The conversion into vitamin A of intravenously administered aqueous dispersions of carotenoids

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During recent years various aspects of the utilization of intravenously administered aqueous dispersions of carotenoids have been investigated by several workers (e.g. Church, MacVicar, Bieri, Baker & Pope, 1954; Bieri & Pollard, 1954; Bieri, 1955; Kon, McGillivray & Thompson, 1955; McGillivray, Thompson & Worker, 1956; McGillivray & Worker, 1957, 1958; Worker, 1956*a*, *b*, 1957*a*, 1959). The way in which animals of certain species are able to utilize this highly unphysiological form of the carotenoids is, in itself, interesting, but the subject has no doubt also attracted much attention because of the light it may throw on normal intestinal conversion of orally administered carotene into vitamin A. If, as it appears to be, the mechanism is similar after oral and intravenous administration, studies by the intravenous technique may prove particularly useful in identifying those factors having a direct effect on the conversion mechanism as distinct from indirect effects that may be exerted on the absorption of carotenoids or on their stability in the digestive tract. Indeed some progress has already been made in this direction, thyroid function, for example, having been shown to exert its effect only on absorption of carotenoids (McGillivray *et al.* 1956; Worker, 1956*b*), whereas tocopherol has a direct effect on the conversion mechanism (McGillivray & Worker, 1957, 1958).

With orally administered carotene, it is now clearly established that the wall of the intestine is the main, if not the only, site of conversion into vitamin A (cf. Thompson, Ganguly & Kon, 1949). Studies with intravenously administered carotene have, however, shown that the enzyme systems involved are not necessarily located specifically in the intestinal wall. Indeed, early work on the site of conversion of intravenously administered carotene led to the conclusion that perhaps many tissues have the ability to convert carotene (Bieri & Pollard, 1954; Kon *et al.* 1955), and subsequent work has supported this view. At the same time there is a growing weight of evidence suggesting that the conversion of the carotene molecule is initiated in the blood, where there is a rapid breakdown to an intermediate, which is then taken up by the tissues and more slowly converted into vitamin A (McGillivray & Worker, 1958). Unlike the intestinal wall, most tissues lack the ability to esterify this vitamin A, which is released into the blood in the alcohol form. It is then taken up by the liver and esterified in the normal way. The mechanism of the conversion has not yet been

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elucidated, but the evidence so far available is not inconsistent with the view that the pathway could be similar to that suggested by Glover & Redfearn (1954). The initial breakdown in the blood could be a type of β-oxidation, through a series of carotenals to a substance such as retinene, which Glover, Goodwin & Morton (1948) have shown can be readily converted into vitamin A in the tissues.

Recently Pollard & Bieri (1958) have extended earlier observations (Kon *et al.* 1955) on the rapid destruction of vitamin A from intravenously administered aqueous dispersions. They have shown that the destruction takes place in the blood and consider that the reticulocytes may be involved. As they suggest, it is possible that when the carotene is present as an aqueous dispersion, the molecule may be attacked in the same way. If the conversion of intravenously administered carotene into vitamin A is initiated in the blood, it is significant that it is only during the period immediately after injection that this type of breakdown occurs and that 15 min or so after injection, although high levels of carotene remain in the blood, this material is no longer susceptible to the type of breakdown that leads to the formation of vitamin A. McGillivray & Worker (1958) suggest that the explanation may lie in the rapid change in the physical state of the carotene after injection.

The study reported here was undertaken to investigate further the site of conversion of intravenously administered carotene into vitamin A and the mechanism of the reaction involved, particularly changes that may occur immediately after injection of the aqueous dispersions into the blood stream and factors that may influence these changes. A preliminary report on this work has already been published (McGillivray, 1960).

EXPERIMENTAL AND RESULTS

General. The materials and methods used were essentially as described by Kon *et al.* (1955), with the modifications discussed by McGillivray *et al.* (1956).

Aqueous dispersions of carotenoids. Except when other Tweens were used, these were all prepared with 20% (v/v) solutions of Tween 40 (polyoxyethylenesorbitan monopalmitate, Atlas Powder Co., Wilmington, Delaware), as described by Bieri & Pollard (1954) and as used in our previous investigations. β-Carotene was obtained from L. Light and Co. Ltd; α-tocopheryl acetate from Glaxo Laboratories (N.Z.) Ltd; the carotenals from Hoffmann-La Roche and Co., and methyl linoleate from the Hormel Institute, University of Minnesota; retinene was prepared from vitamin A alcohol (L. Light and Co. Ltd) by the method of Ball, Goodwin & Morton (1948). The 2,4-dinitrophenylhydrazones were prepared by the standard method, as used by Ball *et al.* (1948) for the preparation of the retinene derivative. Substances to be administered simultaneously with carotenoids were mixed with the carotenoid before preparation of the dispersion.

Rats. The rats were albinos of a Wistar strain rendered partially deficient in vitamin A, as described previously (McGillivray *et al.* 1956). In an attempt to reduce tissue levels of tocopherol, wheat germ was omitted for 2–3 months from the basal diet of the rats to be used for studying the effect of tocopherol on the conversion of carotene into vitamin A. Unless otherwise stated, all injections were

into the jugular vein exposed by ventral neck incision of animals under diethyl-ether anaesthesia.

Determination of vitamin A, carotene and tocopherol. Carotene and vitamin A were determined in individual samples of blood and tissue as described by Thompson *et al.* (1949) and McGillivray & Worker (1957). Tocopherol was determined by the method of Worker (1957*b*).

Determination of fatty acids and esters. Blood samples were extracted with an alcohol–diethyl-ether mixture, as described by Stern & Shapiro (1953), but with ten times the quantities of blood and alcohol–ether mixture. A portion of this extract was used for colorimetric determinations of esters, as described by these workers. Free fatty acids were determined in the remainder of the extract by direct titration with 0·02N alkali.

Electrophoretic measurements. Electrophoretic measurements were carried out by the standard technique in an LKB (Produkter Fabriksaktiebolag, Sweden) moving-boundary type electrophoresis apparatus.

Effect of tocopherol in blood and tissues. In previous work on the effect of tocopherol on the conversion of carotene into vitamin A, various blood levels of tocopherol were achieved by the intravenous injection of aqueous dispersions of tocopherol (McGillivray & Worker, 1958). This tocopherol also accumulated rapidly in certain other tissues, giving levels closely parallel with blood levels, so that the inhibitory effect of the tocopherol on the conversion mechanism could not be used to distinguish unequivocally between the blood and these tissues as possible sites of conversion. However, preliminary experiments showed that the relative levels of tocopherol in different tissues at various times after dosing of rats with tocopherol depended on the mode of administration, and in this investigation it was found possible to achieve a wide range of blood tocopherol levels bearing little or no relation to the corresponding tissue levels. Rats were dosed orally or intramuscularly with various amounts of α-tocopheryl acetate in arachis oil or intravenously or intraperitoneally with an aqueous dispersion of α-tocopheryl acetate in 20% Tween 40. These animals were then killed at various times after dosing, and tocopherol was determined in blood, liver, kidneys and lungs. Other rats were then similarly dosed with tocopherol and at appropriate intervals after dosing were injected intravenously with 400 μg β-carotene as an aqueous dispersion in 0·4 ml 20% Tween 40. The animals were slaughtered 24 h after the carotene injection, and vitamin A in their livers was determined. The range of tocopherol levels found in their tissues at the time of carotene injection is shown in Table 1, together with the amounts of vitamin A present in the livers 24 h after carotene injection. Twenty different levels of blood and tissue tocopherol were obtained within the ranges shown. Each tocopherol value is a mean for two rats, and each vitamin A value is a mean for three rats. It is apparent that only for blood tocopherol levels was there any statistically significant correlation with vitamin A formation.

Effect of reticulocytosis. As described by Pollard & Bieri (1958) in their work on the rate of destruction of vitamin A in the blood, reticulocytosis was induced in a group of 2- to 3-month-old rats by injecting 8 mg neutralized phenylhydrazine hydro-

chloride into each rat on each of 2 alternate days (Wintrobe, 1956). Seven days after the last injection the rats were injected intravenously with an aqueous dispersion of β-carotene in the normal manner. Liver and blood levels of vitamin A ½ and 24 h after this carotene injection are shown in Table 2 (Expt 1). Although the differences in the total amount of vitamin A were not great, it would seem that the phenylhydrazine treatment resulted in slightly greater conversion of carotene into vitamin A.

Table 1. *Effect of level of tocopherol in blood and tissues of rats at time of injection of 400 μg β-carotene as aqueous dispersion in Tween 40 on vitamin A storage in the liver 24 h later*

Vitamin	Range	Correlation coefficient (*r*) for vitamin A with tocopherol
Vitamin A† (total)	11–35 μg	—
Tocopherol‡		
Blood	0·09–12·2 mg	−0·72**
Liver	0·10–9·2 mg	−0·20 N.S.
Lungs	2·5–700 μg	−0·41 N.S.
Kidneys	8·5–110 μg	−0·25 N.S.

N.S., not significant at 5 % level.
** Highly significant (1 % level).
† Each value is a mean for three rats.
‡ Each value is a mean for two rats.

The breakdown of intravenously administered Tween and changes in the physical form of dispersed carotene. In attempts to follow the rate of metabolism of Tween after intravenous administration, no suitable analytical method specific for Tween could be found. However, preliminary experiments with physical methods, such as surface-tension measurements, did indicate a fairly rapid breakdown of the Tween. Hydrolysis appeared a likely reaction, and free fatty acids were therefore determined in the blood of rats killed at various times after the injection of 20% aqueous solutions of Tween 40. Mean results for a number of such determinations are shown in Table 3 and indicate a rapid hydrolysis of the Tween, more than four-fifths of the dose usually being broken down in the first ½ h. It is also apparent that the rate of Tween breakdown greatly exceeded the rate of carotene destruction. That the reaction takes place in the blood was confirmed by in vitro experiments in which Tween solutions were incubated with whole blood (Table 4).

The hydrolysed Tween is apparently no longer able to hold the carotene in dispersion, and electrophoretic studies on blood serum after injections of aqueous dispersions of carotene in Tween 40 revealed a change in the physical form of the carotene soon after injection. Carotene in aqueous dispersion, in the absence of blood serum, showed no movement in the electrical field. In contrast, the carotene in the blood serum, obtained from rats after the injection of an aqueous dispersion of carotene, showed some movement with the serum proteins. As soon after injection as blood samples could be withdrawn (about 2 min), it was found that a small proportion of the carotene present had moved with the proteins, the bulk remaining at the starting

Table 2. *Effect of reticulocytosis induced by phenylhydrazine (Expt 1) and of various Tweens used as dispersing agents (Expt 2) on the utilization of aqueous β-carotene dispersions (0·4 ml) injected intravenously into female rats of mean weight 210 g*

Expt no.	No. of rats	Preliminary treatment	Dose: Vehicle	Dose: Carotene injected (μg)	Time between injection and killing (h)	Blood plasma: Vitamin A alcohol (μg/100 ml)	Blood plasma: Carotene (μg/100 ml)	Liver: Vitamin A: Alcohol (μg)	Liver: Vitamin A: Ester (μg)	Liver: Vitamin A: Total (μg)	Liver: Carotene (μg)
1	2	None	—	—	—	12	—	0·4	0·6	1·0	—
	4	None	Tween 40	400	½	27	3600	4·7	1·2	5·9	77
	4	None	Tween 40	400	24	14	17	5·8	22	28	65
	2	Subcutaneous injections of phenylhydrazine	Tween 40	—	—	11	—	0·3	0·6	0·9	—
	4	Subcutaneous injections of phenylhydrazine	Tween 40	400	½	43	3200	8·1	1·1	9·2	69
	4	Subcutaneous injections of phenylhydrazine	Tween 40	400	24	13	15	6·0	31	37	60
2	2	None	Tween 20	400	24	14	17	5·5	25	31	62
	4	None	Tween 40	400	24	12	19	5·6	19	25	38
	4	None	Tween 60	400	24	13	21	7·4	12	19	28
	2	None	Tween 80	400	24	13	29	6·2	21	27	50

boundary. By ½ h after injection virtually the whole of the carotene present was found to move with the proteins. It appeared that more than one protein component carried a portion of the carotene (or possibly similarly coloured breakdown products), but that most of it was associated with β-globulins, a clearly defined coloured boundary being apparent in this region of the pattern.

Table 3. *Mean amounts of free fatty acid, fatty-acid ester and carotene in the blood of rats at various intervals after intravenous injection of an aqueous dispersion of carotene in Tween* 40

(All injections were made on the basis of body-weight and were calculated to be equivalent to approximately 1 m-equiv. Tween 40/100 ml whole blood)

	Controls		Amount at time after injection of			
	Blood alone	Tween solution + blood*	1/12 h	¼ h	½ h	1 h
Free fatty acid (m-equiv./100 ml)	0·14	0·14	0·29	0·54	0·23	0·14
Fatty-acid ester (m-equiv./100 ml)	0·62	1·63	1·47	1·00	0·81	0·72
Excess free acid (m-equiv./100 ml.) (presumably from Tween)	0	0	0·15	0·40	0·09	0
Excess ester (m-equiv./100 ml) (presumably Tween)	0	1·01	0·85	0·38	0·19	0·10
Excess acid + excess ester (m-equiv./100 ml)	0	1·01	1·00	0·78	0·28	0·10
Unchanged Tween (%)	0	100	84	38	19	10
Carotene (μg/100 ml)	0	4500	4150	3210	2600	1850
Unchanged carotene (%)	0	100	92	72	58	41

* Values in this column were calculated on the assumption that a rat contains 6·7 ml blood/100 g body-weight (Cartland & Koch, 1928).

Table 4. *Free fatty acid and fatty-acid ester (m-equiv./100 ml whole blood) produced by in vitro hydrolysis of Tween* 40 *solutions incubated at* 37° *with rat's whole blood*

(Concentration of Tween 40 was approximately 1 m-equiv./100 ml whole blood)

	Controls		Amount after incubation for			
	Blood alone	Tween solution + blood	¼ h	½ h	1 h	3 h
Free fatty acid	0·10	0·08	0·30	0·35	0·45	0·55
Fatty-acid ester	0·50	1·40	1·15	1·05	0·95	0·80
Excess free acid, presumably from Tween	0	0	0·20	0·25	0·35	0·45
Excess ester, presumably Tween	0	0·90	0·65	0·55	0·45	0·30
Excess acid + excess ester	0	0·90	0·85	0·80	0·80	0·75
Unchanged Tween (%)	0	100	72	61	50	33

Different rates of hydrolysis of the Tweens may explain the differences in liver storage of vitamin A resulting from the injection of carotene dispersions in the various Tweens (Table 2, Expt 2), Tween 60 (polyoxyethylene sorbitan monostearate) in particular giving significantly lower results than the other Tweens.

Conversion of carotenals into vitamin A. Table 5, Expt 1, shows the effect of in-

jecting dispersions of various carotenals. With the C_{30}, C_{27} and C_{25} carotenals (β-apo-8′-, 10′- and 12′-carotenals) the vitamin A liver storage 24 h after injection was less than from the same quantity of β-carotene. The C_{25} carotenal was also less efficiently utilized than the C_{27} and C_{30}. As with β-carotene, the simultaneous injection of

Table 5. *Utilization of aqueous dispersions in Tween 40 of β-carotene and of various carotenals and their hydrazones injected intravenously (400 μg of each substance in 0·4 ml aqueous dispersion) into male rats of mean weight 250 g*

Expt no.	No. of rats	Dose	Time between injection and killing (h)	Blood plasma vitamin A alcohol (μg/100 ml)	Liver vitamin A		
					Alcohol (μg)	Ester (μg)	Total (μg)
Control	2	—	—	10	0·3	0·5	0·8
1	4	β-carotene	24	14	6·6	21	28
	2	β-carotene + 20 mg tocopheryl acetate	24	16	3·5	3·5	7·0
	4	C_{30}-carotenal	24	19	7·6	12	20
	2	C_{30}-carotenal + 20 mg tocopheryl acetate	24	14	2·4	3·6	6·0
	4	C_{27}-carotenal	24	11	5·4	15	20
	2	C_{27}-carotenal + 20 mg tocopheryl acetate	24	12	4·3	3·1	7·4
	4	C_{25}-carotenal	24	10	3·4	11	14
	2	C_{25}-carotenal + 20 mg tocopheryl acetate	24	13	2·0	2·9	4·9
	3	Retinene	24	15	18	136	154
	3	Retinene + 20 mg tocopheryl acetate	24	18	17	118	135
2	3	Retinene	¼	27	52	24	76
	3	Retinene	½	31	26	68	94
	3	Retinene	1	27	22	76	98
	3	Retinene	2	28	23	96	119
	3	Retinene	4	25	18	108	126
3	4	C_{30}-carotenal 2,4-dinitrophenylhydrazone	24	20*	2·5*	2·5*	< 5
	4	C_{27}-carotenal 2,4-dinitrophenylhydrazone	24	22*	3·8*	3·6*	< 7
	4	C_{25}-carotenal 2,4-dinitrophenylhydrazone	24	19*	2·9*	4·7*	< 8
	4	Retinene 2,4-dinitrophenylhydrazone	24	14†	16†	108†	< 124
4	2	β-carotene	24	13	6·1	22	28
	2	β-carotene + 150 μg 2,4-dinitrophenylhydrazine	24	10	4·9	25	30

* Colour developed with antimony trichloride was atypical of vitamin A.

† Colour developed with antimony trichloride was slightly atypical, but probably mainly due to vitamin A.

tocopherol reduced the conversion of these carotenals into vitamin A. Retinene, on the other hand, was well and rapidly utilized (Table 5, Expts 1 and 2), and its conversion into vitamin A was unaffected by tocopherol.

In contrast to the carotenals themselves, 2,4-dinitrophenylhydrazones of the C_{30}, C_{27}, and C_{25} carotenals were poorly utilized. As is shown in Table 5, Expt 3, the

antimony-trichloride colour was not typical of vitamin A, and interfering substances for which no satisfactory allowance could be made contributed to at least a portion of the vitamin A values recorded for liver and blood samples. Retinene 2,4-dinitrophenylhydrazone was converted into vitamin A, but not as efficiently as retinene itself, and again the colour was somewhat atypical.

Intermediates in the conversion of carotene into vitamin A. Attempts were made to isolate from the blood of rats substances that may be intermediates in the conversion of intravenously administered aqueous dispersions of carotene into vitamin A. Since the dinitrophenylhydrazones of the higher carotenals are so poorly utilized, experiments were carried out in which 2,4-dinitrophenylhydrazine was injected simultaneously with the carotene dispersion in the hope that it would act as a trapping reagent for any aldehydic or ketonic intermediates and, through the formation of hydrazones, prevent their further breakdown to vitamin A. From the typical results shown in Table 5, Expt 4, it is apparent that the nitrophenylhydrazine had no effect on the efficiency of conversion of β-carotene into vitamin A. Further, extracts of blood samples taken at intervals after the injection of carotene and the dinitrophenylhydrazine showed no absorption peaks typical of carotenal hydrazones.

Table 6. *Effect on the utilization of intravenously administered aqueous dispersions in Tween 40 of β-carotene (400 μg in 0·4 ml dispersion) of incorporation of methyl linoleate in the dispersion*

(Each set of results relates to one dispersion injected into female rats of mean weight 240 g, killed 24 h after injection)

No. of rats	Dose	Blood plasma		Liver			
				Vitamin A			
		Vitamin A alcohol (μg/100 ml)	Carotene (μg/100 ml)	Alcohol (μg)	Ester (μg)	Total (μg)	Carotene (μg)
2	None	10	—	0·6	0·5	1·1	—
4	β-carotene	16	25	5·2	29	34	56
4	+1 mg methyl linoleate	18	20	18	92	110	50
2	+1 mg methyl linoleate	17	16	7·2	40	47	62
3	+1 mg methyl linoleate	14	22	12	60	72	68
3	+8 mg methyl linoleate	16	30	14	91	105	65
3	+8 mg methyl linoleate	18	18	10	35	45	75
4	+10 mg methyl linoleate	12	20	13	61	74	70

Extracts of blood samples taken from 5 min to 3 h after intravenous injection of aqueous dispersions of carotene alone were examined spectroscopically. The absorption curves indicated the rapid formation of products absorbing at wavelengths between $_{max.}$ for carotene and for vitamin A, but the amounts present were small, and it was not possible to effect a satisfactory separation by standard chromatographic methods. The same extracts when treated with 2,4-dinitrophenylhydrazine failed to show changes in absorption that might indicate the formation of hydrazones. In vitro experiments, in which blood samples were incubated with aqueous dispersions of carotene alone or in the presence of 2,4-dinitrophenylhydrazine, gave similar negative results.

Effect of methyl linoleate. The effect of methyl linoleate on the conversion of intravenously administered aqueous dispersions of carotene into vitamin A is shown in Table 6. It is apparent that all concentrations of methyl linoleate increased the efficiency of conversion of the carotene into vitamin A as judged by liver storage of the vitamin. Although individual rats were found to be relatively consistent in their response to one particular dispersion, the effect of methyl linoleate varied considerably from one dispersion to another, despite the fact that all were made under apparently identical conditions. This variability is shown in the results quoted in Table 6; for example, the three sets of results relating to 1 mg methyl linoleate were obtained with three different dispersions. Two of the dispersions were prepared at the same time and appeared to be identical, but produced mean liver stores of 47 and 72 μg vitamin A/rat. The third dispersion, prepared on a different occasion, gave liver stores of 110 μg vitamin A/rat.

DISCUSSION

The results presented here support previous suggestions that the conversion of intravenously administered aqueous dispersions of carotene is initiated in the blood. They also confirm the rapid change in the physical state of the carotene that inhibits, or at least markedly reduces, its conversion into vitamin A. In a previous investigation (McGillivray & Worker, 1958) tocopherol was injected intravenously at the same time as, or at intervals before or after, the carotene dispersions, in order to investigate the effect of different levels of tocopherol in the blood and tissues on the conversion mechanism. With this one method of administration, however, tissue levels tended to parallel blood levels, a situation which was prevented in the investigation now described by use of various routes of administration, a range of blood levels having been obtained, at different times after dosing and at different dosage levels, unrelated to tissue levels.

Considered with the other evidence available (Kon *et al.* 1955; McGillivray *et al.* 1957; McGillivray & Worker, 1957, 1958), the fact that only the levels of tocopherol in the blood showed a significant correlation with subsequent liver storage of vitamin A would seem to establish that the conversion of intravenously administered carotene is at least initiated in the blood immediately after injection.

The rapid change in the physical state of the carotene after injection is also in line with the findings of the above-mentioned workers as to the rapid breakdown, with subsequent formation of vitamin A, of a small proportion of the injected carotene and the apparent stability of the relatively large amounts of carotene persisting in the blood for some considerable time after injection. Kon *et al.* (1955) have already shown that the utilization of intravenously administered carotene is intimately related to its physical state, and it appears that once the Tween has been hydrolysed the carotene becomes associated with blood-plasma proteins and acquires the degree of stability normally associated with similar protein-bound carotenoids that occur naturally in, for example, the blood of cattle. Similar reactions after the intravenous injection of vitamin A dispersions would explain the initial rapid destruction of the

vitamin as well as the relative stability of that fraction of the dose persisting in the blood for some considerable time after injection (cf. Kon *et al.* 1955).

The evidence presented here is not inconsistent with the view that the conversion mechanism after intravenous administration may be similar to that postulated by Glover & Redfearn (1954) for intestinal conversion. Intermediates in this scheme, the C_{30}, C_{27}, and C_{25} carotenals, are all converted into vitamin A when dispersions are administered intravenously, and their utilization is inhibited by tocopherol in the same way as it influences carotene utilization. If, however, the mechanism of conversion does involve a type of β-oxidation, with the formation of a series of carotenals, the quantities present in the blood at any time after injection of carotene must be small, since neither the carotenals nor their hydrazone derivatives could be isolated from blood extracts. It is surprising, too, that the carotenals are quantitatively less efficiently utilized than β-carotene itself. If these carotenals are intermediates in the conversion of carotene into vitamin A, the opposite would have been expected, liver storage of vitamin A increasing with decreasing chain-length from β-carotene to the C_{25} carotenal.

The conversion of the higher carotenals into vitamin A was markedly reduced when they were converted into 2,4-dinitrophenylhydrazones, suggesting that the conversion of carotenoids into vitamin A involves a stepwise oxidation from one end of the molecule, the presence of the hydrazone grouping protecting the normally susceptible end from oxidation. In contrast, hydrazone formation does not greatly interfere with the reduction of retinene to vitamin A. Differences between the metabolism of β-carotene and the higher carotenals and that of retinene are further shown by the action of tocopherol, which, as might be expected, does not inhibit the reduction of retinene. This would seem to indicate further that, in reducing the conversion of aqueous dispersions of carotene into vitamin A, tocopherol is acting as an anti-oxidant and that its effect is not an indirect one associated with, for example, differences in the physical state of the carotene in dispersions containing added tocopherol.

As judged by liver stores and blood levels of vitamin A, reticulocytosis induced in rats by phenylhydrazine did appear to increase the efficiency of conversion into vitamin A of carotene administered intravenously as a dispersion. Although the increased amount of vitamin A was not great, this finding is in agreement with the report by Pollard & Bieri (1958) on the increased destruction of vitamin A in aqueous dispersions under similar conditions. The increased conversion of carotene into vitamin A could be associated with particular enzyme systems present in the reticulocytes, but, as Pollard & Bieri point out, the blood of rabbits made anaemic by injection of phenylhydrazine contains, in addition to more reticulocytes, more unsaturated fatty acids and peroxides, and these substances undergo autoxidation, which is accelerated by the presence of haemoglobin from haemolysed red cells (Rapoport, Gerischer-Mothes & Nieradt, 1955).

On the assumption that phenylhydrazine produced similar abnormalities in the blood of the rat, it seemed possible that the initial breakdown of the carotene might be coupled with this oxidation of unsaturated fatty acids and that similar increases in the amount of carotene converted into vitamin A might be obtained in normal

animals by increasing blood levels of unsaturated lipids. This was clearly confirmed in the experiments in which methyl linoleate was dispersed with the carotene, intravenous injections of these dispersions resulting in liver stores of up to about three times those obtained with the same quantity of carotene dispersed without methyl linoleate. This experiment would seem to provide strong evidence for postulating that the initial reaction in the conversion, in the blood, of carotene into vitamin A is coupled with oxidation of unsaturated lipids. In seeking the mechanism of such a reaction it seems unnecessary to postulate a specific enzyme system. Indeed it would seem surprising to find in the blood of a carotenoid non-accumulator, such as the rat, an enzyme system specifically capable of acting on carotene, especially in the unphysiological form of a Tween dispersion, and converting it into a substance such as retinene, which can then be reduced to vitamin A. The rapid oxidation of vitamin A itself, when injected as a Tween dispersion, and the breakdown at similar rates of all carotenoids so far investigated, whether they are precursors of vitamin A or not, is further evidence of the non-specific nature of the reaction.

It seems more probable that the carotenoids or vitamin A, while in dispersed forms, merely function as chance substrates for some non-specific type of oxidation. From a consideration of possible reactions of this type that could take place under the conditions obtaining in the blood immediately after injection of the aqueous dispersions, attention naturally focuses on haematin compounds, which, in the presence of oxygen, are active catalysts in effecting the coupled oxidation of the carotene molecule with the unsaturated lipids. The catalytic activity of haematin compounds has been extensively investigated by Tappel (1954, 1955*a*, *b*). It is inhibited by tocopherol, as in the reaction considered here, and would seem to be sufficiently rapid to explain the appearance of vitamin A almost immediately after injection of carotene. It seems significant that haematin compounds are only effective as catalysts in heterogeneous systems. Aqueous dispersions of carotenoids in Tween are of this type; once the Tween has been hydrolysed and the carotenoids have become protein-bound, the same type of lipid–water system is no longer present.

In considering the possibility that haemoglobin is involved in the reaction, it may be of note that injections of Tweens, in the order of concentration used in these experiments, may produce some haemolysis (cf. Sobel, Rosenberg & Engel, 1952). Although slight, this haemolysis has been observed in a number of instances when blood samples were drawn soon after injection of Tween dispersions. Haemoglobin in this form would be a much more effective catalyst than when present in intact red blood cells. As already noted reticulocytosis induced by phenylhydrazine is also associated with haemolysis of the blood cells, and it, as well as increased levels of unsaturated fatty acid in the blood, could explain the more rapid destruction of vitamin A (Pollard & Bieri, 1958) and the slightly increased conversion of carotene into vitamin A reported here.

No explanation can be advanced for the large differences in the utilization of carotene from the dispersions containing methyl linoleate. Differences have been noted in the past between dispersions of individual carotenoids, but they have been relatively small and influenced mainly the amount of carotene taken up by the liver

and other tissues. The larger differences found now with dispersions containing methyl linoleate must be due to undetected differences in the physical state of the dispersions or to oxidative changes in the ester during preparation of the dispersion.

Whatever the mechanism may be, the evidence presented here, and the earlier findings already discussed, suggest that, after intravenous injection of aqueous dispersions of carotene, there is a rapid breakdown of the carotene molecule through a coupled oxidation with unsaturated lipids, possibly with haemoglobin as a catalyst, to retinene, which is then removed by the tissues from the oxidizing environment of the blood and reduced through the already established enzyme system to vitamin A. This vitamin A is then released slowly from the tissues into the blood, from which it is removed by the liver. In this connexion it is perhaps significant to note the similar relatively slow release of vitamin A alcohol from the tissues, and its uptake by the liver, after intravenous injections of aqueous dispersions of retinene. If subsequent work should indeed establish that haemoglobin is involved in the initial oxidation of the carotene molecule, the marked similarities between the mechanism of conversion of carotene into vitamin A after oral or intravenous administration would lead one to ponder whether haematin compounds, either haemoglobin itself or myoglobin of the intestinal wall, may not also play a key part in intestinal conversion.

SUMMARY

1. The conversion into vitamin A of intravenously administered aqueous dispersions of carotenoids has been further investigated in rats.

2. Additional evidence has been obtained to show that the reaction is initiated in the blood immediately after injection. The simultaneous injection of methyl linoleate with the carotene increased up to threefold the liver storage of vitamin A, and it is suggested that the carotene undergoes a rapid coupled oxidation with unsaturated lipid with haemoglobin as a possible catalyst and that this non-specific oxidation leads to the formation of a compound, such as retinene, which is then reduced to vitamin A in the tissues.

3. A number of carotenals can be converted into vitamin A when administered intravenously as aqueous dispersions, but not as efficiently as would be expected if these compounds were intermediates in the conversion of β-carotene into vitamin A.

4. It has been shown that the Tween 40 used as dispersing agent is rapidly hydrolysed, with release of the carotene, which then becomes associated with plasma proteins. In this form it is apparently no longer susceptible to the type of oxidation that leads to the formation of vitamin A.

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