Ganguly, J., Kon, S. K. & Thompson, S. Y. (1947). *Brit. J. Nutrit.* **1**, iii.
Gillam, A. E. (1935). *Biochem. J.* **29**, 1831.
Hogness, T. R., Zscheile, F. P. & Sidwell, A. E. (1937). *J. phys. Chem.* **41**, 379.
Koehn, C. J. & Sherman, W. C. (1940). *J. biol. Chem.* **132**, 527.
Kon, S. K., Mawson, E. H. & Thompson, S. Y. (1944). *Nature, Lond.*, **154**, 82.
Mattick, A. T. R., Hiscox, E. R., Crossley, E. L., Lea, C. H., Findlay, J. D., Smith, J. A. B., Thompson, S. Y., Kon, S. K. & Egdell, J. W. (1945). *J. Dairy Res.* **14**, 116.
Moore, T. (1929). *Biochem. J.* **23**, 1267.
Notevarp, O. & Weedon, H. W. (1936). *Biochem. J.* **30**, 1705.
Preston, J. S. & Cuckow, F. W. (1936). *Proc. R. phys. Soc. Lond.* **48**, 869.
Society of Public Analysts, Publication Committee (1942). *Analyst*, **67**, 164.
Thompson, S. Y. (1942). *Chem. & Ind.* **61**, 170.
Thompson, S. Y., Ganguly, J. & Kon, S. K. (1947). *Brit. J. Nutrit.* **1**, v.
Thompson, S. Y., Ganguly, J. & Kon, S. K. (1949). *Brit. J. Nutrit.* **3**, 50.
Thompson, S. Y., Kon, S. K. & Mawson, E. H. (1942). *Biochem. J.* **36**, xvii.

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

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When Moore (1930) first showed that vitamin A is formed from carotene in the animal body he assumed (Moore, 1931) that the conversion takes place in the liver, and this view was generally held until quite recently. It never explained satisfactorily why the blood of many mammals, including the rat, pig, sheep and goat, contains only traces of carotenoids or how carotene reaches the liver in these animals. A possible explanation is that it travels only through the portal vein to be at once removed by the liver and thus never reaches the general circulation, but Goodwin, Dewar & Gregory (1946) found recently no carotene in blood samples from the portal vein of sheep and goats taken at intervals after the introduction of carotene into the abomasum, though vitamin A similarly given appeared in large quantities.

At about the same time Sexton, Mehl & Deuel (1946) observed that carotene, introduced parenterally into vitamin A-deficient rats, accumulated in the liver without being converted into vitamin A or relieving the symptoms of deficiency. In commenting on these findings Sexton *et al.* said: 'One explanation of these phenomena would be that carotene is transformed into vitamin A in the rat before reaching the blood stream. A possible site for the transformation of carotene to vitamin A might be the intestinal wall.'

For some years now we have been working on the metabolism of carotenoids and of vitamin A in rats and in pigs, and the observations of Goodwin *et al.* (1946) and of Sexton *et. al.* (1946) underlined our growing doubts about the part played by the liver in the formation of vitamin A. The work now described, of which, so far, only a preliminary note has been published (Thompson, Ganguly & Kon, 1947), fully supports

* Read in part before The Nutrition Society on 31 May 1947 (Thompson, Ganguly & Kon, 1947).

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the opinion of Sexton *et al.* (1946), and agrees with the findings of Glover, Goodwin & Morton (1947), of Mattson, Mehl & Deuel (1947) and of Wiese, Mehl & Deuel (1947), published at the same time as our preliminary note.* Quite recently, through the courtesy of Prof. B. C. P. Jansen, we were able to see a report from Germany by Wagner (1939) on work done by him at the Whale Catching Station at Lopra in the Faeroes. Wagner studied the conversion in the whale of carotene derived from krill ('Gattung der Euphansia superba Dana') to vitamin A, and came to the conclusion that 'Das Vitamin A wird nach diesen Befunden nicht in der Leber umgewandelt, sondern in den Darmabschnitten selbst. Der Ort der Umwandlung wird nicht in die Darmwand verlegt, sondern in den Darminhalt.' Wagner was thus first to demonstrate the intestinal conversion of carotene in a mammal.†

EXPERIMENTAL

Sources of vitamin A and carotene

Carotene

At the beginning carrot oil was used, prepared by extracting carrots with acetone, transferring the acetone extract to light petroleum, washing out the acetone with water and evaporating off the solvent. From ten carrots 1·72 g. of oil was obtained containing 7080 μg./g. of α-carotene and 23,200 μg./g. of β-carotene. Later, a carotene concentrate containing 3·5 % of β-carotene was obtained from the British Chlorophyll Co. Ltd., and finally crystalline β-carotene was obtained from the same source. Most of the work was done with this crystalline carotene dissolved in arachis oil.

Preparation of carotene for feeding. Carotene solutions were made fresh for each experiment. The quantity varied with the need but the following example illustrates the procedure:

About 50 mg. of β-carotene were accurately weighed out and dissolved in 30–40 ml. of diethyl ether, and 5 g. of arachis oil stabilized with 0·1 % hydroquinone were added. The ether was then evaporated off in vacuo on the steam bath.

Vitamin A

Vitamin A ester. Usually a fish-liver oil containing *c.* 80,000 i.u./g. of vitamin A was used. This liver oil was diluted at the time of feeding with the requisite amount of arachis oil stabilized with hydroquinone.

Vitamin A alcohol. The fish-liver oil used as a source of ester was saponified (Gillam, Henry & Kon, 1937), purified by chromatography (see p. 57) and the concentration of vitamin A was determined. A stock solution of the substance in *n*-hexane was kept in cold storage.

It is our experience that vitamin A alcohol prepared and stored in this way keeps remarkably well for at least 3–4 months. For feeding, requisite quantities were taken from this stock solution, arachis oil was then added and the hexane evaporated off in vacuo on the steam bath.

* The full reports of the findings of the Liverpool workers (Goodwin & Gregory, 1948; Glover *et al.* 1948) have just appeared (January 1949).

† See *Note added in proof* (p. 77).

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Rats

Hooded Norwegian rats of our own breeding were used.

Rats deficient in vitamin A

Normal stock-colony does were mated as usual (Folley, Ikin, Kon & Watson, 1938; Folley, Henry & Kon, 1947). During the first 15 days after littering they received the ordinary stock diet (Folley *et al.* 1938) but without milk, liver or carrot. On the 16th day, i.e. when the pups usually start eating solid food, their diet was changed to one, of the following percentage composition, which is almost devoid of vitamin A and contains only 3 μg. of carotene/100 g.: dried separated milk 20, whole wheat 55, linseed cake 11, crude casein 5, dried yeast 4, ground-nut oil 4, calcium carbonate 0·5, sodium chloride 0·5. They were kept thereafter on the vitamin A-deficient diet. They usually grew well on this diet without any supplement of vitamin A or carotene but, if growth was retarded, they were given 70 i.u. of vitamin A weekly in two doses. All rats received 35 i.u./week of vitamin D in the form of calciferol in arachis oil in one dose.

Table 1. *The vitamin A content of the small intestine, blood and liver of rats which had received for* 8–10 *weeks the diet deficient in vitamin A*

(Values expressed per organ and, for blood, per rat)

Rat no.	Small intestine Total vitamin A (i.u.)	Blood		Liver Total vitamin A (i.u.)
		Alcohol* (i.u.)	Ester* (i.u.)	
1	1·3	0·7	0·6	0·8
2	1·5	0·9	0·6	0·7
3	1·4	1·4	0·3	0·7
4	1·5	0·6	0·2	1·0
Mean	1·4	0·9	0·4	0·8

* See p. 57.

Under these conditions there was no storage of vitamin A in the liver. The blood and intestine were also devoid of this factor, as shown in Table 1. In general the vitamin A-deficient rats were 12–15 weeks old and weighed 200–250 g. when used for experiments.

Normal rats

For certain experiments stock-colony rats of both sexes were used. Their ages varied from 12 to 15 months. If necessary, they were given for 1 week the vitamin A-deficient diet. This treatment removed almost entirely the vitamin A present in the gut without appreciably affecting the liver stores or concentration in the blood (Table 2).

Preparation and dosing of rats

The rats received only water for 24 hr. before dosing, and skim milk only for the preceding 24 hr. In this way, when they were killed, little solid material was present in the stomach and small intestine, and the analysis of the contents was facilitated.

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The solution of carotene or vitamin A in arachis oil of desired strength (usually 4 mg. of carotene or 500 i.u. of vitamin A in 400 mg. of arachis oil) was mixed with 1 g. of the vitamin A-deficient diet made fat-free by extraction with diethyl ether in a Soxhlet extractor, and subsequently passed through a 40-mesh sieve. The mixture was offered in Petri dishes in individual cages to the rats, prepared as described above,

Table 2. *Changes in the vitamin A content of the small intestine, blood and liver of stock-colony rats when given a diet deficient in vitamin A*

(Values expressed per organ and, for blood, per rat)

Rat no.	Time on deficient diet (days)	Small intestine		Blood		Liver	
		Alcohol* (i.u.)	Ester* (i.u.)	Alcohol* (i.u.)	Ester* (i.u.)	Alcohol* (i.u.)	Ester* (i.u.)
1	0	2·4	18	2·5	1·4	175	6514
2	0	5·6	13	2·5	1·2	165	7430
3	5	1·8	3·6	4·6	1·0	69	4570
4	5	1·6	1·6	4·0	1·0	72	3771
5	9	1·2	2·4	3·0	1·0	79	5943
6	9	1·4	2·4	3·6	1·0	73	5257
7	13	1·3	2·8	4·0	1·0	114	6630
8	13	1·2	2·4	3·4	1·0	125	4800

* See p. 57.

and was, as a rule, consumed in less than 5 min. In general there was little spilling. When necessary, the spillings were collected on blotting paper and weighed back. Under these conditions some of the carotene mixed with the solid food passed very quickly from the stomach to the intestine. In fact, carotene in most cases travelled half-way down the small intestine within 15 min. of being offered in the diet, and reached the caecum in less than 2 hr.

Killing of rats

The animals were anaesthetized with a 1:2:3 mixture of alcohol, chloroform and ether, the chest was rapidly opened, and as much blood as possible taken in an oxalated syringe directly from the heart. The required organs were then removed for analysis.

Pigs

Large White baconers bred and fattened in the piggery of the Institute were used. They were starved for 24 hr. before dosing and received the dose of vitamin A or carotene either directly into the mouth or mixed with a small quantity of meal. A sample of blood was obtained at slaughter and the necessary organs were removed for analysis.

Measurement of vitamin A and carotene in different tissues

General

All manipulations were carried out in subdued light and as rapidly as possible. In our experience, speed of operation is one of the most important factors for the successful measurement of vitamin A in natural products. All solvents and reagents were tested before use.

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Blood

Usually 2–4 ml. of plasma were obtained, without difficulty, from each rat. The plasma was made up with distilled water to 10 ml. and the vitamin A, and carotene if present, were extracted by the method of Kimble (1939). The final light petroleum extract was transferred to 1–2 ml. of *n*-hexane, and chromatographed to separate the vitamin A ester and carotene (if present) from the vitamin A alcohol as described on p. 57. The same procedure was used for pig's blood, but no dilution with water was necessary since larger quantities of plasma were available.

The vitamin A fractions were then evaporated in vacuo in a 50 ml. flask with ground glass connexions, and taken up in suitable quantities of chloroform for measurement of vitamin A by the antimony-trichloride reaction in the photoelectric spectrophotometer of Thompson (1942) as described by Thompson (1949). As a rule the blood-plasma extract of one rat was taken up in 0·2 ml. of chloroform and quantitatively transferred to the reaction cell in a volume of 0·5 ml. The total amount of vitamin A in the blood of a rat was calculated by assuming that a rat contains 6·7 ml. of blood/100 g. (Cartland & Koch, 1928), of which plasma constitutes 50 %.

Other tissues

Removal of intestinal contents. At first the contents of the small intestine were separated from the wall by squeezing out. Later, for fixing the villi, 30 % (v/v) alcohol followed by absolute alcohol was used and treatment with diethyl ether completed the washing out. This technique was discontinued on the advice of Prof. A. C. Frazer, who had shown that it extracted some fat from the gut wall and presumably also some vitamin A and carotene. At his suggestion a 0·9 % (w/v) solution of sodium chloride in distilled water was used for washing out the contents. The pyloric end of the small intestine was tied firmly to an injection needle of wide bore, and the contents were forced out at the caecal end into a beaker by means of a 20 ml. syringe filled with saline. Three 20 ml. lots of saline were used for washing, and contents and washings were combined.

As judged by the naked eye, this method of washing removed most of the contents. Traces were undoubtedly left adhering firmly to the wall and could, for instance, after large doses of carotene, be seen on splitting the intestine. From our point of view it was more important to avoid stripping off any of the material contained in the wall itself than to remove the last traces.

Extraction of gut wall and other tissues. The organ was cut into small pieces, 30 ml. of absolute alcohol, 15 ml. of distilled water and 100 ml. of light petroleum (b.p. 40–60°) were added, and the mixture was transferred to a small flat-sided brown glass jar 6·5 × 10 cm., into which the knife assembly of a Waring Blendor was fitted. Nitrogen was blown in to displace the air in the jar, as otherwise there was some loss of vitamin A (Table 3), and the mixture was homogenized for 2–3 min. Entry of air into the jar was prevented by pressing a rubber bung into its mouth.

The mixture was then transferred to a separating funnel and allowed to settle for 10–15 min. The bottom layer was run off and re-extracted with another 100 ml. of

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light petroleum. The two extracts were combined, made to volume, carotene, if any, was measured (Thompson, 1949), and the extract was then treated to separate the vitamin A and carotene as described on p. 57; the amount of vitamin was then determined as for blood, but the extracts of organs were taken up in 1·5 ml. of chloroform, and were then, if necessary, suitably diluted for the antimony-trichloride reaction.

Table 3. *Comparison for calf-liver tissue of the effectiveness of the solvent extraction method, with and without displacement of air by nitrogen in the Waring Blendor jar, with the alkali-digestion method*

(For each experiment separate 5 g. samples were taken from a thoroughly minced whole calf liver kept in cold storage.)

	Alkali-digestion method Vitamin A			Solvent-extraction method: Without displacing air by nitrogen Vitamin A			Solvent-extraction method: With air displaced by nitrogen Vitamin A		
	Alcohol* (i.u.)	Ester* (i.u.)	Total (i.u.)	Alcohol* (i.u.)	Ester* (i.u.)	Total (i.u.)	Alcohol* (i.u.)	Ester* (i.u.)	Total (i.u.)
	5·6†	10·0†	15·6†	4·0†	8·0†	12·0†	4·2‡	12·8‡	17·0‡
	6·0†	11·2†	17·2†	3·2†	9·2†	12·4†	3·6‡	12·8‡	16·4‡
	6·0†	9·6†	15·6†	3·2†	8·4†	11·6†	3·6‡	12·4‡	16·0‡
	5·2†	12·0†	17·2†	4·0†	8·0†	12·0†	3·8‡	9·6‡	13·4‡
Mean	5·7	10·7	16·4	3·6	8·4	12·0	3·8	11·9	15·7

* See p. 57. † Done on 4. ii. 48. ‡ Done on 11. ii. 48.

Extraction of intestinal contents. To the combined saline washings of the contents were added 60 ml. of absolute alcohol and 200 ml. of light petroleum, and the mixture was transferred to an ordinary Waring Blendor jar (p. 54); a second extraction was done with a further 200 ml. of light petroleum and the further procedure was then as for the intestinal wall.

Investigation of the adequacy of the method of extraction, and comparison of it with the alkali-digestion method

The usual alkali-digestion method (Davies, 1933), though used at the beginning, was later abandoned, not only because it proved troublesome in the presence of starch, but also because it led to some hydrolysis of vitamin A ester as shown in Table 3, in which results obtained with a calf liver are given.

In Table 4 results are given for the analysis of the livers and small intestines of vitamin A-deficient rats each dosed with 4 mg. of β-carotene in 400 mg. of arachis oil and killed 2–3 hr. after dosing. Values are given also for rats dosed only with arachis oil. Findings with a pig liver of high vitamin A content are given in Table 5. It will be seen from the tables that there was invariably some increase in the alcohol form when the alkali treatment was used, due undoubtedly to the hydrolysis of the ester form.

Values obtained by the extraction method were, on the whole, higher than those given by the alkali-digestion method. Moreover, the residue of tissue left after extraction, when digested by alkali, yielded further, though relatively small, quantities of carotene and vitamin A. It appeared, therefore, that neither method was quite

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Table 4. *Comparison of the alkali-digestion method and of the solvent-extraction method with nitrogen in the jar of the Waring Blendor, as applied to the extraction of vitamin A and carotene from the liver, small intestine, and contents of the small intestine, of rats. The values are for the blended livers and small intestines (wall and contents) of four vitamin A-deficient rats dosed with 4 mg. β-carotene in 400 mg. arachis oil 2 hr., and of four dosed 3 hr., before they were killed, and for each individual of two rats dosed with arachis oil alone, and of two others dosed with arachis oil containing 4 mg. β-carotene, and killed 2 hr. after dosing*

				Alkali digestion				Solvent extraction: Original extract*				Solvent extraction: Residues*			
					Vitamin A				Vitamin A				Vitamin A		
Treatment	No. of rats	Interval after dosing (hr.)	Organ	Carotene (μg.)	Alcohol† (i.u.)	Ester† (i.u.)	Total (i.u.)	Carotene (μg.)	Alcohol† (i.u.)	Ester† (i.u.)	Total (i.u.)	Carotene (μg.)	Alcohol† (i.u.)	Ester† (i.u.)	Total (i.u.)
Arachis oil + β-carotene	4	2	Liver	—	—	—	30	—	—	—	38	—	—	—	1·4
				—	—	—	24	—	—	—	31	—	—	—	1·9
			Wall and contents of small intestine	600	—	—	17	653	—	—	18	20	—	—	2·0
				547	—	—	18	587	—	—	17	34	—	—	1·8
	4	3	Liver	—	31	114	145	—	22	223	245	—	—	—	—
				—	34	112	146	—	22	210	232	—	—	—	—
			Wall and contents of small intestine	—	15	39	54	—	21	54	75	—	—	—	—
				—	26	30	56	—	21	54	75	—	—	—	—
Arachis oil only	2	2	Liver	—	—	—	—	—	1·4	3·0	4·4	—	—	—	1·0
				—	—	—	—	—	0·4	2·8	3·2	—	—	—	1·0
			Wall of small intestine	—	—	—	—	3·0	0·3	0·9	1·2	0·8	—	—	0·6
				—	—	—	—	3·0	0·3	1·2	1·5	0·9	—	—	1·0
			Contents of small intestine	—	—	—	—	27	0·2	0·4	0·6	0·3	—	—	0·3
				—	—	—	—	20	0·4	0·8	1·2	0·2	—	—	0·5
Arachis oil + β-carotene	2	2	Liver	—	—	—	—	—	30	56	86	—	—	—	4·8
				—	—	—	—	—	42	55	97	—	—	—	5·2
			Wall of small intestine	—	—	—	—	27	15	34	49	4·0	—	—	2·8‡
				—	—	—	—	27	15	39	54	3·5	—	—	2·8‡
			Contents of small intestine	—	—	—	—	1080	6·8	7·2	14	11	—	—	2·2‡
				—	—	—	—	1140	6·8	7·2	14	9·0	—	—	2·4‡

* See p. 55. † See p. 57.
‡ High readings caused by artifact formed from carotene.
A dash indicates that no analysis was made.

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quantitative, but that the extraction method was, in this respect also, superior to the digestion method. It should be noted that the extraction method consisted of only two extractions; possibly a third extraction would make it quantitative, but for practical purposes the procedure adopted was quite satisfactory.

Table 5. *Comparison for pig-liver tissue of the solvent-extraction method, with nitrogen in the Waring Blendor jar, with the alkali-digestion method for extracting vitamin A*

(For each experiment separate 5 g. samples were taken from a thoroughly minced whole pig liver.)

Solvent-extraction method, with air displaced by nitrogen Vitamin A			Alkali-digestion method Vitamin A		
Alcohol* (i.u.)	Ester* (i.u.)	Total (i.u.)	Alcohol* (i.u.)	Ester* (i.u.)	Total (i.u.)
8·0	246	254	32	224	256
8·8	252	261	14	234	248
9·2	246	255	16	240	256
Mean 8·7	248	257	21	233	254

* See this page.

Chromatographic separation of vitamin A and carotene

General

Carotene gives a blue colour with the antimony-trichloride reagent and, when present in large quantities, interferes with the measurement of vitamin A.

Many methods are available for the separation of the ester and alcoholic forms of vitamin A by phase separation, molecular distillation and chromatography. Hardly any of these are concerned with the removal of carotene and many are too complicated for routine analysis. The simple and convenient chromatographic method developed by us is based on the experience of other workers and on our own. It allows the separation of vitamin A alcohol from carotene and vitamin A ester and, subsequently, the separation of carotene from the ester after saponification, and is suitable for routine analysis.

Description of method

Reagents. Alumina: British Drug Houses Ltd. for chromatographic adsorption analysis.

Solvent: *n*-hexane, boiling range 67–69°.

Eluents: (*a*) 2 % (v/v) acetone in *n*-hexane prepared by adding 2 ml. of acetone to 98 ml. of *n*-hexane; (*b*) 20 % (v/v) acetone in *n*-hexane prepared by adding 20 ml. of acetone to 80 ml. of *n*-hexane; (*c*) 8 % (v/v) ethyl alcohol in *n*-hexane prepared by adding 8 ml. of ethyl alcohol to 92 ml. of *n*-hexane.

Preparation and use of adsorption column. The alumina as purchased is placed for a few hours in a steam-heated oven to make it more active. It then retains this activity for at least 1 month when kept in a screw-cap bottle.

The chromatographic columns are formed in test-tubes 13 cm. in length and 1·3 cm. in internal diam. with a hole blown in the base, to which is joined a 5 cm. length of glass tubing of 0·4 cm. bore. A plug of cotton wool at the bottom of the test-tube supports the column of alumina. This is formed by pouring a suspension of

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alumina in *n*-hexane into the tube. Best results are obtained when the whole column of 3–4 cm. height is formed in one pouring.

The *n*-hexane is allowed to run out and the column is weakened by pouring on to it 2–3 ml. of eluent (*c*) followed by 5 ml. of *n*-hexane, when the column is ready for use. The sample to be treated chromatographically is taken up in the smallest quantity of *n*-hexane, usually 2–3 ml. A receiver is placed under the column and the solution is poured on to the column followed by about 20 ml. of eluent (*a*), which completely elutes carotenes (mainly β-carotene) and vitamin A ester. The receiver is then changed and the alcoholic form of vitamin A is eluted with about 20 ml. of eluent (*c*).

Table 6. *The separation and recovery of vitamin A ester and alcohol on the chromatographic column*

No. of exp.	Vitamin A ester		Vitamin A alcohol	
	Added (i.u.)	Found (i.u.)	Added (i.u.)	Found (i.u.)
1	0	0	58	57
	0	0	58	57
	0	0	58	57
2	60	58	0	0
	60	60	0	0
	60	58	0	0
3	12	12	12	12
	12	12	12	12
	12	12	12	12

If carotenes, or fat, in appreciable quantities are present in the ester fraction, it is saponified (as described on p. 51), extracted and taken up again in *n*-hexane. The solution in *n*-hexane is poured on to a fresh column and treated as before; this time, however, the eluent (*a*) fraction contains carotenes only, if any were present, whereas the ester form of vitamin A, now converted to the alcohol, appears in the eluent (*c*) fraction.

No suction is applied to the columns at any stage, and the eluents are allowed to percolate slowly through them. By using a suitable stand, eight to ten chromatographic separations can be conveniently done at the same time. All operations are done in rapid succession, and care is taken to avoid undue exposure of the top of the column to air. The columns are used only once.

The eluent (*c*) fraction of tissue extracts gives sometimes a pink or orange colour with the antimony-trichloride reagent in addition to the blue of vitamin A. As these interfering substances are held on the column more firmly than vitamin A this interference can be eliminated by using eluent (*b*), which is a weaker eluting agent, instead of eluent (*c*). At least 50–60 ml. of eluent (*c*) are required for complete elution of vitamin A alcohol.

Tests of the validity of the chromatographic method as applied to various materials

Pure solutions. Solutions in *n*-hexane of vitamin A alcohol, vitamin A ester and a mixture of both were passed in triplicate through chromatographic columns and the columns were eluted with eluent (*a*) followed by eluent (*c*). Table 6 shows that the recovery and separation of the two forms of vitamin A were quantitative.

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The vitamin A alcohol was prepared as described on p. 51, and the vitamin A ester was obtained by chromatographic removal of any vitamin A alcohol present in the oil used for feeding to animals (p. 51). By means of the capacity of vitamin A to fluoresce under ultraviolet light or by painting the extruded column with antimony trichloride and noting the blue colour, it was shown that the vitamin A alcohol is held firmly at the top of the column after the removal of the vitamin A ester by treatment with eluent (*a*).

Table 7. *Recovery and separation by the chromatographic method of the vitamin A alcohol and vitamin A ester in rat's blood in the presence and absence of added vitamin A alcohol prepared from fish-liver oil*

Treatment	Rat no.	Volume of plasma (ml.)	Vitamin A alcohol: Added (i.u.)	Vitamin A alcohol: Found (i.u.)	Vitamin A ester found (i.u.)
Vitamin A-deficient, dosed with 400 mg. arachis oil 2 hr. before killing	1	2·6	0	0·8	0·3
	2	3·6	8·5	9·5	0·3
Vitamin A-deficient, dosed with 4 mg. β-carotene in 400 mg. arachis oil 2 hr. before killing	3	3·4	0	5·1	3·4
	4	3·7	8·5	14	4·1

Rat's blood. Two vitamin A-deficient rats were dosed with arachis oil only, and two with 4 mg. of β-carotene in arachis oil, and all four were killed 2 hr. later. The blood was collected, and the blood plasma was pooled within each group and extracted as described on p. 54. The hexane extracts were each halved, and in both cases 8·5 i.u. of vitamin A alcohol dissolved in hexane was added to one-half. The four solutions were then chromatographed and analysed. The results are shown in Table 7.

Tissue extracts. The walls and contents of the small intestines and the livers of the same four rats, pooled in pairs as for blood, were extracted in the way described on p. 54. The solutions in *n*-hexane of the different parts were divided into two equal portions, and each time 12·8 i.u. of vitamin A alcohol were added to one. All twelve samples were chromatographed and, where necessary to remove carotene, the ester fraction was saponified and chromatographed again. Table 8 shows the results.

Effect of fat on the chromatographic separation of vitamin A alcohol from vitamin A ester and carotene. Some of the materials studied contained fat, and it is known that fat has a tendency to cause the elution of substances adsorbed on the chromatographic column. The results quoted in Tables 7 and 8 indicate that the quantity of fat normally present in extracts of blood, small intestine and liver did not interfere with the separation. Separate tests showed, moreover, that under our conditions the determination of 24 i.u. of vitamin A alcohol is not affected by the presence of 400 mg. of margarine fat.

RESULTS

Preliminary experiments with rats and pigs

In the preliminary tests stock-colony rats were kept on a vitamin A-deficient diet for 2, 7 or 14 days. In this way most of the vitamin A present in the intestine of the normally fed rat was removed (see p. 52). After that some of them were given directly

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into the mouth a solution of β-carotene in arachis oil, and others received, similarly, arachis oil only.

After 2–24 hr. the rats were bled by heart puncture under anaesthesia, and the blood, livers, stomachs, small intestines and large intestines were analysed as already described (p. 54). When there was more than one rat in a group, the corresponding organs

Table 8. *Recovery and separation by the chromatographic method of the vitamin A alcohol and vitamin A ester in the wall and contents of the small intestine, and liver of the rats described in Table 7, with vitamin A alcohol added in certain cases*

Organ	Treatment	Rat no.	Vitamin A alcohol Added (i.u.)	Vitamin A alcohol Found (i.u.)	Vitamin A ester found (i.u.)
Wall of small intestine	Dosed with 400 mg. arachis oil only	1	0	0·3	0·9
		2	13	13	1·2
	Dosed with 4 mg. β-carotene in 400 mg. arachis oil	3	0	15	34
		4	13	27	40
Contents of small intestine	Dosed with 400 mg. arachis oil only	1	0	0·2	0·4
		2	13	13	0·8
	Dosed with 4 mg. β-carotene in 400 mg. arachis oil	3	0	7·2	7·0
		4	13	20	7·4
Liver	Dosed with 400 mg. arachis oil only	1	0	1·4	3·0
		2	13	13	2·8
	Dosed with 4 mg. β-carotene in 400 mg. arachis oil	3	0	32	54
		4	13	42	54

were pooled. Table 9 shows that, in comparison with undosed rats, the vitamin A content of the small intestine of dosed rats was markedly increased, but there was no difference in that of the stomach or large intestine. The ester form of vitamin A, but not the alcoholic form, was increased in the dosed rats.

Preliminary experiments with rats deficient in vitamin A also showed the appearance of vitamin A in the small intestine 2–12 hr. after a meal of carotene, the quantities after 2 hr. being comparable with those found at the same time in the liver (Fig. 1). The vitamin A content of the blood, which was negligible before dosing, also rose markedly after 2 hr., the increase of the ester form being relatively greater than of the alcohol form. The concentration of the alcohol form then continued to increase, whereas that of the ester declined.

Experiments also with bacon pigs slaughtered 3–7 hr. after a meal of carotene showed an increase in the concentration of vitamin A in the wall and contents of the small intestine and in the mesenteric lymphatics, but not in the stomach, kidneys, pancreas or gall (Table 10). There was also a relative increase in the esterified form of vitamin A in the blood.

Main experiments with rats and pigs

The appearance of vitamin A in the small intestine of the rat after a meal of carotene

The preliminary experiments with rats and pigs described on p. 59 showed that after a meal of carotene vitamin A appears in the small intestine, but not in the stomach or large intestine. Further experiments were done to locate more precisely the site of appearance of vitamin A in the small intestine.

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Table 9. *The appearance of vitamin A and of carotene in different organs of stock-colony rats kept for different periods of time on a diet deficient in vitamin A, then given a meal of carotene and killed 2–24 hr. later*

(Values expressed per organ and, for blood, per rat)

						Stomach		Small intestine		Large intestine		Blood Vitamin A		
Exp. no.	No. of rats	Weight of rats (g.)	Period on vitamin A-deficient diet (days)	Dose given	Interval between dosing and killing (hr.)	Carotene (μg.)	Vitamin A (i.u.)	Carotene (μg.)	Vitamin A (i.u.)	Carotene (μg.)	Vitamin A (i.u.)	Alcohol (i.u.)	Ester (i.u.)	Liver Vitamin A (i.u.)
1	1	440	14	Ten drops arachis oil only	2	0	0·7	0	7·0	0·5	1·3	3·1	1·5	58,000
	1	180	14	2·6 mg. β-carotene in	2	100	0·9	703	62	35	1·4	2·5	5·5	23,000
	1	220	14	ten drops arachis oil	2	130	0·9	484	36	19	1·1	2·5	4·8	11,200
2	1	290	7	Ten drops arachis oil only		1·3	0·9	1·0	11	2·5	2·1	3·2	0·7	—
	1	302	7		2	0·7	1·2	1·3	7·4	3·0	3·4	†	†	—
	1	295	7	2·6 mg. β-carotene in	2	84	1·2	587	24	130	3·4	3·5	2·0	—
	1	284	7	ten drops arachis oil	2	7·0	1·3	727	21	375	3·6	8·3	1·7	—
						Stomach + small intestine: Carotene (μg.)		Stomach + small intestine: Vitamin A (i.u.)						
3	3	140*	2	None	—	0		5·2		0	4·8	3·5	0·4	1,670
	3	150*	2	Five drops carrot oil for each rat	24	88		21		330	5·1	4·1	0·8	1,730

* Mean. † Estimation lost. A dash indicates that no analysis was made.

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Early experiments in which the contents of the intestine were removed by washing out after fixation with alcohol (p. 54) showed that after a meal of carotene more vitamin A was found in the contents than in the wall. As explained on p. 54 this method was abandoned as unsatisfactory. To make sure that in the process of separation

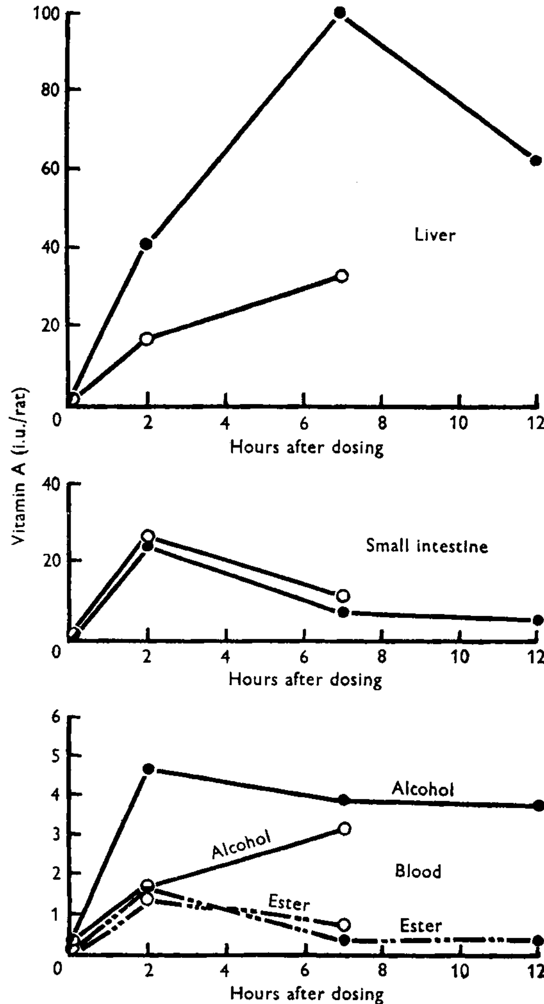

Fig. 1. The rate of appearance of vitamin A in the small intestine, blood and liver of vitamin A-deficient rats each dosed with 2·6 mg. β-carotene in 200 mg. arachis oil and killed after different intervals of time. Mean values for groups of four rats. ○—○ Exp. 1; ●—● Exp. 2.

no vitamin A was removed from the walls of the intestine, rats were intravitally fixed with 5 % formaldehyde in normal saline 2 hr. after a meal of carotene, and the contents were washed out with normal saline in the way described by Frazer (1947) for fixation with Wittmaack's solution, which could not be used as it caused loss of vitamin A (Table 11). The results are shown in Table 11. A large proportion of the vitamin A was found in the wall; nevertheless, about one-quarter of the total quantity was present in the contents. We have found this proportion consistently in the numerous experiments

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Table 10. *Appearance of vitamin A and carotene in different organs of normal bacon pigs given a meal of carotene and killed 3 or 7 hr. later*

Exp. no.	Dose	Pig no.	Time between dosing and slaughter (hr.)	Blood, Vitamin A (i.u./100 ml.), Before dosing, A	Blood, Before dosing, E	Blood, After dosing, 3 hr., A	Blood, After dosing, 3 hr., E	Blood, After dosing, 7 hr., A	Blood, After dosing, 7 hr., E	Stomach, Wall, Vit. A (i.u./g.)	Stomach, Wall, Carotene (μg./g.)	Stomach, Contents, Vit. A (i.u./g.)	Stomach, Contents, Carotene (μg./g.)	Small intestine, Wall, Vit. A (i.u./g.)	Small intestine, Wall, Carotene (μg./g.)	Small intestine, Contents, Vit. A (i.u./g.)	Small intestine, Contents, Carotene (μg./g.)	Lymphatics Vit. A (i.u./g.)	Kidneys Vit. A (i.u./g.)	Pancreas Vit. A (i.u./g.)	Gall Vit. A (i.u./g.)	Liver Vit. A (i.u./g
1	20 g. arachis oil only	1	7	—	—	—	—	84	1·6	0·2	—	0·2	0·2	0·2	—	0·3	0	0·5	—	—	—	—
	20 g. carotene concentrate containing 3·5 % β-carotene	2	7	—	—	—	—	112	10·3	0·2	—	1·6*	250	1·0	—	3·1	5·1	4·8	—	—	—	—
2	20 g. arachis oil only	3	7	50	1·0	—	—	50	1·0	—	—	—	—	0·3	0·1	0·1	0·1	0·4	1·4	0·2	0·1	57
	20 g. arachis oil containing 600 mg. β-carotene	4	3	57	2·1	60	5·0	—	—	—	—	0·2	100	0·6	0·1	0·6	3·1	2·0	1·8	0·3	0·1	39
		5	7	43	2·0	46	6·0	46	7·2	—	—	0·4	160	2·0	0·3	1·1	22	2·2	1·0	0·3	0·1	62

A = alcohol; E = ester. * Probably artifact. A dash indicates that no analysis was made.

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Table 11. *Vitamin A and carotene in the walls and contents of the small intestine of rats. Comparison of values obtained with and without intravital fixation of the animals. Results for eight treated and six untreated rats 2 hr. after dosing with 2 or 4 mg. β-carotene in arachis oil*

		Intestinal wall				Intestinal contents				Total/organ	
		Vitamin A/organ		Carotene/organ		Vitamin A/organ		Carotene/organ			
Fixative used	Dose of carotene (mg.)	(i.u.)	Percentage of total	(μg.)	Percentage of total	(i.u.)	Percentage of total	(μg.)	Percentage of total	Vitamin A (i.u.)	Carotene (μg.)
None	4*	20	70	88	9	9	30	840	91	29	928
	4*	48	69	82	6	22	31	1320	94	70	1402
	4*	34	62	95	5	22	38	1880	95	55	1975
	4*	40	63	81	4	23	37	2184	96	63	2265
	2·6†	18	78	37	2	5	22	1700	98	23	1737
	2·6†	18	62	20	1	11	38	1600	99	29	1620
Formol saline	4*	38	84	88	7	7	16	1178	93	45	1266
	4*	41	72	87	7	16	28	1116	93	57	1203
	4*	77	88	153	8	10	12	1584	92	87	1737
	4*	54	80	171	10	13	20	1612	90	67	1783
	2·6†	26	76	116	8	8	24	1400	92	34	1516
	2·6†	19	77	79	23	3	23	260	77	22	339
Wittmaack's solution	2·6†	11	61	30	4	7	39	790	96	18	820
	2·6†	6	75	200	26	2	25	570	74	8	770
Mean for unfixed intestine		30	67	67	5	15	33	1587	95	45	1654
Mean for fixed intestine		34	77	116	11	8	23	1064	89	42	1180

* Vitamin A-deficient rats.
† Stock-colony rats kept for 1 week on the vitamin A-deficient diet.

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in which intestines were fixed in this way before the contents were removed. Table 11 shows also that washing out with normal saline without the preliminary intravital fixation strips off from the wall only a relatively small proportion of vitamin A. We have, therefore, used this method in routine work.

Examination of different portions of the small intestine of the rat

Subdivision into four equal parts. Vitamin A-deficient rats received 4 mg. of β-carotene as solution in arachis oil, control rats received twenty drops of arachis oil only and all were killed 2 hr. after dosing. The small intestine was arbitrarily divided into four parts

Table 12. *Concentration of vitamin A and carotene in different segments of the small intestine of vitamin A-deficient rats 2 hr. after dosing with 4 mg. β-carotene in 400 mg. arachis oil*

			Intestinal wall		Intestinal contents	
Treatment	Rat no.	Quarter of the small intestine*	Vitamin A (i.u./quarter)	Carotene (μg./quarter)	Vitamin A (i.u./quarter)	Carotene (μg./quarter)
Undosed	1	1	2·8	3·0	0·5	6·5
		2	4·0	0	0·8	4·5
		3	2·0	0	0·8	8·5
		4	2·4	0	0·8	6·5
Dosed	2	1	6·0	4·0	4·8	35
		2	11	10	8·4	83
		3	2·5	3·0	6·6	454
		4	1·8	4·0	4·8	950
	3	1	5·2	5·0	3·6	41
		2	14	29	14	360
		3	3·6	28	6·7	1475
		4	1·8	2·0	4·0	885
	4	1	4·0	1·0	6·4	43
		2	10	20	16	208
		3	5·6	21	6·8	550
		4	2·0	18	4·0	949

* Starting from stomach.

of equal length. The contents of each part were separately washed out with a 0·9 % solution of sodium chloride in water, and the contents and the walls were analysed separately. The results are given in Table 12.

It will be seen that in both wall and contents of the small intestine the highest concentration of vitamin A was in the second quarter. The same was true of carotene in the wall, but in the contents most of it was present in the third and fourth quarters. The carotene in the wall was, in all probability, mechanically adsorbed (p. 54).

Subdivision into eight equal parts. Vitamin A-deficient rats were each dosed with 4 mg. of β-carotene in arachis oil and killed 0·5, 1 and 2 hr. later. Control rats received arachis oil only. The intestines were divided into eight equal lengths. This time the contents were not separated from the walls. The results are given in Fig. 2.

It will be seen that the highest concentration of vitamin A was in the fourth segment, corresponding to the second segment of the previous experiment. This peak was

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independent of the time interval between dosing and killing of the rats, whereas with carotene the peak shifted towards the large intestine with the progress of time.

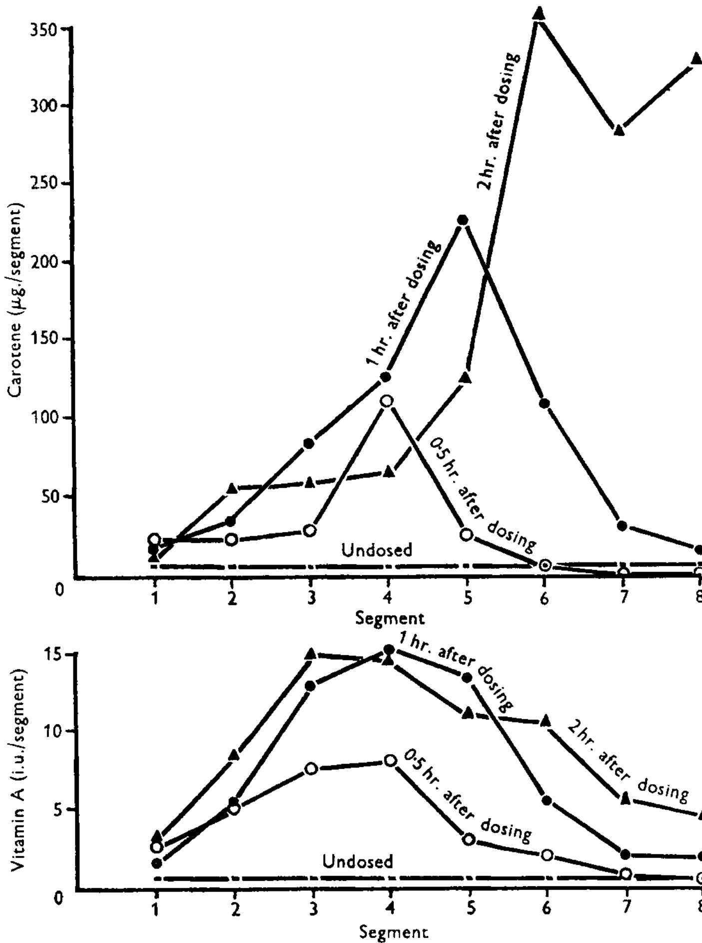

Fig. 2. The appearance of vitamin A and carotene in different segments of the small intestine of vitamin A-deficient rats 30, 60 and 120 min. after a meal of 4 mg. β-carotene in 400 mg. arachis oil mixed with 1 g. food. The 'undosed' animals received only arachis oil and food. Mean values for groups of two rats.

The time of appearance of vitamin A and carotene in different parts of the body of vitamin A-deficient rats after a meal of carotene

The experiments described on p. 52 showed that, when vitamin A and carotene were removed from the diet, the vitamin A content of the intestinal tract of rats was reduced within a few days to very small quantities.

We showed further that after a meal of carotene vitamin A appeared rapidly in the small intestine but not elsewhere in the alimentary tract of stock-colony rats prepared in this way or of rats deficient in the factor. Though this in itself suggested that conversion of carotene takes place in the intestine it remained possible that vitamin A is

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in fact produced in another part of the body and later carried to the intestine. Further experiments were, therefore, done to determine the time of appearance of vitamin A in different organs.

Groups of vitamin A-deficient rats were given carotene, and the rats were then killed at intervals for analysis of the organs. Each time undosed animals also were

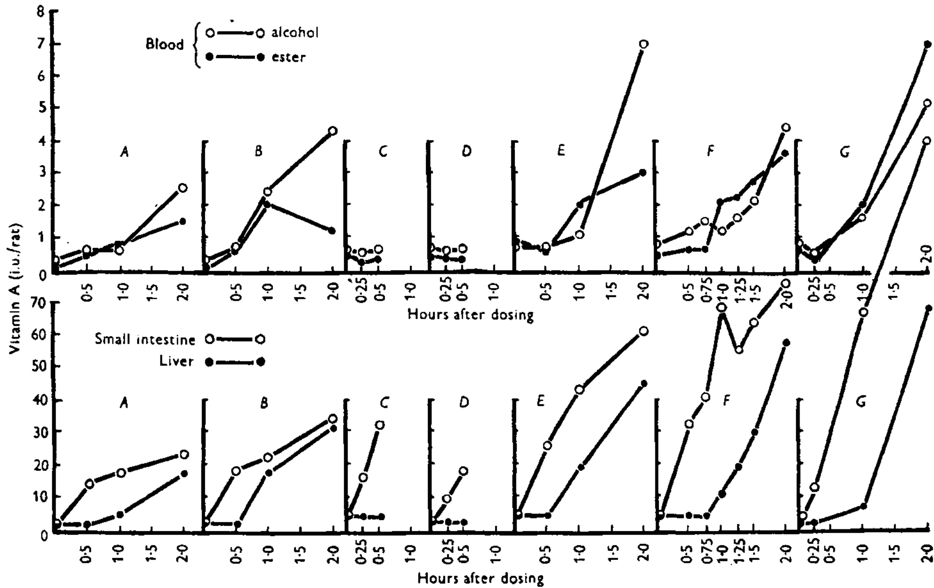

Fig. 3. The appearance of vitamin A in the wall and contents of the small intestine, in the blood and in the liver of vitamin A-deficient rats at different time intervals after a meal of carotene. Mean values for groups of (*A*) two rats dosed with 2·6 mg. β-carotene dissolved in 200 mg. arachis oil or with arachis oil alone, (*B*) two rats treated as in (*A*) but given 1 g. food with dose, (*C*) three rats given 4 mg. β-carotene in 400 mg. arachis oil or 400 mg. arachis oil alone mixed with 1 g. food, (*D, E, F, G*) two rats treated as in (*C*). In each experiment the values for time 0 are those for animals dosed with arachis oil alone.

Table 13. *Appearance of vitamin A in the wall and contents of the small intestine, in the blood and in the liver of vitamin A-deficient rats killed at intervals of* 0·5, 1 *and* 2 *hr. after a meal of carotene (Exp. E of Fig.* 3)

(Values expressed per organ and, for blood, per rat.)

Dose	Rat no.	Time after carotene feeding (hr.)	Small intestine Total vitamin A (i.u.)	Blood Alcohol (i.u.)	Blood Ester (i.u.)	Liver Total vitamin A (i.u.)
400 mg. arachis oil	1	0	3·1	0·8	1·1	2·6
	2	0	2·8	0·7	0·7	3·8
4 mg. β-carotene in 400 mg. arachis oil	3	0·5	19	0·8	0·6	3·1
	4	0·5	32	0·8	0·7	4·0
	5	1	46	1·7	2·5	24
	6	1	39	0·9	2·0	13
	7	2	59	6·0	5·0	37
	8	2	65	8·0	3·0	54

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examined. In all, seven experiments involving about fifty rats were done. The results are presented in graph form in Fig. 3, and values obtained for individual rats in one experiment are given in Table 13 as an illustration.

It will be seen that in all seven experiments the content of vitamin A in the intestine was the first to increase. A definite rise occurred within 15 min. of dosing, that is, in the shortest interval used. No increase took place in the liver within 30 min., and the first sign of a change could be noticed between 45 and 60 min. It is remarkable that

Table 14. *The amount, in relation to the size of the dose of carotene, of vitamin A found in the small intestine, blood and liver, and of carotene in the stomach and small and large intestine, of vitamin A-deficient rats, 2 hr. after a meal of carotene*

(Mean values expressed per organ and, for blood, per rat.)

Dose	No. of rats	Stomach Carotene (μg.)	Small intestine Total vitamin A (i.u.)	Small intestine Carotene (μg.)	Large intestine Carotene (μg.)	Blood Vitamin A Alcohol (i.u.)	Blood Vitamin A Ester (i.u.)	Liver Total vitamin A (i.u.)	Proportion of carotene dose recovered (%)
400 mg. arachis oil only	3	3·3	0·6	24	3·7	3·9	0·4	4·0	—
0·8 mg. β-carotene in 80 mg. arachis oil	3	99	16	155	211	10	4·3	35	58
4 mg. β-carotene in 400 mg. arachis oil	3	1447	38	1130	137	7·8	4·4	49	68

within the first 2 hr. after dosing the absolute quantity of vitamin A found in the small intestine was consistently greater than that in the liver. After longer intervals the liver reserves naturally outstripped those in the intestine. This is shown in Fig. 1. The concentration in the blood also rose more slowly after dosing than in the intestine, though probably the blood just kept ahead of the liver in this respect. At first both forms increased to about the same extent, though the relative increase was greater for the ester form. With intervals between dosing and sampling greater than 2 hr. the ratio of the two forms tended towards that normally found in blood (Fig. 1). The size of the dose did not affect the sequence of events, but with the larger dose of 4 mg. more vitamin A appeared in the organs studied than with 2·6 mg. The same relationship between size of dose and quantity of vitamin A recovered was observed also in simultaneous experiments with different doses. This is shown in Table 14.

Appearance and distribution of vitamin A alcohol and vitamin A ester in different organs

In the vitamin A-deficient rat after a meal of carotene. Table 15 shows the appearance of vitamin A alcohol and of vitamin A ester in the wall and in the contents of the small intestine, in the blood and in the liver. The intestinal contents were separated from the wall by washing out with normal saline as described on p. 52. In agreement with observations already described (p. 62) about 30 % of the vitamin A present in the gut was found in the contents. Vitamin A was there mainly in the alcohol form, whereas in the wall the ester form eventually predominated. The amounts present increased rapidly between the first appearance 15 min. after dosing and the last reading at 2 hr. In the blood the usual increase took place at 1 hr., the ester form gaining relatively more. The first increase in the liver stores took place also after 1 hr. with both forms

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present in nearly equal amounts. After 2 hr., however, there was more of the ester form, the normal storage form in the liver.

So far, with the exception of those in Table 15, values for the content of vitamin A alcohol and ester have been quoted only for blood. Though in most experiments the two forms of vitamin A were measured also in the intestine and in the liver, only the

Table 15. *The appearance of vitamin A alcohol and ester in the wall and contents of the small intestine, the blood and the liver of vitamin A-deficient rats* 15 *min.–2 hr. after a meal of carotene*

(Values expressed per organ and, for blood, per rat.)

			Small intestine							
		Time after carotene	Wall		Contents		Blood		Liver	
Dose	Rat no.	feeding (min.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u.)	Ester (i.u.)	Alcohol (i.u.)	Ester (i.u.)
400 mg. arachis oil	1	0	1·2	1·0	0·8	1·6	1·0	0·6	1·6	1·4
	2	0	1·2	0·8	1·0	0·8	0·7	0·7	1·8	1·2
4 mg. β-carotene in 400 mg. arachis oil	3	15	3·8	2·2	2·4	1·3	0·5	0·4	1·6	1·4
	4	15	6·0	4·0	4·8	2·4	0·6	0·3	1·4	1·2
	5	60	24	24	10	3·2	1·2	2·0	4·0	4·4
	6	60	29	32	10	2·0	2·0	2·0	2·6	2·8
	7	120	32	48	21	9·6	3·4	7·0	25	30
	8	120	43	51	26	8·0	7·0	7·0	26	52

total vitamin A was given for these organs. One reason was that on those occasions when the alkali-digestion method was used, the proportion of ester to alcohol was distorted by partial saponification (see p. 55). Another reason was to simplify the presentation of already complicated data. If the values for wall and contents given in Table 15 are added together, it will be seen that both forms of vitamin A were present in roughly equal amounts. The two forms were measured in the intestine simultaneously, by the solvent-extraction method, on thirty-nine occasions. Though the conditions and the length of time between dosing and killing varied from experiment to experiment, in each experiment the two values were strictly comparable and the findings are given in Fig. 4. On the average the ester form amounted to 43 % and the alcohol form to 57 % of the total, but the paired t test of 'Student' (1908, 1925) showed that the difference was not significant in the conventional sense ($P = 0{\cdot}06$). It is evident from Fig. 4 that the proportion of alcohol to ester was not dependent on the time which elapsed between dosing and killing.

The appearance of both forms of vitamin A in the liver after a meal of carotene is shown in Fig. 4. As would be expected the proportion of ester to alcohol increased with the time elapsing between dosing and killing. Whereas roughly equal quantities of the two forms were found shortly after dosing, much more ester was found after longer intervals.

In the vitamin A-deficient rat after a meal of vitamin A alcohol or vitamin A ester. Table 16 shows the appearance of the two forms of vitamin A in different organs of deficient rats 0·5–4 hr. after a meal of vitamin A alcohol, and Table 17 similar findings after a meal of vitamin A ester.

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In the stomach vitamin A was present almost exclusively in the form in which it was fed, and there was no evidence of esterification or hydrolysis, as the case may be, for up to 4 hr. after dosing. As would be expected, the total quantity of either form present in the stomach decreased gradually. In the contents of the small intestine both forms were present, the form fed clearly preponderating, but in the wall the ester and alcohol were found in very nearly equal amounts irrespective of the form consumed. The

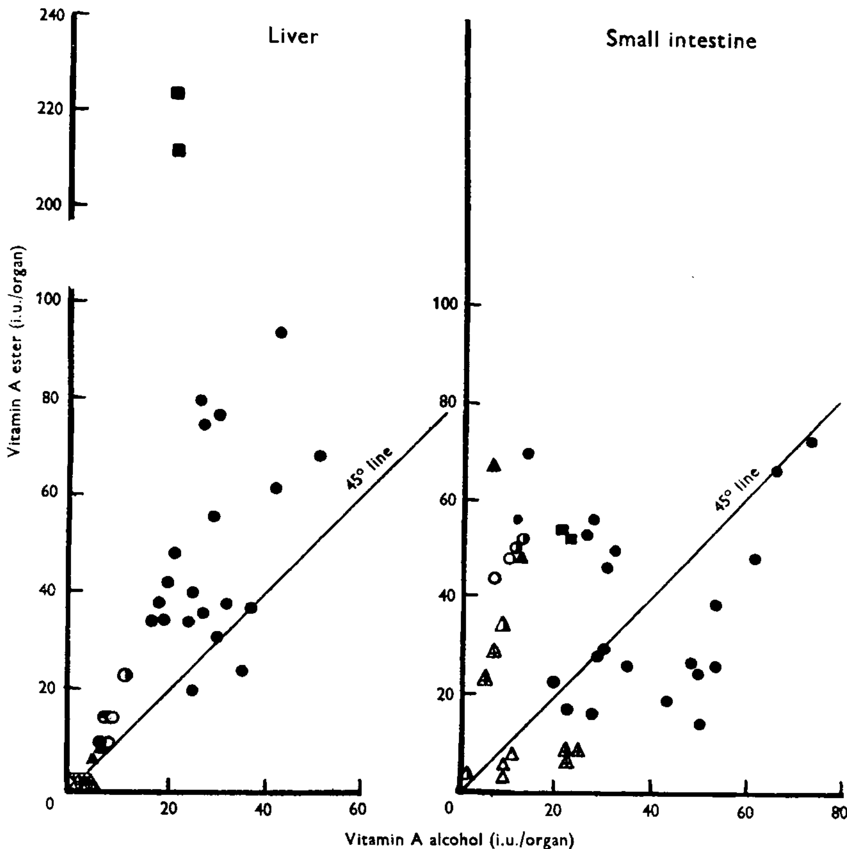

Fig. 4. The relation between the content of vitamin A alcohol and vitamin A ester in the small intestine and liver of the vitamin A-deficient rat at different stages after a meal of 4 mg. β-carotene in 400 mg. arachis oil mixed with 1 g. food. △ ¼ hr., ◬ ½ hr., ▲ ¾ hr., ▲ 1 hr., ○ 1¼ hr., ◐ 1½ hr., ● 2 hr., ■ 3 hr.

quantities present varied but little 0·5–4 hr. after feeding. No vitamin A was detected in the large intestine in either experiment. In blood both forms increased markedly within 2·5 hr. after dosing, and the effects were very similar in the two experiments. In the liver more ester was deposited than alcohol, whatever the form eaten by the animal.

In normal bacon pigs after a meal of vitamin A ester. Large White baconers received at intervals before slaughter fish-liver oil mixed with a small quantity of food. The results of the analysis of blood and lymphatics are given in Table 18. As many mesenteric lymph nodes as possible were collected and carefully separated from the

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Table 16. *The appearance of vitamin A alcohol and ester in the stomach, small intestine, large intestine, blood and liver of vitamin A-deficient rats 0·5–4 hr. after a meal of vitamin A alcohol*

(Values expressed per organ and, for blood, per rat.)

Dose	Rat no.	Time after vitamin A feeding (hr.)	Stomach Alcohol (i.u.)	Stomach Ester (i.u.)	Small intestine Wall Alcohol (i.u.)	Small intestine Wall Ester (i.u.)	Small intestine Contents Alcohol (i.u.)	Small intestine Contents Ester (i.u.)	Large intestine Total vitamin A (i.u.)	Blood Alcohol (i.u.)	Blood Ester (i.u.)	Liver Alcohol (i.u.)	Liver Ester (i.u.)	Proportion of vitamin A dose recovered (%)
400 mg. arachis oil	1	0	2·0	1·3	1·3	3·2	1·4	1·2	1·4	0·5	0·2	1·0	2·0	—
400 mg. arachis oil containing 500 i.u. vitamin A alcohol	2	0·5	218	2·4	17	6·6	14	2·4	1·9	0·6	0·6	2·0	3·2	54
	3	0·5	294	2·8	7·0	4·4	10	4·0	1·4	0·6	0·6	1·4	2·8	66
	4	1	270	2·6	14	10	7·0	1·6	1·1	0·7	1·0	4·0	4·2	63
	5	1	236	2·3	16	8·4	13	4·4	1·1	0·8	1·2	2·4	4·0	58
	6	2·5	199	1·4	12	10	15	6·0	1·6	4·0	6·0	9·1	25	58
	7	2·5	147	3·0	17	14	16	10	1·6	5·0	6·0	18	26	53
	8	4	122	2·1	10	10	13	3·2	1·9	7·0	3·5	11	27	42
	9	4	86	1·4	14	18	14	3·6	2·4	7·0	4·0	6·0	45	40

Table 17. *The appearance of vitamin A alcohol and ester in the stomach, small intestine, large intestine, blood and liver of vitamin A-deficient rats 0·5–4 hr. after a meal of vitamin A ester*

(Values expressed per organ and, for blood, per rat.)

Dose	Rat no.	Time after vitamin A feeding (hr.)	Stomach Alcohol (i.u.)	Stomach Ester (i.u.)	Small intestine Wall Alcohol (i.u.)	Small intestine Wall Ester (i.u.)	Small intestine Contents Alcohol (i.u.)	Small intestine Contents Ester (i.u.)	Large intestine Total vitamin A (i.u.)	Blood Alcohol (i.u.)	Blood Ester (i.u.)	Liver Alcohol (i.u.)	Liver Ester (i.u.)	Proportion of vitamin A dose recovered (%)
400 mg. arachis oil	1	0	1·0	2·1	2·0	2·0	0·9	0·7	1·6	1·0	0·2	2·0	2·1	—
500 i.u. vitamin A ester as fish-liver oil in 400 mg. arachis oil	2	0·5	4·3	294	10	10	14	18	1·4	1·5	0·5	2·0	4·3	72
	3	0·5	3·5	320	8·4	10	8·8	7·6	0·9	1·4	0·7	2·0	3·1	73
	4	1	3·2	187	14	13	12	15	1·4	2·0	1·0	2·8	7·0	52
	5	1	4·0	221	14	18	6·4	7·6	1·6	1·5	0·5	2·8	6·4	57
	6	2·5	2·8	50	11	16	18	27	1·6	8·0	2·3	18	30	37
	7	2·5	2·1	60	15	19	4·8	7·9	2·0	6·2	2·0	12	26	31
	8	4	2·3	44	15	15	18	22	1·4	9·0	4·0	17	37	37
	9	4	3·6	94	14	12	9·6	10	1·2	6·0	3·0	12	32	39

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adhering fat. After mincing and blending in a Waring Blendor, 10 g. portions were taken for analysis by the method described for liver on p. 54, but the vitamin A ester was determined as the difference between the total value for vitamin A obtained after saponification and that for vitamin A alcohol measured on the non-saponifiable, light-petroleum extract of another 10 g. portion of the lymph nodes. A recovery test with added vitamin A alcohol showed that the fat present in the extract (0·2–0·5 g./10 g. of

Table 18. *The appearance of vitamin A in the blood and mesenteric lymph nodes of bacon pigs after the administration of 50,000 i.u./pig of vitamin A ester in the form of fish-liver oil*

		Blood Vitamin A/100 ml. plasma		Lymphatics			
						Vitamin A/10 g. tissue	
Pig no.	Time after feeding (min.)	Alcohol (i.u.)	Ester (i.u.)	Total weight of lymphatics collected from individual pigs (g.)	Fat/10 g. tissue (g.)	Alcohol (i.u.)	Ester (i.u.)
1	0	51	2·5	89	0·340	1·4	1·8
2		66	4·0	55	0·346	1·6	1·0
3	75	66	2·0	110	0·257	1·8	2·0
4		69	2·5	92	0·228	2·0	2·4
5	90	62	2·5	94	0·360	2·4	2·3
6		62	3·5	75	0·415	2·2	1·8
7	105	60	13	46	0·470	4·8	11
8		69	8·5	66	0·381	3·6	8·3
9	120	50	9·0	87	0·330	4·9	17
10		61	13	50	0·504	6·4	15
11	150	60	11	48	0·397	5·6	16
12		51	19	61	0·466	7·6	34

tissue) did not interfere with the chromatography. The consumption of vitamin A ester by the pigs resulted in the simultaneous and progressive increase, after 105 min., of the ester fraction of vitamin A in the lymph nodes and circulating blood. There was also some slight increase in the vitamin A alcohol in the lymphatics.

Demonstration of vitamin A in organs by fluorescence in ultraviolet light

Vitamin A fluoresces in ultraviolet light, and the property has been repeatedly used for its demonstration in tissues. Popper (1941) has notably developed the technique, and it proved useful in our hands in following the intestinal conversion of carotene to vitamin A. Some vitamin A-deficient rats were dosed with carotene in the usual way, and others were dosed with vitamin A ester as fish-liver oil, or received arachis oil only. They were killed in trios at intervals after feeding, suitably dissected, and examined and photographed in ultraviolet light (Pl. 1).

Half an hour after feeding, the mesenteric lymph ducts of rats of all three types showed by their milky-white appearance in visible light that active fat absorption was taking place (Pl. 1). In the rat dosed with carotene, carotene could be seen to have travelled almost to the caecum. In the rat given vitamin A, the stomach, small intestine and mesenteric lymph duct, but not the thoracic duct, showed the characteristic

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yellow fluorescence in ultraviolet light. The appearance of the rat given carotene was similar except that the stomach did not fluoresce. Two hours after dosing, vitamin A was present in the sites already mentioned and, in addition, was plainly visible in the thoracic duct.

The small intestine of one of the rats given carotene was frozen 2 hr. after dosing and cut in half longitudinally. Inspection under ultraviolet light showed vitamin A fluorescence starting at the point of entry of the bile and pancreatic ducts, becoming gradually brighter in the next 5 cm. and then decreasing again.

Incubation experiments with whole bodies of rats

Attempts were made to obtain conversion of carotene to vitamin A by incubating at 37° for 3 hr. the whole bodies of rats killed 0·5 hr. after the administration of carotene in arachis oil. The livers were removed immediately after killing. No increase in the vitamin A content of the small intestine was detected; on the contrary, the amounts found were less than in similar rats analysed immediately after killing.

Perfusion experiments with intestines of rats and pigs

Several attempts were made to obtain conversion of carotene in the surviving isolated intestine of rats and pigs, perfused with Ringer-Locke solution. These were uniformly unsuccessful, though on occasions the intestine was kept alive for up to 3 hr. The failure may have been due, partly at least, to the effect of anaesthetics.

Effect of anaesthetics on the intestinal conversion of carotene

It was planned to obtain frequent samples of portal and systemic blood from pigs after a meal of carotene to study the appearance of vitamin A in the blood and the possible transfer of carotene to the liver. Preliminary experiments showed, however, no conversion of carotene to vitamin A in the intestine of a pig maintained for 6 hr. after a carotene meal under cyclopropane anaesthesia, whereas abundant quantities appeared 6 hr. after a similar meal in the intestine of a non-anaesthetized pig.

Rats also, when placed under soluble pentobarbitone (Nembutal, Abbott Laboratories) anaesthetic, did not convert carotene to vitamin A in the intestine within 2 hr. of dosing, though in non-anaesthetized rats conversion was evident within 15 min. (see p. 68).

This inhibitory effect of anaesthetics made it likely that nervous control was involved and Prostigmin (Roche Products Ltd.), a synthetic peristaltic stimulant, and amphetamine, a sympathetic stimulant, were given to rats to see whether they could influence the conversion of carotene. These preliminary experiments indicated slight depression with amphetamine and slight enhancement with Prostigmin. Further work is planned.

DISCUSSION

Our observations showed that when a vitamin A-deficient rat was given a meal of vitamin A as either alcohol or ester, the vitamin rapidly appeared in the wall of the small intestine, but that it took something like 1–2 hr. before appreciable quantities could be detected in the liver or were found in the blood. Very much the same happened when carotene was given instead of vitamin A. Thus, vitamin A appeared in the small

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intestine of a vitamin A-deficient rat within 15 min. after a meal of carotene but could not be detected in appreciable amounts in the liver or in the blood for another 45 min. It is striking that for the first hour the total quantity present in the intestine markedly exceeded that found in the rest of the body including the liver. Later, as the interval after the meal of carotene or of vitamin A increased, the vitamin A content of the intestine gradually decreased, whereas the liver reserves continued to accumulate. On the other hand, in the absence of vitamin A or carotene from the diet the vitamin A of the small intestine soon became depleted and only traces of it could be found even in the intestine of stock-colony rats a few days after they had been placed on a vitamin A-deficient diet, despite their very high liver reserves. It is thus clear that the intestine is not a storage place for vitamin A and that the factor is found there only at the stage of active absorption. If that is so, the rapid appearance of vitamin A after a meal of carotene in the gut of the vitamin A-deficient rat can be explained only by assuming that the conversion of carotene takes place there.

The similarity between the behaviour of vitamin A arising from carotene and that administered in the preformed state went still further, as was shown by separate observation of the appearance of the esterified and alcoholic forms in different organs. Whether carotene, or vitamin A alcohol or ester was given, the sequence of events was much the same. The vitamin A present in the lymphatic system was almost exclusively in the ester form (cf. also Thompson, Braude, Cowie, Ganguly & Kon, 1949) although in the intestinal wall both forms were present side by side in roughly equal amounts. Again, irrespective of whether carotene or vitamin A alcohol or ester was given, the increase in the blood of vitamin A-deficient rats was at first mainly in the ester form, but the amount of the alcohol form also was built up rapidly towards the normal concentrations. With normal animals the increase was mostly in the ester form, and the alcohol form changed but little.

The early appearance of vitamin A in the lymphatic system after a meal of carotene or of vitamin A speaks strongly in favour of the intestinal conversion of carotene. It will be recalled that in the pig appreciable quantities of vitamin A could be detected in the mesenteric lymphatics at the same time as it appeared in increased quantities in the blood after administration of vitamin A, and that vitamin A was detected there also after a meal of carotene. With vitamin A-deficient rats the characteristic fluorescence of vitamin A in ultraviolet light was seen within 30 min. after a carotene meal in the mesenteric lymph nodes and ducts and, within 2 hr., the vitamin A was obviously travelling through the thoracic duct.

Carotene is not normally present in the blood or milk of the rat or pig, nor could we detect it in measurable quantities in the blood at any stage after the administration of relatively large doses of carotene by mouth; yet, if transformation into vitamin A took place in the liver, one would expect to find carotene in the blood very soon after feeding, at any rate in the portal circulation. Goodwin *et al.* (1946) found no carotene in samples of the portal blood taken through a London cannula from a sheep given carotene. Our experience with pigs, which will be reported in a separate paper, and is mentioned in the preliminary communication of Thompson *et al.* (1949), was quite similar.

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Though the bulk of the intestinal vitamin appeared in the wall of the intestine, some was found in the contents. Its presence there can be readily explained on the basis of intestinal conversion of carotene, but is much more difficult to explain if conversion in the liver is accepted. It might conceivably be carried to the lumen of the intestine with digestive juices, and especially bile, but we have found no vitamin A in pig bile after a meal of carotene.

The results of the experiments in which different segments of the small intestine of rats were analysed for vitamin A and carotene at different intervals of time after a meal of carotene also support the view that carotene is converted in the intestine. The concentration of vitamin A was consistently highest in the middle portion of the small intestine, whereas the wave of concentration of carotene moved with time along the small intestine and into the caecum. This peak of concentration of vitamin A in the intestine indicates probably the main seat of conversion, and it would seem far-fetched to assume that the carotene has to be taken into the liver to be then returned preferentially as vitamin A to a circumscribed portion of the gut.

All the experiments in which we observed the appearance of vitamin A in the intestine were done, with intact animals, in the presence of the liver, and we cannot exclude the liver by direct evidence either as an organ essential to the conversion of carotene, or, for that matter, as the actual site of conversion. We believe, however, that the indirect evidence quoted by us is sufficient to build an overwhelming case in support of the hypothesis of intestinal conversion of carotene.

We are unable at present to decide whether this conversion takes place in the wall of the gut, in the contents or in both. Quantitatively, the wall seems by far the more important site, as some three-quarters of the vitamin A formed were found there, and it might be argued that what vitamin A was present in the contents was derived from the wall. It is interesting to recall that Wagner (1939) who, as already mentioned, observed the intestinal conversion of carotene to vitamin A in the whale, was convinced that the process occurred in the contents. So huge was the amount converted that large quantities of vitamin A were found even in the faeces in the rectum.*

Whatever the position, our experiments have shown that in the rat after a meal of carotene the proportion of vitamin A alcohol to ester in the contents was different from that in the wall, and that after a meal of one form of vitamin A some of the other form appeared in the contents. To explain this it is necessary to assume either that the contents have the power rapidly to esterify vitamin A alcohol and de-esterify vitamin A ester, or that the wall of the intestine is capable of even more rapid secretion into the lumen of either form of vitamin A, or that both these processes may take place simultaneously. On the evidence available we are unable to choose between these possibilities.

SUMMARY

1. Carotenoids and vitamin A alcohol and ester were extracted from animal tissues in a Waring Blendor with light petroleum as solvent in the presence of ethanol.

2. β-Carotene and the two forms of vitamin A were then separated by chromatography on alumina by a method suitable for routine work with small quantities.

* See *Note added in proof* (p. 77).

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3. When β-carotene was given by mouth to vitamin A-deficient rats, vitamin A appeared within 15 min. in the wall and contents of the small intestine, whereas the first increase in the blood and in the liver did not occur before 45–60 min. had elapsed. Up to 2 hr. after dosing there was more vitamin A in the small intestine than in the liver.

4. Of the vitamin A appearing in the intestine some 25–30 % was found in the contents when these were removed by washing out with saline after, or without, intravital fixation.

5. No vitamin A appeared in the stomach or in the large intestine, and within 15 min. to 2 hr. after dosing the highest concentration of vitamin A was in the middle of the small intestine, whereas that of carotene progressively shifted towards the caecum with the passage of time.

6. The alcohol form of vitamin A predominated in the contents of the small intestine, but in the wall the two forms were present in roughly equal quantities.

7. The rapid appearance of vitamin A in the small intestine after a meal of carotene was demonstrated also with normal pigs. In addition, a similar increase, mainly in the ester form, was noted in the mesenteric lymphatics and, by fluorescence in ultraviolet light, in the mesenteric lymph duct of the rat.

8. When stock-colony rats were given a diet deficient in vitamin A, the intestine became depleted of the factor within a few days, though the high liver stores and the amount circulating in the blood were hardly affected, indicating that the intestine of the rat is not a storage place for vitamin A.

9. When vitamin A alcohol or ester was given to vitamin A-deficient rats, the sequence of appearance of vitamin A in the intestinal wall, in the blood and in the liver were much the same as after a meal of carotene, and in the wall both forms of vitamin A appeared in roughly equal proportions, irrespective of the form fed.

10. It is concluded that the small intestine is the site of conversion of carotene to vitamin A and that the lymphatic system is a route of transport of the vitamin so formed.

We are much indebted to Prof A. C. Frazer for advice on the intravital fixation of rat intestine and the removal of contents, to Miss M. E. Coates for help in the fixation and perfusion experiments, to Dr A. T. Cowie for operations on, and to Dr R. Braude for help with, the pigs.

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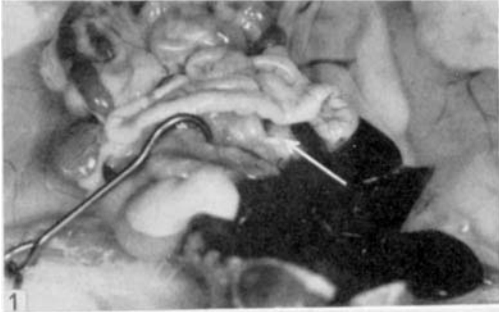
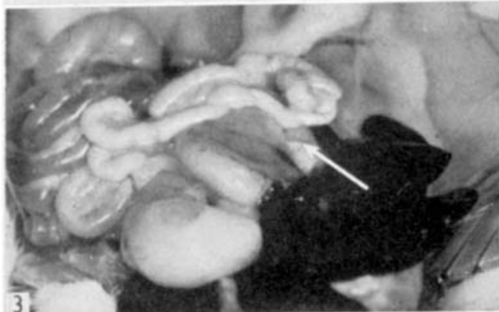
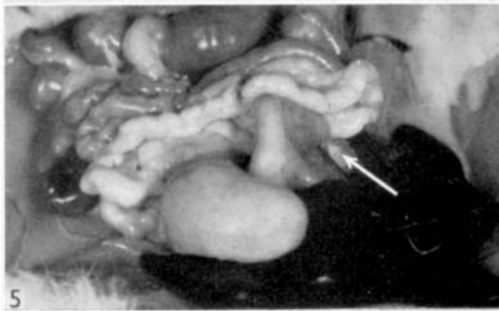
REFERENCES

Cartland, G. F. & Koch, F. C. (1928). *Amer. J. Physiol.* **85**, 540.
Davies, A. W. (1933). *Biochem. J.* **27**, 1770.
Folley, S. J., Henry, K. M. & Kon, S. K. (1947). *Brit. J. Nutrit.* **1**, 39.
Folley, S. J. Ikin, E. W., Kon, S. K. & Watson, H. M. S. (1938). *Biochem. J.* **32**, 1988.
Frazer, A. C. (1947). Private communication.
Gillam, A. E., Henry, K. M. & Kon, S. K. (1937). *Milk and Nutrition*, part 1, p. 45. Reading: National Institute for Research in Dairying.
Glover, J., Goodwin, T. W. & Morton, R. A. (1947). *Biochem. J.* **43**, xlv.

https://doi.org/10.1079/BJN19490008 Published online by Cambridge University Press







https://doi.org/10.1079/BJN19490008 Published online by Cambridge University Press

Glover, J., Goodwin, T. W. & Morton, R. A. (1948). *Biochem. J.* **43**, 512.
Goodwin, T. W., Dewar, A. D. & Gregory, R. A. (1946). *Biochem. J.* **40**, lx.
Goodwin, T. W. & Gregory, R. A. (1948). *Biochem. J.* **43**, 505.
Kimble, M. S. (1939). *J. Lab. clin. Med.* **24**, 1055.
Mattson, F. H., Mehl, J. W. & Deuel, H. J. Jr. (1947). *Arch. Biochem.* **15**, 65.
Moore, T. (1930). *Biochem. J.* **24**, 692.
Moore, T. (1931). *Biochem. J.* **25**, 275.
Popper, H. (1941). *Arch. Path. Lab. Med.* **31**, 766.
Sexton, E. L., Mehl, J. W. & Deuel, H. J. Jr. (1946). *J. Nutrit.* **31**, 299.
'Student' (1908). *Biometrika*, **6**, 1.
'Student' (1925). *Metron*, **5**, 105.
Thompson, S. Y. (1942). *Chem. & Ind.* **61**, 170.
Thompson, S. Y. (1949). *Brit. J. Nutrit.* **3**, 43.
Thompson, S. Y., Braude, R., Cowie, A. T., Ganguly, J. & Kon, S. K. (1949). *Biochem. J.* **44**, ix.
Thompson, S. Y., Ganguly, J. & Kon, S. K. (1947). *Brit. J. Nutrit.* **1**, v.
Wagner, K. H. (1939). *Vitamin A und β-Carotin des Fin-, Blau- und Spermwals.* Leipzig: Johann Ambrosius Barth.
Wiese, C. E., Mehl, J. W. & Deuel, H. J. Jr. (1947). *Arch. Biochem.* **15**, 75.

EXPLANATION OF PLATE

Photographs in visible and ultraviolet light of intestines of vitamin A-deficient rats 2 hr. after a meal of 4 mg. β-carotene in 400 mg. arachis oil or of 4000 i.u. vitamin A in 400 mg. arachis oil, or of 400 mg. arachis oil only.

1. Rat given vitamin A; visible light; the arrow points to the mesenteric lymph duct; the milky appearance indicates active absorption of fat.
2. Same rat; ultraviolet light. The fluorescence of the stomach and small intestine indicates the presence of vitamin A. The arrow points to the mesenteric lymph duct fluorescing because of the presence of vitamin A in the lymph.
3. Rat given arachis oil only; visible light. The arrow indicates active fat absorption in the lymphatics.
4. Same rat; ultraviolet light. No indication by fluorescence of vitamin A in stomach, intestine or lymphatic system.
5. Rat given β-carotene; visible light. Arrow indicates active absorption of fat in mesenteric lymph duct.
6. Same rat; ultraviolet light. No vitamin A in stomach; vitamin A present in small intestine and clearly visible in mesenteric lymph duct, indicated by arrow.

Note added in proof. Through the courtesy of Dr R. Robinson of the whaling expedition of the *Balaena* to the Antarctic we obtained recently samples of food from the stomach and samples of intestine and intestinal contents of two Fin Whales. Our findings with these specimens are sharply at variance with the observations of Wagner (1939) with whales from northern waters mentioned on p. 51.

It should be pointed out that *Euphausia superba* Dana is exclusively antarctic in distribution and that Wagner's krill must have consisted of other crustacea, probably *Meganyctiphanes norvegica*. We hope to study this species shortly to see whether we can detect in it the large quantities of β-carotene (14.5 mg./kg. krill) found by Wagner. Be that as it may, our krill, identified as *Euphausia superba* Dana contained only small traces of carotene together with the usual large quantities of astaxanthin pigments characteristic of crustacea. Preformed vitamin A, identified by chromatography, the antimony-trichloride test, absorption at 328 mμ. and liver-storage tests with rats depleted of vitamin A, was present, however, in appreciable amounts, in

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one of the whales to the extent of about 6 i.u./g. stomach contents, with smaller concentrations further down the intestine. The astaxanthin pigments were present in the intestinal contents in increasing concentration along the gut but no carotene was detected there.

Though we do not deny that with the whales caught at the Lopra station the position may have been as described by Wagner, our findings indicate that the antarctic whale at any rate may derive its vitamin A from vitamin A itself rather than from carotene. This observation may throw new light on the still baffling question of the source of the large stores of vitamin A in whale livers.

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