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

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

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

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REFERENCES

- Drummond, J. C., Bell, M. E. & Palmer, E. T. (1935). *Brit. med. J.* i, 1208.
- Eden, E. & Sellers, K. C. (1948). *Biochem. J.* **42**, xlix.
- Glover, J., Goodwin, T. W. & Morton, R. A. (1947a). *Biochem. J.* **41**, xlv.
- Glover, J., Goodwin, T. W. & Morton, R. A. (1947b). *Biochem. J.* **41**, xxiv.
- Glover, J., Goodwin, T. W. & Morton, R. A. (1947c). *Biochem. J.* **41**, 97.
- Glover, J., Goodwin, T. W. & Morton, R. A. (1948a). *Biochem. J.* **43**, 109.
- Glover, J., Goodwin, T. W. & Morton, R. A. (1948b). *Biochem. J.* **43**, 512.
- Goodwin, T. W., Dewar, A. D. & Gregory, R. A. (1946). *Biochem. J.* **40**, lx.
- Goodwin, T. W. & Morton, R. A. (1946). *Analyst*, **71**, 15.
- Goodwin, T. W. & Morton, R. A. (1948). *Spec. Rep. Ser. med. Res. Council, Lond.*, no. 264. In the Press.
- Gregory, R. A. (1946). *J. Physiol.* **105**, 39 P.
- Gregory, R. A. (1947). *Vet. Rec.* **59**, 377.
- London, E. S. (1928). *Ergebn. Physiol.* **26**, 320.
- Mattson, F. H., Mehl, J. W. & Deuel, H. J., jun. (1947). *Arch. Biochem.* **14**, 65.
- Mitchell, J. H. & Wise, G. H. (1944). *J. Dairy Sci.* **27**, 189.
- Popper, H. & Volk, B. W. (1944). *Arch. Path.* **38**, 71.
- Rea, J. & Drummond, J. C. (1932). *Z. Vitaminforsch.* **41**, 177.
- Sexton, E., Mehl, J. W. & Deuel, H. J., jun. (1946). *J. Nutrit.* **31**, 299.
- Thompson, S. Y., Ganguly, J. & Kon, S. K. (1947). *Brit. J. Nutrit.* **1**, v.
- Wald, G., Carroll, W. R. & Sciarra, D. (1941). *Science*, **94**, 95.
- Wiese, C. E., Mehl, J. W. & Deuel, H. J., Jnr. (1947). *Arch. Biochem.* **14**, 75.
- Woolf, B. & Moore, T. (1932). *Lancet*, ii, 13.

Studies in Vitamin A

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

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

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

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

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

to mincing and incubating. In all the experiments for which positive results were claimed, at most only traces of vitamin A appear to have been formed. Again, it is now well known that under the action of heat β -carotene yields *cis*-isomerides, solutions of which in saturated-hydrocarbon solvents exhibit an absorption maximum c. 335 m μ ., which is difficult to distinguish from the vitamin A maximum when other absorbing substances are present. Hence the results of *in vitro* experiments are not considered unequivocal proof that the conversion takes place in the liver.

The *in vitro* technique having failed to solve the problem, workers turned their attention to studies on the living animal. If carotene administered by a parenteral route shows vitamin A activity, then the liver is the probable site of conversion of the provitamin. Carotene in colloidal suspension has been administered parenterally by various groups of workers and again the results were conflicting or indefinite. Using the intravenous route, Wolff, Overhof & van Eekelen (1930) and Ahmad, Grewal & Malik (1934), obtained positive results for the rabbit, but Rea & Drummond (1932) and Drummond & MacWalter (1933) could not confirm the transformation of carotene to vitamin A in the liver of the cat or the rat.

More recently, using a technique of partial hepatectomy similar to that of Drummond & MacWalter (1935), Vinet, Plessier & Raoul (1943) claim to have demonstrated the conversion of carotene to vitamin A in the liver of the rabbit. The analysis of small specimens of this tissue taken at intervals after injection of a colloidal suspension of carotene showed an increase in vitamin A in less than 15 min., whereas controls showed a drop in vitamin A content in that period. The carotene content of the specimens was determined at the same time. No values greater than 2.16 μ g. were recorded, suggesting that an immediate conversion of the carotene had occurred on reaching the liver. This observation is in conflict with the fact that the transformation of carotene into vitamin A is a comparatively slow process, and also with the observations of van den Bergh, Müller & Brockmeyer (1920), Drummond, Gilding & MacWalter (1934) and many others, that carotene introduced into the circulation intravenously is stored in the liver, particularly in the Kupffer cells. So, apart from the difficulties of partial hepatectomy (both as to technique and interpretation) emphasized by Drummond & MacWalter (1935), the evidence of Vinet *et al.* is not satisfactory in establishing the liver as the site of conversion.

Using curative or growth tests on vitamin A-depleted animals as the criterion of activity, Chu & Coady (1934), With & Wanscher (1939) and Tomarelli, Charney & Bernhart (1946) have claimed that carotene possesses vitamin A activity when

administered by the intramuscular route. Yet Rokhlina, Balakhovski & Bodrova (1943) came to the conclusion that carotene administered parenterally is completely lacking in vitamin A effect, regardless of whether an oil or an aqueous colloidal solution is used. Again, the evidence is conflicting.

Recently, Sexton, Mehl & Deuel (1946) have carefully compared the biological activity of carotene administered to the rat orally and parenterally. When carotene was given by the parenteral route moderate amounts were stored in the liver but only traces of vitamin A were found there. When given by the oral route, however, in smaller doses spread over a period, moderate amounts of vitamin A, but little carotene, appeared in the liver. Furthermore, vitamin A-deficient rats were unable to utilize carotene given by intrasplenic injection, for the symptoms of vitamin A deficiency persisted, although substantial amounts of carotene had reached the rats' livers. The authors suggested that this negative evidence excluded the liver and pointed to the intestine as the site of conversion.

It appears from these results that the biological activity of carotene is at best very much less when given by the parenteral route than when fed orally, while direct analysis of the liver tissue shows that substantial amounts of β -carotene may be stored there (in spite of the fact that the rat is an efficient converter of carotene to vitamin A) and yet not be utilized by the animal to alleviate symptoms of vitamin A deficiency. Thus, even if the liver can produce traces of vitamin A from the provitamin, it can be neither the main nor the only locus of the transformation.

The next organ to consider as the site of conversion is the intestine, which has not hitherto been examined so extensively. Moore (1931) carried out various colorimetric tests on intestinal lipids. He used a ratio expressing the intensity of light absorption due to visible pigment relative to that of the blue colour produced with the antimony trichloride reagent to detect the presence of vitamin A. The observed change in the ratio (a fall) was not, however, sufficient to establish the presence of vitamin A. Using the same criterion for vitamin A, Vinet *et al.* (1943) examined the intestinal contents and mucosa of vitamin A-depleted guinea pigs 2-6 hr. after oral administration of carotene, and obtained a similarly indefinite result. Sexton *et al.* (1946) dosed vitamin A-depleted rats with carotene and then removed the intestinal tracts and incubated them for 6-24 hr. at 37°. No evidence was obtained either of conversion of carotene to vitamin A or of its destruction.

Popper (1941) studied the problem using the method of fluorescence microscopy. By examining the various organs of a series of vitamin A-depleted rats at intervals after administering carotene orally

and parenterally, he attempted to find the organ in which fluorescence first appeared. He never detected fluorescence due to vitamin A in the liver of the rat following parenteral administration of carotene; after oral administration he sometimes detected fluorescence first in the intestine and later in the liver, but usually first in the latter. Since he was occasionally able to detect vitamin A fluorescence first in the intestine Popper suggested that this organ could convert carotene into vitamin A, though he believed the liver to be the main site of conversion.

It appears likely that the conversion can take place in the intestine, but the fact that the reaction is slow probably accounts for the lack of absolute proof. The mechanism of the transformation reaction is not known. Hunter (1946), following the work of Morton & Goodwin (1944) on retinene, suggested that oxidative attack upon the central double bond occurs resulting (possibly with intermediate stages) in formation of vitamin A aldehyde, which in turn is reduced to vitamin A alcohol. He has shown that such a scheme can be realized *in vitro*, albeit with very small yields. Glover, Goodwin & Morton (1948) have shown that retinene, (vitamin A aldehyde) fed to rats is reduced immediately on entering the intestinal wall. This strengthens the hypothesis that the aldehyde is a link in the conversion of β -carotene to vitamin A in the intestine.

In the light of the foregoing considerations, the aim of the present work was to examine more closely the possibility that the intestinal wall is the site of the transformation. A preliminary report of the work has already been published (Glover, Goodwin & Morton, 1947*a*). The following possible reactions were considered: (a) breakdown of β -carotene to vitamin A aldehyde; (b) reduction of the aldehyde to the alcohol; and (c) absorption and esterification of vitamin A alcohol followed by its removal from the intestine into the blood stream. If (b) and (c) take place more rapidly than (a), no appreciable amount of vitamin A will be found in the intestine even at the height of absorption of β -carotene. Compared with vitamin A, carotene is poorly absorbed even under the most favourable circumstances. The results on the absorption of vitamin A aldehyde (Glover *et al.* 1948) indicate that processes (b) and (c) are very rapid indeed, but little is known about (a). With (1941) compared the rate of deposition of vitamin A in the liver after feeding carotene and vitamin A. He found that vitamin A appeared in the liver not earlier than 2 hr. after ingestion of carotene, whereas only 0.5 hr. was required for the preformed vitamin to reach that organ. This suggests a moderate time lag for process (a). Rea & Drummond (1932) and Drummond & MacWalter (1933) have come to a similar conclusion. Furthermore, many workers have been unable to

observe any appreciable rise in the plasma level of carotene or vitamin A in human subjects given moderate doses of carotene, e.g. von Drigalski (1934), With (1941), and Steigmann & Popper (1944). Similarly, Goodwin, Dewar & Gregory (1946) and Goodwin & Gregory (1948) could not detect a rise in carotene or vitamin A in portal or systemic blood when large doses of carotene were fed to goats through a duodenal fistula. The above observations can only be satisfactorily explained if process (a) were much slower than (b) or (c). Hence one cannot expect to find much vitamin A in the rat intestinal wall unless a moderately high concentration of carotene can be obtained there. It is hoped to revert to the dynamics of the processes at a later date.

EXPERIMENTAL

Preparation of carotene. In the preliminary experiments, the carotene used was prepared from dried grass, which was extracted with acetone in a large Soxhlet extractor. The solvent was removed from the extract and the residue, taken up in light petroleum, was chromatographed on a column of alumina (Savory and Moore) to remove chlorophylls and xanthophylls. The light petroleum eluate was made up to a known volume and the carotene content of the solution determined by measuring its extinction at 448 $m\mu$., using the Beckman quartz photoelectric spectrophotometer.

To a known volume of this solution a measured quantity of arachis oil was added and the solvent removed, so that the desired amount of carotene was contained in 1 ml. of the oil. For the later more quantitative experiments, in which the absorption spectra of the tissue lipids were recorded, crystalline β -carotene was used. The requisite amount was dissolved in diethyl ether (freshly distilled over reduced Fe) and again a quantity of arachis oil containing a little lecithin (0.1 g./ml.) was added and the solvent removed *in vacuo* at room temperature leaving a solution of β -carotene of known concentration in the oil (2–5 mg./ml.). Tocopherols or other anti-oxidants were not added to this solution because their presence would complicate the ultra-violet absorption spectra of the lipid extracts from the various tissues.

Animals. Two groups of adult rats (5 males and 5 females) were prepared as controls by feeding them on the normal diet of cubes (Lever Bros. and Unilever Ltd.) supplemented with carrots and cabbage for approximately 3 months. On this diet the rats build up a moderate liver reserve of vitamin A solely from a provitamin source, as the cubes when used contain no detectable vitamin A but a certain amount of carotene (c. 5 $\mu\text{g./g.}$). At the end of this period the rats were taken off the carotene diet and placed on a vitamin A-free diet (Glover, Goodwin & Morton, 1947*b*) for 4 days to allow the residual carotene in the intestine to disappear. They were then killed and their livers and small intestines removed for examination.

Leaving out preliminary experiments, a further group of 28 newly weaned rats were placed on a vitamin A-free diet for about 5 weeks, by which time growth had ceased. A group of 4 were killed as controls. The pooled livers and pooled intestines were separately saponified. Each

non-saponifiable extract was tested for vitamin A with SbCl_3 . The negative results obtained confirmed the absence of detectable amounts of vitamin A.

Except for 3 which were examined singly at higher dose levels, the remainder of the rats were divided into groups of 3-5. The animals were fasted for 15 hr. prior to feeding β -carotene. The solution of β -carotene in oil (2-5 mg./ml.) was taken up in a syringe and 1 ml. administered by stomach tube to each animal in the group. For the single animals fed at higher dose levels (one at 10 mg. and two at 14 mg. each) the β -carotene was contained in 2 ml. oil. A little of the β -carotene in the oil used at the highest dose level did not appear to be in true solution.

Preparation of tissue extracts and analytical methods

At various intervals (5-24 hr.) after administering the β -carotene, the animals were anaesthetized and killed. The liver and intestinal tract, excluding the large intestine, were removed for examination. The former was ground with anhydrous Na_2SO_4 and extracted with freshly distilled ether. The extract was then examined for vitamin A and carotene by the methods to be described. The small intestine was separated from the stomach at the pyloric sphincter. The stomach was cut open and the contents removed with a little ethanol and ether. The mixture was transferred to a separatory funnel and after the addition of a suitable quantity of water, extracted with ether. This extract was washed with water and dried over Na_2SO_4 . The solvent was then removed, and the residue dissolved in light petroleum and chromatographed on bone-meal to remove the residual β -carotene. The adsorbate was removed with acetone and the lipid residue was recovered and examined for vitamin A.

The contents of the small intestine were flushed out with 0.9% NaCl and the mixture collected in a beaker. Approximately 0.5 vol. of ethanol was added and the whole triturated in a mortar to break up any solid material. The mixture was then transferred to a separating funnel and extracted with ether until free from colour, the ether extract being then treated as described for the stomach extract. The intestinal tissue was saponified and extracted with ether. The washed extract was dried over Na_2SO_4 , filtered and the solvent removed; the residue was taken up in 20 ml. light petroleum and chromatographed on a bone-meal column. The light petroleum eluate was made up to a known volume, usually 100 ml. The adsorbate was eluted with acetone, the solvent removed and the lipid residue taken up in 10 ml. light petroleum or methanol in a 25 ml. flask. The flask was placed in a previously prepared ethanol- CO_2 mixture at -60° for 1 hr., during which time a large proportion of the sterols was precipitated. This was filtered off on a small G. 4 sintered glass funnel containing and surrounded by pieces of solid CO_2 . The clear filtrate was collected in a small filter tube. Suitable samples were taken for determination of the ultraviolet absorption spectrum and of vitamin A.

Incubation of intestines. Rats were anaesthetized 8 hr. after being dosed with carotene and the small intestines ligatured at the pyloric sphincter and at the caecum. The closed intestines were then immediately removed and placed in Ringer solution and incubated at 37° . At the end of the required period (3-20 hr.) they were removed and treated as described for the normal intestine.

Determination of carotene and vitamin A. The solutions of carotene were examined in the Beckman quartz photoelectric spectrophotometer. The extinction of the solutions was measured at 448-450 $\text{m}\mu$., and accepting the $E_{1\%}^{1\text{cm.}}$ (450 $\text{m}\mu$.) for β -carotene in light petroleum as 2500, the amount present was calculated.

Vitamin A was estimated by two methods: (1) a 3-5 ml. portion of the filtrate after removal of sterols was taken to dryness *in vacuo* at room temperature in a hard glass test tube. 0.5 ml. chloroform was added to the residue, 4.0 ml. SbCl_3 reagent run in and the mixture placed immediately in a 2 cm. cell on the Hilger-Nutting spectrophotometer and the extinction of the blue solution (maximum observed at 617 $\text{m}\mu$.) measured, from which the concentration in i.u. could be calculated using a conversion factor of 550. (2) The ultraviolet absorption spectrum was recorded and using the three-point method of correction for irrelevant absorption (Morton & Stubbs, 1946) the true absorption due to vitamin A was calculated, from which the concentration in i.u. was calculated, using a conversion factor of 1800.

RESULTS

Preliminary qualitative experiments with the SbCl_3 reagent on the non-saponifiable matter from intestinal extracts indicated that vitamin A was present in the intestine during the absorption of carotene and that the maximum amount of vitamin A occurred 6-8 hr. after dosing. No detectable quantities of vitamin A were found in the contents of the lumen of the small intestine or stomach. The quantitative results for the young rats fed with a solution of crystalline β -carotene and for the control animals given the carotene-rich diet are shown in Table 1.

The small intestines of the vitamin A-depleted animals and of the controls (Exps. II and III), which had a high liver reserve of vitamin A formed entirely from a carotenoid source, were examined for vitamin A. Both the SbCl_3 test and the correction procedure failed to demonstrate its presence, even in traces. In the remaining experiments (IV-XI), where β -carotene was in process of absorption, vitamin A, characterized by its ultraviolet absorption spectrum and by the colour test with SbCl_3 , was always found in the gut wall. A typical absorption curve and the corrected vitamin A curve are shown in Fig. 1. The extracts from all seven groups of animals gave similar curves capable of correction by the three-point fixation method, and also the blue colour (absorption max., 617 $\text{m}\mu$.) characteristic of vitamin A with the SbCl_3 reagent.

The vitamin was determined by both methods of analysis and in general, close agreement (columns 7 and 8, Table 1) was obtained for the amount present in spite of the large corrections which had to be applied to the ultraviolet absorption curves in the second method. The amount of vitamin A found was very small. In an attempt to increase the yield of vitamin A, the small intestines of the remaining rats were allowed to take up β -carotene

Table 1. *Vitamin A formed in the intestine of the rat during absorption of carotene*

Exp.	No. of rats	β -Carotene given (mg./rat)	Time after dosing (hr.)	Incubation time (hr.)	Intestine						
					Lumen		Gut wall			Liver	
					Carotene	Vitamin A	Carotene (μ g.)	Vitamin A (i.u.)		Carotene (μ g.)	Vitamin A (i.u./rat)
								U.V.	SbCl ₃		
I	4	0	—	—	—	—	0	0	—	0	
II	5	(i)*	—	—	—	0	(ii)*	0	0	(iii)* 6644	
III	5	(i)	—	—	—	0	(ii)	0	0	(iii) 2465	
IV	5	2.5	20	—	+ve	Nil	3	—	2	5 360	
V	3	2.5	16	—	+ve	Nil	2	—	3	5 70	
VI	4	5.0	7	—	+ve	Nil	5	10	20	-ve 49	
VII	4	5.0	7	—	+ve	Nil	34	35	28	2 108	
VIII (a)	4	5.0	6	—	+ve	—	30	6	6	1 25	
VIII (b)	5	5.0	7.5	3	+ve	—	35	10	12	11 80	
IX	1	10.0	6	15	+ve	—	20	10	—	—	
X	1	14.0	7.8	20	+ve	—	—	22	21	18 360	
XI	1	14.0	8.0	20	+ve	—	—	20	18	16 270	

* (i) Diet containing mixed carotenoids; (ii) trace of yellow pigment not carotene; (iii) trace of yellow pigment.

under physiological conditions. They were then removed intact from the animal at the peak period of absorption (6–8 hr. after dosing) and incubated in Ringer solution. In Exp. IX the vitamin A

Fig. 1. (a) Ultraviolet absorption spectrum of the gut-wall extract (in methanol) of the carotene-fed rat; and (b) the corrected vitamin A curve. ($E_{\text{corr. } 325 \text{ m}\mu} = 0.238$ equivalent to a total of 175 i.u.)

content of the incubated gut walls is greater than that of the non-incubated, but the increase is not sufficiently marked to assert definitely that *in vitro* conversion had taken place.

DISCUSSION

The preparation of an extract from the small intestine which gives both a correctable ultraviolet absorption curve for vitamin A and a strong colour test at 617 m μ , compared with control extracts which gave neither, is taken as unequivocal proof of the presence of vitamin A in the intestinal wall of the rat during the absorption of β -carotene. Furthermore, in Exps. VI–VIII c. 10–30 i.u. of vitamin A were found in the intestine of each animal killed 6–8 hr. after dosing with β -carotene, and the liver reserves of vitamin A were low (25–108 i.u./liver), whereas in Exps. IV and V on rats killed 20 and 16 hr. respectively after dosing, the amount of vitamin A in the intestines was just detectable, i.e. 2 and 3 i.u., and the liver reserves were 360 and 70 i.u. respectively. These results considered together with those of Exps. II and III* can only be satisfactorily explained on the basis that vitamin A is formed from the β -carotene in the intestine and does not originate in the liver. The result obtained in Exp. VIII where vitamin A in the intestines was greater after incubation compared with control tissue from similarly dosed animals supports this view.

The absence of detectable quantities of vitamin A from the contents of the lumen and stomach during

* Although in the final analysis no vitamin A was detected in these tissue extracts, it is possible that the original extracts contained traces (<5 i.u.), but the process of saponification and particularly that of chromatography of the vitamin A under the best conditions always involves a slight loss of vitamin. The loss, however, ought to be of the same order for each tissue examined as the technique was always the same.

the absorption of the β -carotene implies that the latter is converted into vitamin A on entering the gut wall. However, Thompson, Ganguly & Kon (1947) have reported vitamin A in the contents of the small intestine of the carotene-fed rat in amounts comparable to that observed in the gut wall. In contrast to our result above, this could indicate that the carotene is broken down to vitamin A before it reaches the gut wall. It is, perhaps, possible that the vitamin found in the lumen contents arises from mucosal cells removed in the washings by friction when the intestines were being flushed out with ether. On the other hand, the observation (Glover *et al.* 1948) that vitamin A aldehyde, a possible intermediate in the breakdown of β -carotene, is reduced to vitamin A immediately on entering the gut wall, supports the suggestion that this organ is responsible for the breakdown of β -carotene. Furthermore, the coexistence of β -carotene and vitamin A in the gut wall, coupled with the fact that much less β -carotene is detected in the liver than in the wall of the small intestine during the absorption of the provitamin, strongly indicates that the latter organ is responsible for the conversion of β -carotene to vitamin A.

The above work, with that of Mattson, Mehl & Deuel (1947) and Wiese, Mehl & Deuel (1947) on the rat, Thompson *et al.* (1947) on the rat and pig, and Goodwin & Gregory (1948) on the goat, establishes the important fact that the main site of conversion of the provitamin A, carotene, to vitamin A is the small intestine of the animal. The results bear out in full the idea that the failure of earlier workers to detect vitamin A in the intestine after feeding β -carotene was due to the relatively slow rate of conversion and rapid removal of the vitamin A formed from the intestine.

One main problem which has so far defied satisfactory explanation is the variation in the relative amounts of carotene and vitamin A in the blood and liver of different species. The differences can, however, be considered as dependent on the rate of intake of carotene and the efficiency or abundance of an enzyme system effecting the conversion in the intestinal wall. If the carotene intake is large, the

system will not be able to break down all the carotene and some will pass into the bloodstream and hence to the liver. Once it has passed the intestinal wall further conversion of carotene to vitamin A is improbable. It would seem that the widely varying ratios of carotene to vitamin A in the blood serum or liver of different animals are due to several factors: (a) varying levels of intake and variable success in presenting food carotene for assimilation; (b) variable efficiency* for its conversion into vitamin A in the intestinal wall; and (c) differences in the rates of withdrawal† of the provitamin and the vitamin from the blood.

SUMMARY

1. The small intestine of the rat fed on a carotene-rich diet for 3 months and then placed on a vitamin A-free diet for 4 days prior to examination does not contain a detectable amount of vitamin A, even though there is a moderate reserve of the vitamin in the liver.

2. When the absorption of a single dose of carotene by vitamin A-depleted animal is maximal (approx. 6 hr. after dosing), vitamin A, demonstrated by two independent methods, is present in the gut wall in an amount comparable to that in the liver, and the gut wall contains an appreciable quantity of carotene, although there is only a small or negligible amount in the liver.

3. These facts establish the wall of the small intestine as the locus of the transformation of carotene into vitamin A.

4. An explanation is suggested for the differences observed in the relative amounts of vitamin A and carotene in the blood and livers of different animal species.

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* As the reaction may be an oxidation process, the efficiency will depend not only on the abundance of the enzyme present, but also on the hormonal activation of the enzymes responsible for the oxidation.

† Withdrawal includes physiological utilization and oxidative wastage.

REFERENCES

- Ahmad, B. (1931). *Biochem. J.* **25**, 1195.
 Ahmad, B. (1934). *Curr. Sci.* **2**, 477.
 Ahmad, B., Grewal, K. S. & Malik, K. S. (1934). *Indian med. Gaz.* **69**, 320.
 Chu, F. T. & Coady, H. (1934). *J. Pediat.* **5**, 75.
 Drigalski, W. von (1934). *Z. Vitaminforsch.* **3**, 37.
 Drummond, J. D., Gilding, H. P. & MacWalter, R. J. (1934). *J. Physiol.* **82**, 75.
 Drummond, J. C. & MacWalter, R. J. (1933). *Biochem. J.* **27**, 1342.
 Drummond, J. C. & MacWalter, R. J. (1935). *J. Physiol.* **83**, 236.
 Euler, H. von (1932). *Ergebn. Physiol.* **34**, 367.
 Euler, H. von & Klussman, E. (1932). *Hoppe-Seyl. Z.* **213**, 21.
 Glover, J., Goodwin, T. W. & Morton, R. A. (1947a). *Biochem. J.* **41**, 94.
 Glover, J., Goodwin, T. W. & Morton, R. A. (1947b). *Biochem. J.* **41**, 97.
 Glover, J., Goodwin, T. W. & Morton, R. A. (1948). *Biochem. J.* **43**, 109.

- 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.
- Hunter, R. H. (1946). *Nature, Lond.*, **158**, 257.
- Mattson, F. H., Mehl, J. W. & Deuel, H. J., Jr. (1947). *Arch. Biochem.* **14**, 65.
- Moore, T. (1930). *Biochem. J.* **24**, 692.
- Moore, T. (1931). *Biochem. J.* **25**, 275.
- Morton, R. A. & Goodwin, T. W. (1944). *Nature, Lond.*, **153**, 405.
- Morton, R. A. & Stubbs, A. L. (1946). *Analyst*, **71**, 348.
- Olcott, H. S. & McCann, D. C. (1931). *J. biol. Chem.* **94**, 185.
- Parienti, A. C. & Ralli, E. P. (1932). *Proc. Soc. exp. Biol., N.Y.*, **29**, 1209.
- Popper, H. (1941). *Arch. Path. Lab. Med.* **31**, 766.
- Rea, J. L. & Drummond, J. C. (1932). *Z. Vitaminforsch.* **1**, 177.
- Rokhlina, M. L., Balakhovski, S. C. & Bodrova, A. A. (1943). *Biochimia*, **8**, 168. (*Chem. Abstr.* 1944, **38**, 1608.)
- Sexton, E. L., Mehl, J. W. & Deuel, H. J. (1946). *J. Nutrit.* **31**, 299.
- Steigmann, F. & Popper, H. (1944). *Amer. J. med. Sci.* **207**, 468.
- Thompson, S. Y., Ganguly, J. & Kon, S. K. (1947). *Brit. J. Nutrit.* **1**, v.
- Tomarelli, R. M., Charney, J. & Bernhart, F. W. (1946). *Proc. Soc. exp. Biol., N.Y.*, **63**, 108.
- Van den Bergh, H., Müller, P. & Brockmeyer, J. (1920). *Biochem. Z.* **108**, 279.
- Vinet, A., Plessier, M. & Raoul, Y. (1943). *Bull. Soc. Chim. biol., Paris*, **25**, 87.
- Wiese, C. F., Mehl, J. W. & Deuel, H. J., Jr. (1947). *Arch. Biochem.* **14**, 75.
- With, T. K. (1941). *Absorption, Metabolism and Storage of Vitamin A and Carotene*. Oxford University Press.
- With, T. K. & Wanscher, O. (1939). Quoted by With (1941).
- Wolff, L. K., Overhof, J. & Eekelen, M. van (1930). *Dtsch. med. Wschr.* **56**, 1428.

The Use of Radioactive Isotopes in Immunological Investigations

2. THE FATE OF INJECTED ^{32}P -CONTAINING PROTEINS

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Isotopic tracers can be of considerable value in immunochemical investigations. ^{15}N has been used, for example, in studies on the formation and circulation of antibodies (Schoenheimer, Heidelberger, Rittenberg & Ratner, 1941; Heidelberger, Treffers, Schoenheimer, Ratner & Rittenberg, 1942). Radioactive isotopes of P and S have been used to determine the amount of tracer-containing antigen in antigen-antibody precipitates (Bournsnel, Dewey, Francis & Wormall, 1947), and in investigations on the lipid P and vitellin P in lipovitellin-antilipovitellin precipitates (Francis & Wormall, 1948). Libby (1947) and Libby & Madison (1947) have recently used ^{32}P -containing tobacco mosaic virus to study the rate of breakdown of this antigen in the animal body. There are obviously many other ways in which labelled antigens and antibodies can be used in the field of immunology.

In the present investigation, a preliminary study has been made of the fate of injected ^{32}P -containing antigens, phosphorylated human serum proteins and vitellin, to determine the length of time these antigens remain in the blood stream after injection.

METHODS

Phosphorylated proteins

Preparation of phosphoryl chloride containing ^{32}P . Radioactive sodium phosphate was diluted with ordinary phosphate and precipitated as $\text{Ca}_3(\text{PO}_4)_2$ (0.9 g.). This was dried and intimately ground with 0.9 g. of HCl-extracted charcoal (Merck). The mixture was thoroughly dried at 150° and then heated in a dry all-glass Pyrex apparatus in a metal bath at 300° , with dry air drawn through the $\text{Ca}_3(\text{PO}_4)_2$ -charcoal mixture to remove traces of moisture. A weighed receiver was connected and cooled in a CCl_4 -solid CO_2 mixture at about -25° and dry COCl_2 was passed through the mixture at 300 – 360° until the reaction ceased. The residual $\text{Ca}_3(\text{PO}_4)_2$ -charcoal mixture was removed and finely ground to break up the CaCl_2 layer which had formed over the remaining $\text{Ca}_3(\text{PO}_4)_2$; it was then reheated in a current of dry air and COCl_2 passed through as before. When this reaction had ceased, the POCl_3 in the receiver was warmed to remove COCl_2 and distilled. (Yield 0.44 g.; 49% of theory.)

Preparation of phosphorylated human serum proteins. Human serum (6.5 ml.) was phosphorylated with 0.229 g. of $^{32}\text{POCl}_3$ (i.e. a POCl_3 /protein ratio of 1:2) as described previously (Bournsnel, Dewey & Wormall, 1948). In this