only, are presented in Table 8.

In the first experiment the cannulated rat was allowed 3 hr. to recover from the operation and was then given carotene, whereas the control rat received arachis oil only. Both were killed 75 min. after the cannulated rat had taken its dose, that is, as soon as the vitamin A fluorescence was noticed in the lymph. It may be taken that vitamin A began to appear in increased quantities some time after the collection of the '60 min.' sample. It will be seen that the increase in the lymph was again exclusively in the ester form. Appreciable quantities of vitamin A were present also in the contents and wall of the small intestine but the amount of vitamin A in the blood and liver was no greater than in the undosed rat.

In the second experiment three rats were used, one cannulated, and two not cannulated, of which one was dosed and the other given arachis oil only. Again 3 hr. were allowed between operation and dosing, but this time the rats were not killed until 2 hr. later, that is, at a time when normally, in rats given carotene, vitamin A appears in appreciable amounts not only in the intestine but also in the blood and liver (cf. Thompson, Ganguly & Kon, 1949). Vitamin A ester began to appear in the lymph between 1 and 1·5 hr. after the carotene meal, some 13 i.u. altogether being collected before the rat was killed. Another 13 i.u. were found in the intestine but the quantities present in the blood and in the liver differed only a little from those in the undosed animal. In contrast, in the rat uncannulated but dosed, the vitamin A stores in the liver and in the blood were markedly increased and some 35 i.u. were found in the intestine. The uncannulated rat had obviously been more efficient in conversion than the cannulated one, but this is probably what should be expected, since the uncannulated rat had not been exposed to anaesthetic and operation and, moreover, took more of its dose than the cannulated one. The main significance of the experiments lies in the finding that by tapping and removing the lymph flowing from the intestine the vitamin was prevented from reaching the blood or liver.

Exps. 3 and 4 differed from the preceding ones in that the uncannulated rats were exposed to a sham operation (p. 399) and that all the rats, whether cannulated or not,

Table 8. *Appearance of vitamin A in the small intestine, in the lymph from the lymphatic draining the small intestine, in the blood and in the liver (and in the mesenteric lymph nodes in Exps. 3 and 4) of vitamin A-deficient rats after a meal of 4 mg. β-carotene in 400 mg. arachis oil. In Exps. 1 and 2 the carotene solution was offered on a piece of bread about 3 hr. after cannulation of lymphatic. In Exps. 3 and 4 the carotene solution was injected into the stomach by syringe through the wall immediately after cannulation or sham operation*

Exp. no.	Rat No.	Rat Weight (g.)	Treatment	Time after dosing (min.)	Small intestine Wall* Vitamin A (i.u./rat)	Small intestine Contents* Vitamin A (i.u./rat)	Small intestine Contents* Carotene† (μg./rat)	Lymph Fluorescence in ultraviolet light	Lymph Yield (ml.)	Lymph Vitamin A Alcohol (i.u.)	Lymph Vitamin A Ester (i.u.)	Blood Vitamin A Alcohol (i.u./rat)	Blood Vitamin A Ester (i.u./rat)	Liver Total vitamin A (i.u./rat)	Mesenteric lymph nodes Total vitamin A (i.u./rat)
1	1	348	Cannulated and dosed	−210– 0	—		—	Bluish	0·9	0·3	0·3	—	—	—	—
				0– 30	—		—	Bluish	0·5	0·3	0·2	—	—	—	—
				30– 60	—		—	Bluish	0·3	0·2	0·3	—	—	—	—
				60– 75‡	12		65	Yellow	0·5	0·3	1·5	1·8	0·8	1·4	—
	2	283	Not cannulated, arachis oil only	75	1·8		—	—	—	—	—	1·8	1·4	0·9	—
2	1	336	Cannulated and dosed	−200– 0	—		—	Bluish	2·0	0·2	0·2	—	—	—	—
				0– 30	—		—	Bluish	0·5	0·2	0·1	—	—	—	—
				30– 60	—		—	Bluish	0·7	0·2	0·1	—	—	—	—
				60– 90	—		—	Yellow	0·7	0·4	4·0	—	—	—	—
				90–120‡	13		102	Yellow	0·8	0·4	8·6	1·4	0·9	2·1	—
	2	326	Not cannulated, dosed	120	35		86	—	—	—	—	13·7	7·5	24·0	—
	3	323	Not cannulated, arachis oil only	120	0·9		—	—	—	—	—	1·1	0·2	0·4	—
3	1	320	Cannulated and dosed	0– 30	—	—	—	Bluish	0·2	0·2	0·2	—	—	—	—
				30– 60	—	—	—	Bluish	0·3	0·1	0·1	—	—	—	—
				60– 90	—	—	—	Bluish	0·2	0·2	0·1	—	—	—	—
				90–120	—	—	—	Bluish	0·7	0·2	0·3	—	—	—	—
				120–150	—	—	—	Yellow	0·6	0·1	0·6	—	—	—	—
				150–180	—	—	—	Yellow	0·4	0·2	0·5	—	—	—	—
				180–210‡	15	0·3	140	Yellow	0·4	0·2	0·6	2·6	0·1	0·4	0
	2	349	Sham operation, not cannulated, dosed	0	0	0·3	375	—	—	—	—	—	—	0·8	0
4	1	293	Cannulated and dosed	0–180	—	—	—	Bluish	0·4	0·3	0·6	—	—	—	—
				180–210	—	—	—	Yellow	0·4	0·3	1·0	—	—	—	—
				210–240‡	20	4·5	240	Yellow	0·6	0·3	1·4	3·1	0·2	2·2	4·8
	2	258	Sham operation, not cannulated, dosed	240	53	7·7	1650	—	—	—	—	7·6	5·3	31	2·1

* Contents removed as described on p. 400, but immediately after and not before the removal of blood.
† In the contents unless the wall and contents were analysed together.
‡ The time interval applies to the collection of lymph; the second value denotes the time of killing.

received their dose of β-carotene under anaesthesia, at the end of the operation, directly injected by syringe into the stomach through the wall. Under these conditions the flow of lymph in the cannulated rats was rather slower and the concentration of vitamin A in it decidedly less than in Exps. 1 and 2. However, the main trend was the same, in that, despite good conversion in the intestine, interruption of passage of lymph to the blood stream resulted in the non-appearance of vitamin A ester in the blood, and of vitamin A in the liver, of the cannulated animal, whereas relatively large quantities of the ester were circulating at the same time in the blood, and also much vitamin A had reached the liver, of the control which experienced the sham operation. When the two rats in Exp. 4 were killed, the mesentery of the cannulated rat was engorged with lymph, and analysis showed that it contained twice as much vitamin A as the mesentery of its control.

DISCUSSION

By removal in vivo of the contents of the intestine, our methods of analysis have certainly been improved, and the new technique has given us more definite answers to some points left open in the earlier paper of Thompson, Ganguly & Kon (1949).

We are now satisfied that most, if not all, of the vitamin A arising in the gut from carotene appears in the wall, and that the appreciable quantities of vitamin A detected by us earlier in the contents must have been to a very large extent carried there mechanically from the wall in the process of washing. Our more recent findings agree thus with those of Glover *et al.* (1948*b*). We now know that handling the dead intestine, even when quite fresh, may easily lead to such mechanical losses.

The washing out of the intestine during life, as well as yielding more accurate results, is also much easier and removes the contents more completely. A further benefit of the method is that its speed prevents hydrolytic cleavage of vitamin A ester. We should, however, point out that even with the new technique we consistently find in the intestinal contents traces of apparent vitamin A alcohol amounting, after all necessary corrections, to some 3–5% of the total vitamin A in the intestine.

Repetition of our earlier tests with administration of vitamin A alcohol or ester to vitamin A-deficient rats has shown again that the distribution and form of vitamin A in different sites are the same as after a meal of carotene, though possibly after the meal of carotene the proportion of vitamin A alcohol in the intestinal wall is less than after a meal of either form of preformed vitamin A. With all three, however, at least two-thirds of the vitamin A in the wall appears as the ester. Eden & Sellers (1949, 1950) found with preformed vitamin A very similar proportions in the intestine of calves and sheep.

As a rule no carotene can be detected by the naked eye on the mucosa of the intestine washed out in vivo after a meal of carotene. We have, so far, no other proof that none is left there adhering mechanically, but it is interesting to note that the curve of concentration of carotene in the wall (see Fig. 3) follows closely that found earlier (Thompson, Ganguly & Kon, 1949) for the contents. We realize that such similarity of behaviour would be expected with carotene merely mechanically held on the surface of the intestine. Be it as it may, 2 hr. after a carotene meal the peak for

carotene in both wall and contents is much farther down the small intestine than that for vitamin A.

We have found vitamin A in the intestine of previously depleted animals within 5 min. after a carotene meal and the extreme rapidity of the process is, to our mind, a valuable proof, if indeed further proof is needed, that the intestine is the primary site of conversion. Observations with mesenteric lymph, and especially those in which diversion of the flow prevented the appearance of vitamin A in the liver, make the evidence wellnigh conclusive. Our findings with lymph are in full agreement with those of the Liverpool workers published in the paper which follows this one (Alexander & Goodwin, 1950).

The time lag between the formation of vitamin A from carotene in the wall of the intestine and its appearance in the mesenteric lymph is noteworthy. The former can occur within 5 min., but we have never detected vitamin A in the flowing lymph in less than 1 hr., though we have seen it (Thompson, Ganguly & Kon, 1949) fluorescing in a lymph node after 30 min. We have at present no explanation of the delay.

However strong the proof that there is intestinal conversion, the evidence for excluding the liver as another, or for that matter the only, active site is still only circumstantial. All our experiments and, as far as we know, those of the other workers in this field, were made in the presence of the liver. Even the valuable study of Krause & Pierce (1948), in which the hepatic circulation was blocked, cannot, on their own admission, be taken to settle the point. We are at present working on this problem.

We think it right that tribute be paid where it is due and that emphasis be laid on the bearing of the early work of Drummond *et al.* (1935) on the absorption of carotene and vitamin A in man, and on the problem of intestinal conversion. The importance of the findings was realized at the time by few, though, arising from them, Verzár & McDougall (1936) were prompt to suggest that carotene might be converted to vitamin A in the intestinal mucosa. It will be recalled also, as already pointed out by Glover *et al.* (1948*b*), that nearly 10 years ago Popper & Greenberg (1941) offered histological evidence of intestinal conversion of carotene.

SUMMARY

1. Further work on the intestinal conversion of carotene to vitamin A in the rat and in the pig was done by methods already largely described by Thompson, Ganguly & Kon (1949).

2. In the present experiments, however, when rats were used, the intestinal contents were washed out from the living intestine while it was still connected with the blood supply.

3. It was then found that the vitamin A appeared almost exclusively in the wall of the intestine, 75% of it in the ester form.

4. The same proportion appeared as ester when preformed vitamin A was given, whether as alcohol or as ester.

5. The efficiency of conversion of carotene increased with the state of dispersion; powdered carotene in a fat-free diet was least, and colloidal and oily solutions were most, efficiently converted.

6. Vitamin A appeared in the intestine within 5 min. of a meal of carotene in colloidal or oily solution.

7. In the rat 2 hr. after a carotene meal the peak of conversion was just proximal to the middle of the small intestine. No conversion was observed in that part of the intestine preceding the entrance of the common bile duct.

8. After a carotene meal no more than traces of carotene were found at any time in the systemic or portal blood of pigs. An increase in vitamin A ester preceded that in vitamin A alcohol simultaneously in the two circulations.

9. Cannulas were established in the mesenteric lymphatics of pigs and rats.

10. In pigs, vitamin A, exclusively as ester, appeared in high concentration (up to 32 i.u./ml.) in the lymph within 1–2 hr. of a meal of carotene or vitamin A. After the meal of carotene small quantities of carotenoids, apparently not β-carotene, accompanied the vitamin A.

11. In rats vitamin A, also exclusively as ester, appeared in the lymph before it was found in the blood or liver. Diversion of the lymph flow prevented its appearance in either place.

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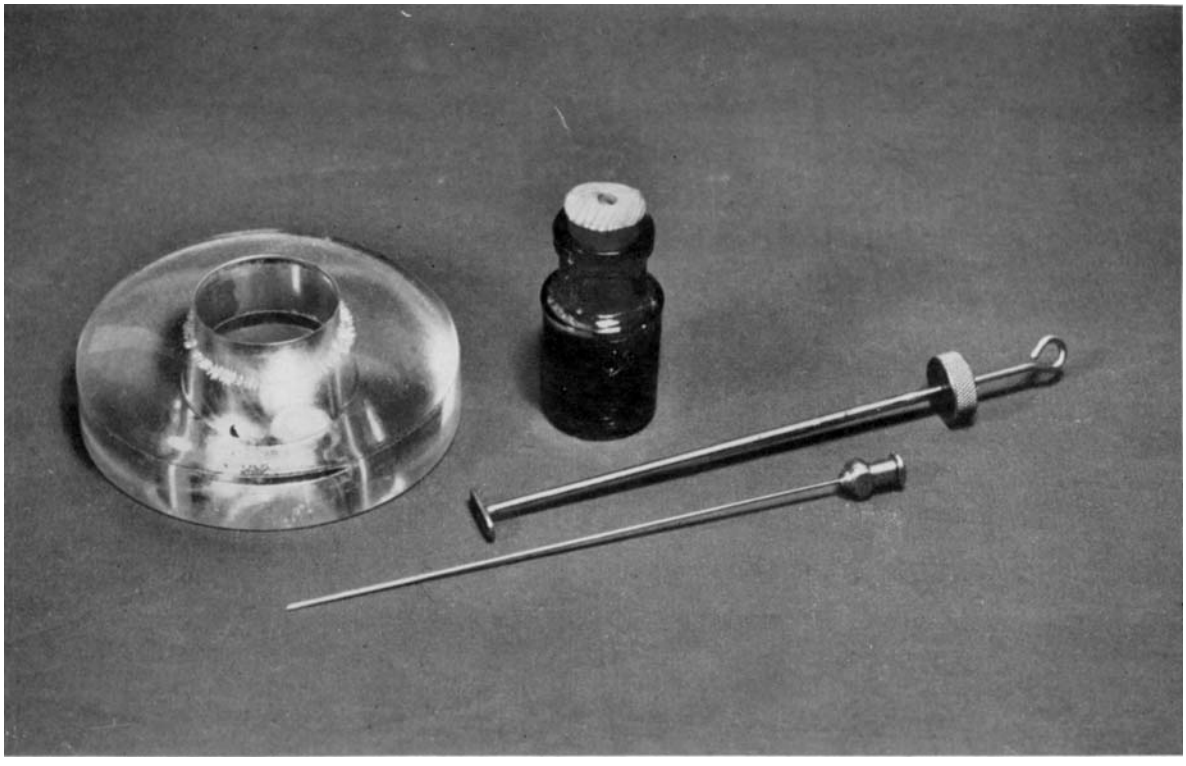

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EXPLANATION OF PLATE

Pl. 1. London's cannula, protective Perspex dome and collecting bottle.

A Demonstration of the Conversion of Carotene into Vitamin A in Conscious Rats

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There is now considerable evidence that in animals carotene is converted into vitamin A in either the lumen or the wall of the intestine, most probably in the latter. This has been proved in many ways by experiments both in vivo and in vitro on a variety of animals (Glover, Goodwin & Morton, 1947, 1948; Goodwin & Gregory, 1948; Wiese, Mehl & Deuel, 1947; Mattson, Mehl & Deuel, 1947; Thompson, Ganguly & Kon, 1947, 1949; Mattson, 1948; Krause & Pierce, 1948; Thompson, Coates & Kon, 1950; Lane, 1950). In particular, Goodwin & Gregory (1948) showed that carotene, introduced into the intestine of conscious goats provided with an abomasal or duodenal fistula and a thoracic duct fistula, gave rise to increased amounts of vitamin A in the lymph but no carotene.

Although the conversion in the rat's intestine has been well established, it has not been confirmed in conscious animals by the method applied to goats and since, primarily for another purpose, the intestinal lymphatics of a large number of rats were being cannulated, the opportunity was taken of using some of the animals to follow the changes in the vitamin A content of the lymph after oral administration of β-carotene. These experiments were in progress when Coates, Thompson & Kon (1950) gave a preliminary report of the results of their extensive investigation carried out along similar lines. Our own was then discontinued, but the results obtained are briefly reported here because they completely confirm those of the Reading investigators, which are published in full in the paper just preceding this one (Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950).

EXPERIMENTAL

Preparation of rats. The intestinal lymphatic vessel was cannulated with fine polythene tubing in an aseptic operation under ether anaesthesia by the technique devised by Bollman, Cain, Grindlay & van Hook (1948). In their original description