CLXXXI. THE BIOLOGICAL RELATION BETWEEN CAROTENE AND VITAMIN A.

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THE experiments of Moore [1929], since amply confirmed by numerous workers, clearly established the appearance in the liver of vitamin A following the oral administration of carotene. It has been rather generally assumed that the conversion occurs in that organ, but direct evidence of a satisfactory character has not yet been advanced. Wolff *et al.* [1930] removed a portion of the liver from rabbits which had been fed on a diet devoid of both carotene and the vitamins. Following an injection of carotene into the circulation the vitamin A content of the residual liver tissue, determined by the increase in the antimony trichloride colour reaction, showed a considerable increase at the end of 3 days.

The first claim to have brought about the conversion *in vitro* was made by Olcott and McCann [1931]. They incubated buffered suspensions of liver and of an enzyme preparation, "carotenase," made from that tissue with colloidal solutions of carotene dissolved in ethyl laurate. A loss of colour was observed and after 24 hours the unsaponifiable fraction extract prepared from the digests showed on spectroscopic examination a discrete absorption band at 3280 Å. The changes were not observed when the "carotenase" had been inactivated by heating.

The disappearance of the colour of the carotene in such incubation experiments, is, as Woolf and Moore [1932] point out, of no particular significance, but serious attention must be given to what appears to be clear evidence of the formation of a product with selective absorption at 3280 Å. and to the statement that no such substance is formed when the liver extracts have been heated.

One confirmation of the claims of Olcott and McCann has been reported by Parienti and Ralli [1932]. They detected an increased response to the antimony trichloride reaction after incubating colloidal solutions of carotene with minced dog liver, buffered at p_H 7.4. They also state that "carotenase" is inactivated by heating. Other investigators have failed to confirm these observations and the position at the moment is not satisfactory. Von Euler and Klusmann [1932] claimed that the absorption at 3280 Å. of cow-liver extracts was increased threefold after incubation with colloidal carotene, but in another communication [v. Euler, 1932] it is stated that "die Versuche von Klusmann mit kolloidalem Carotin zum Leberbrei in Boratpuffer konnten die Ergebnisse von Olcott und McCann nicht bestätigen."

In this laboratory Ahmad [1931] and Rea and Drummond [1932] failed to detect the production of a substance resembling vitamin A from carotene either by incubating with liver preparations *in vitro* or by perfusing into the portal circulation of surviving cats.

The failure of the latter technique was rather surprising and suggested that the tissues of a typical carnivore might not be well adapted for efficiently performing the conversion; it must be remembered that evidence is accumulating to show that the carotene molecule may be degraded or modified in a variety of ways in the animal organism.

In the work to be described the investigation has been extended to cover a typical example of the herbivores. In general the procedure has been similar to that already described.

EXPERIMENTAL.

Preliminary experiments to determine the distribution of vitamin A throughout the liver were made, and it soon became apparent that rather large variations were to be expected.

The liver samples were dried by intimate mixing with anhydrous sodium sulphate, and the fatty material was rapidly extracted in a continuous extractor by hot chloroform. The extracts were examined spectroscopically for pigments and for vitamin A. For this work a new form of photometer recently placed on the market by Adam Hilger Ltd., was used—the Notched Echelon Wedge Cell. This instrument possesses the advantage of enabling the estimations to be made with great rapidity; a matter of great importance when dealing with such labile substances as carotene and vitamin A which are easily decomposed photochemically.

The experiments on the distribution of vitamin A in the liver of rabbits were made on two different groups of animals. The first had been maintained for some 8 weeks on a diet of oats and bran in order to lower their tissue reserves of that substance. Those in the second group had received the usual stock laboratory rabbit diet containing ample supplies of green leaves.

In Tables I and II the figures referring to vitamin A concentration are expressed as the extinction coefficient ($\log_{10} \frac{\text{light transmitted}}{\text{light incident}}$ referred to 1 cm. of solution) at 3280 Å., at such a concentration that 1 g. of liver is contained in 1 cc. of the chloroform extract.

Table I.

Number	I	II	III	IV	V	VI	VII	VIII	IX
Weight, kg.	1.2	1.8	1.93	—	—	1.4	1.7	—	—
Diet	-A	-A	-A	—	—	Normal		—	—
Left anterior lobe:									
Weight, g.	21.9	3.7	7.6	6.4	43	8.65	21.55	26.4	25.0
Vitamin concentration	2.65	4.5	3.55	8.2	2.5	6.9	2.95	17.1	3.7
Fat concentration	—	—	—	—	0.0173	0.0337	0.0175	0.0264	0.0182
Caudate lobe:									
Weight, g.	3.1	—	10.9	11.2	30	6.6	17.3	25.1	27
Vitamin concentration	2.0	—	—	12.2	3.4	6.8	3.6	12.20	6.1
Fat concentration	—	—	—	—	0.0104	0.0279	0.0200	0.0344	0.0157
Left posterior lobe:									
Weight, g.	12.8	11.35	9.7	16.90	56.5	9.5	23.60	26.3	23.0
Vitamin concentration	2.2	4.75	3.55	7.1	2.1	7.6	3.05	11.8	5.9
Fat concentration	—	—	—	—	0.0069	0.025	0.0137	0.0351	0.0201
Right lobe:									
Weight, g.	9.6	10.35	12.8	22.6	52.5	9.9	20.25	28.8	26.0
Vitamin concentration	—	5.0	3.0	9.2	3.8	7.2	3.65	17.2	6.6
Fat concentration	—	—	—	—	0.0137	0.0265	0.0172	0.0324	0.0164
Maximum deviation from mean %	16	6	10	31	29	4.2	12	19	33.7

The results show that the distribution of vitamin A in these rabbit livers is far from being uniform. The differences are greater in the normally fed animals than in those which had been deprived of vitamin A in their diet. In most cases they are definitely greater than the experimental error, which we estimate at 5 %. Curiously, no sharp correlation was found between the distribution of fatty material extracted by chloroform and the vitamin, as might reasonably have been expected.

The injection experiments were made by the following procedure. A portion of one lobe of the liver was removed aseptically under ether anaesthesia and a sterile colloidal suspension of carotene in 5 % glucose solution was slowly injected directly into the portal vein. The carotene suspension was prepared according to the directions of Fodor and Schoenfeld [1931]. After the injection the incision was closed and the animal kept for a suitable period before removing the remainder of the liver for examination. These periods extended from 24 hours to 20 days and in most cases care was taken to examine separately the residual portion of the lobe from which the original sample had been removed.

Some of the results are summarised in Table II.

Table II.

Rabbit No.	I	II	III	IV	V
Weight, kg.	1.8	1.57	1.05	2.5	1.97
Duration of experiment, days	1	5	5	8	20
Liver, excised portion:					
Weight, g.	3.30	4.9	2.5	3.7	2.6
Vitamin content	4.8	11.0	2.02	12.0	3.2
Basal portion of same lobe:					
Weight, g.	—	—	4.1	7.64	9.10
Vitamin content	—	—	1.7	15.0	8.7
Residual portion:					
Weight, g.	45.0	65.0	49.8	40.30	54.3
Vitamin content	4.4	9.3	2.0	14.0	6.0
% Increase on mean	-8	-15.4	-8.4	+20.8	+130
Carotene:					
Injected, mg.	0.69	0.70	0.6	1.2	1.12
Recovered, mg.	0.35	0.52	0.42	0.9	0.80

These results provide no evidence of a definite increase in vitamin A in the liver until 8 days have elapsed after the injection of the carotene. Observation showed that the injected pigment is rapidly taken up by the liver after introduction into the circulation. This was also noted by van den Bergh *et al.* [1920] who found that carotene injected in the form of a colloidal suspension into the jugular vein of rabbits was soon removed from the blood and to a large extent was held by the liver.

Our experiments show that some part of the carotene is broken down or disappears from the liver, although the loss is not nearly so large as was observed in our earlier experiments with cats. We cannot exclude the possibility that formation of vitamin A had occurred in all our experiments, for it is conceivable that the depleted organism might immediately utilise the material for resumption of growth or repair of structure. If such were true a measurable increase in the amount present in the liver would, presumably, only be detected after the requirements of the body had been satisfied and storage had become possible.

The resumption of growth shown by the rabbits kept for the longer periods after operation, in which storage of new vitamin A was detected, rather suggests that this may be true (Fig. 1).

Fig. 1.

This work has been supplemented by a new series of experiments made to try and reproduce the conditions under which Olcott and McCann observed conversion of carotene into vitamin A *in vitro*. In every case the results were negative. Another method of attack was therefore devised, which it was thought would lead to success. The liver of a rabbit which had received an injection of colloidal carotene into the portal vein was removed after an interval sufficient to ensure that the pigment had been taken up in a normal manner from the circulation by the hepatic cells. Sterile preparations of the minced tissue suspended in phosphate buffer at p_H 7.3 were incubated at 37° for different periods. Afterwards the materials were extracted by chloroform and the extracts examined spectroscopically. Table III gives the results of four such experiments, but before stating the conclusion to which they lead us it is necessary to explain

Table III.

Number	Weight of liver, g.	Duration hours	Absorption at 3280 Å.	Absorption at 4800 Å.	Correction	Corrected absorption at 3280 Å.
I	2.21	0	5.5	—	—	—
	4.42	0	4.3	—	—	—
	2.17	48	5.75	—	—	—
	2.32	48	5.75	—	—	—
II	3.68	0	4.0	2.7	0.65	3.35
	3.77	24	3.95	2.7	0.9	3.05
	4.43	65	2.9	1.9	1.5	1.4
III	4.28	0	5.1	5.0	0.83	4.27
	0.72	0	5.1	5.0	0.83	4.27
	5.59	48	5.1	5.0	3.6	1.5
	4.16	120	5.1	2.0	2.0	3.1
IV	1.18	168	6.2	2.9	4	2.2
	4.80	0	17.7	1.8	0.3	17.4
	5.18	48	8.2	0.645	0.43	7.77
	7.14	120	7.0	0.645	0.645	6.38
	6.62	168	18.6	0.883	1.26	17.34
	6.53	168	18.6	0.645	0.92	17.68

the correction which it has been found necessary to apply in estimating the absorption in the region 3280 Å. We have confirmed the observations of McNicholas [1931], who found that solutions of carotene in ether and alcohol mixtures exposed to diffuse sunlight undergo oxidative breakdown in two stages. The initial changes are characterised by the appearance of an absorption band in the region of 3200 Å. with a corresponding decrease in the band at 2730 Å. The later stage is marked by a decrease in the colour of the solution and a widening of the ultra-violet bands which finally lose their entities.

A colloidal solution of carotene, such as we employed for our intravenous injections, when incubated at 37° in contact with air shows such changes. In Fig. 2 is represented the relation between the intensity of absorption at 4800 Å. and that at 3280 Å. as it changes during a period of 7 days; by 14 days the colour of the carotene had completely disappeared.

Fig. 2.

It is necessary, therefore, to apply a correction based on such a curve as that presented in Fig. 2 for all estimations of absorption at 3280 Å. made on preparations of carotene incubated in the presence of air. When such corrections are applied our results indicate that the incubated liver containing absorbed carotene shows at first a decreased absorption at 3280 Å. followed by a gradual increase. But a similar increase is shown by liver preparations to which carotene has not been added.

It is, however, impossible to apply a similar correction for the estimations made on the perfusion experiments described earlier in the paper because our observations show that a considerable proportion of the carotene may remain unchanged in the liver for periods as long as 20 days. In this organ the changes appear to be less rapid than in the solutions exposed to air.

SUMMARY.

After injecting carotene into the portal circulation of rabbits no increase in the vitamin A in the liver was observed, using a spectrophotometer method, until 8 days after the experiment. There is of course the possibility that formation of vitamin occurs within much shorter periods, but either that it is utilised and converted into another substance or that the amounts are too small to be detected by our technique. Further attempts to obtain conversion of carotene into vitamin A *in vitro* by incubation of liver tissues have met with no success.

In some of these experiments the conditions were made more "physiological" by permitting the liver cells to take up the pigments from the circulating blood before the minced preparation was incubated.

REFERENCES.

- Ahmad (1931). *Biochem. J.* **25**, 1195.
v. den Bergh, Müller and Brockmeyer (1920). *Biochem. Z.* **108**, 279.
v. Euler (1932). *Ergeb. Physiol.* **367**.
— and Klusmann (1932). *Svensk. Kem. Tidskr.* **44**, 223.
Fodor and Schoenfeld (1931). *Biochem. Z.* **223**, 243.
McNicholas (1931). *Bur. Standards, J. Res.* **7**, 171.
Moore (1929). *Lancet*, ii, 380.
Olcott and McCann (1931). *J. Biol. Chem.* **94**, 185.
Parienti and Ralli (1932). *Proc. Soc. Exp. Biol. Med.* **29**, 1209.
Rea and Drummond (1932). *Z. Vitaminforsch.* **1**, 177.
Wolff, Overhoff and Eckelen (1930). *Deutsch. med. Woch.* No. 34.
Woolf and Moore (1932). *Lancet*, ii, 13.