XXXIV. VITAMIN A AND CAROTENE.
VII. THE DISTRIBUTION OF VITAMIN A AND CAROTENE IN THE BODY OF THE RAT.

By THOMAS MOORE[1].

From the Dunn Nutritional Laboratory, Cambridge.

(*Received January 9th, 1931.*)

In a preceding communication of this series [Moore, 1930] it was shown that the feeding of excess of carotene to albino rats resulted in an accumulation in the liver of large amounts of the colourless vitamin A, and the inference was drawn that the pigment, or some part of it, is the parent substance of the vitamin, capable of conversion in the normal course of metabolism. In this work attention was focused on the liver on account of its importance in the storage of the vitamin, and no attempt was made to examine other organs, or to decide whether the conversion of carotene is effected in the alimentary tract or after absorption into the blood stream. It seemed desirable, therefore, to make a detailed study of the distribution of vitamin A and carotene throughout the body and intestinal contents of rats receiving large amounts of carotene, in the hope of gaining further knowledge on these points.

Since Palmer and Kennedy [1920] have shown that carotene does not appear in the body fat of the rat even when large amounts are introduced into the diet, it is natural that the question of the distribution of the pigment in this animal should have hitherto attracted little attention. The question of vitamin A distribution, on the other hand, has been studied by numerous workers, notably Sherman and Boynton [1925], who used the rat-growth technique in estimating the vitamin, and by Kerppola [1930], who has employed the antimony trichloride reaction. Using tissues from rats deriving their supplies of vitamin A from the inclusion in their diet of 33⅓ % of dried milk powder, the American workers found that daily doses of 0·02 g. liver, 0·1 g. lung, 0·1 g. kidney, or 4·0 g. of muscle were necessary to promote growth in other rats, and it was deduced that under these nutritional conditions nine-tenths of the vitamin A reserves of the body (neglecting those of the adipose tissue and skin) were to be found in the liver. The results of Kerppola, although not reduced to quantitative terms, suggest a similar distribution in regard to the liver and lungs. A positive reaction was also obtained from the intestines and contents, but negative results were given by all other organs examined. The predominance of the liver in the storage of the vitamin has

[1] In the whole time employment of the Medical Research Council.

also been reported in many other animals in addition to the rat; mention may be made of the work of Rosenheim and Webster [1927] on the fulmar petrel, and the comparison by Ahmad and Drummond [1930] of the vitamin A activities of the liver and body oils of fishes.

Experimental.

The experimental procedure was designed to determine the relative distributions of the pigment and vitamin under conditions in which the former was supplied in amounts greatly in excess of the immediate physiological requirements. Albino rats received synthetic diets in which excess of carotene was included, for reasons of convenience and economy, as red palm oil or, in one instance, as carrot-fat. After receiving these diets for periods sufficiently prolonged to ensure that a large superfluity of pigment had been made available certain of the rats were killed by coal gas and examined without further treatment. Others, with a view to the removal of traces of unaltered carotene that might have been present in the blood stream, were transferred to a diet deficient in vitamin A for appropriate periods before killing.

In estimating the amounts of vitamin A and carotene in the various tissues the methods employed were essentially those previously adopted in the extraction and colorimetric examination of liver oils [Moore, 1930]. Some modification, however, was necessary in the procedure adopted in the interpretation of results, since it was found that in red palm oil and carrot-fat the ratio of the natural yellow value to the blue value given in the antimony trichloride reaction was not more than about 4 to 1, as compared with the value of 11 to 1 previously quoted for pure carotene. Although at first sight this low ratio might be taken to imply the presence of carotene and vitamin A simultaneously, it is actually most improbable that this should have been the case, since no evidence of absorption at 610–630 $\mu\mu$, the position characteristic of vitamin A, could be detected when the blue colorations produced by these materials in the $SbCl_3$ reactions were examined spectroscopically. Two alternative explanations of the discrepancy may be put forward. In the first place since determinations of both yellow and blue values by the tintometer technique are subject to large experimental errors, the ratio between them becomes subject to a much greater error, so that the difference observed between the ratios may actually be less significant than it appears. Secondly it is probable that the yellow value of carotene, under certain natural conditions[1], may deteriorate in much the same way as it does under the action of benzoyl peroxide [Moore, 1929] leaving the blue value unchanged. In interpreting the present results confusion will be avoided by basing comparisons upon the yellow/blue ratios actually determined in the red palm oil and carrot-fat.

[1] Evidence of such deterioration has been obtained in the course of experiments in collaboration with Dr Woolf on the aerobic incubation of carotene with tissue preparations.

The distribution of vitamin A and carotene in rats receiving diets rich in carotene.

Rat 1, ♀ (*118 g.*). *Carrot-fat diet* (*results shown in Table I*). After receiving a diet deficient in vitamin A for 29 days this rat was cured by the administration of carrot-fat, admixed with the diet, at the level of about 250 mg.

Table I.

Organ	Wet weight (g.)	Weight of fat (g.)	Yellow units	Blue units	Inference
Rat 1, ♀ (*118 g.*). *Carrot-fat diet.*					
Stomach	3·1	0·048	200	250	Carotene?
Small intestine and contents	3·5	0·055	100	100	Carotene?
Large intestine and contents	3·1	0·147	10,000	3,500	Carotene
Liver	9·1	0·063	280	2,500	Vitamin A
Brain	1·1	0·018	0	0	—
Heart	0·7	0·005	0	0	—
Kidneys	1·5	0·008	0	0	—
Lungs	1·4	0·0111	0	0	—
Pancreas	0·05	0·008	0	0	—
Spleen	0·7	0·0074	0	0	—
Suprarenals	0·046	0·006	0	0	—
Thymus	0·8	0·0054	0	0	—
Intraperitoneal fat	2·7	0·49	0	0	—
Rat 2, ♂ (*370 g.*). *Red palm oil diet.*					
Alimentary tract and contents	13	1·05	2,500	375	Carotene
Liver	15	0·274	250	70,000	Vitamin A
Carcase	342	71·4	140	160	?
Rat 3, ♀ (*197 g.*). *Red palm oil diet.*					
Small intestine and contents	6·2	0·1664	360	100	Carotene
Liver	10·2	0·1616	980	70,000	Vitamin A
Lungs	1·6	0·0268	0	15	Vitamin A
Rat 4, ♀ (*208 g.*). *Red palm oil diet.*					
Liver	7·6	0·1395	570	80,000	Vitamin A
Blood	4·6	0·0195	1	0	—
Kidneys	2·2	0·0886	1	10	Vitamin A?
Lungs	1·3	0·0318	1	10	Vitamin A?
Intraperitoneal fat	11·4	9·84	20	150	Vitamin A

daily. The carotene content of the latter was equivalent to 40 natural yellow units and 15 $SbCl_3$ blue units per mg. The yellow/blue ratio in the ingested fat was therefore about 2·7, and the daily intake of carotene about 20 mg. Carotene feeding was continued for 37 days. Upon *post mortem* examination positive $SbCl_3$ reactions were given by the fats derived from the stomach, small intestine, large intestine, with their respective contents, and by the liver, negative reactions by the fats from the remaining organs and also by the intraperitoneal fat. In the fat from the large intestine a yellow/blue ratio of about 3 was given, suggestive of unchanged pigment, and this conclusion was supported by the observation of an absorption band at 590 $\mu\mu$ in the $SbCl_3$ reaction. The fats from the stomach and large intestine showed a yellow/blue ratio of about unity, but since the characteristic band of vitamin A could not be detected in the $SbCl_3$ colorations it is probable that this low ratio

was essentially due to deterioration of the pigment, possibly on account of an abnormally long period of cold storage before examination. The liver oil contained a small amount of unconverted pigment, but the main chromogen present was vitamin A, as typified by a yellow/blue ratio of 0·1, together with absorption at 610–630 $\mu\mu$ in the $SbCl_3$ reaction.

It may be pointed out that the excess of carrot-fat administered to this rat was probably poorly tolerated, since the rate of growth at the time of killing was not rapid. The vitamin A content of the liver was not so high as in the cases described below, in which the feeding of a lower level of pigment was continued over longer periods.

Rat 2, ♂ (370 g.). Red palm oil diet (Table I). In the course of a separate experiment this rat had received for 119 days a synthetic diet in which vitamin A was included as 0·02 mg. of crystalline carotene daily; it was then transferred to a diet containing 15 % of red palm oil for a further period of 173 days.

The red palm oil employed had a natural yellow colour equivalent to 2·6 units per mg., and gave in the $SbCl_3$ reaction a value of 0·6 B.U. per mg. The yellow/blue ratio thus worked out at about 4.

For *post mortem* examination this rat was dissected into three portions: (1) the alimentary tract and contents, (2) the liver and (3) the rest of the carcase[1]. The fat obtained from the alimentary tract was characterised by a yellow/blue ratio of about 6, indicating the persistence of unconverted pigment. In the liver a very high level of vitamin A was found, characterised by a yellow/blue ratio of about 0·003 and by the appearance in the $SbCl_3$ test of the usual 610–630 $\mu\mu$ absorption band. The carcase fat, on the other hand, gave only a faintly positive reaction, so transient that it was impossible to make spectroscopic measurements. The yellow/blue ratio, moreover, may in this case have been deceptive, since it is probable that the yellow value observed was rendered unduly high through the admission of particles of diet adhering to the skin. All that can be said with safety, therefore, is that the colour value of this fat, whether attributable to vitamin A or carotene, was of a very low order in comparison with the values given by the fats of the liver and alimentary tract.

Rat 3, ♀ (197 g.). Red palm oil diet (Table I). The preparatory feeding of this rat was exactly similar to that adopted in the previous case, except that the red palm oil diet was given for the slightly longer period of 273 days. Only the small intestine, liver, and lungs were examined, positive $SbCl_3$ reactions being given in all three cases. In the intestine fat a yellow/blue ratio of about 3·6 was observed, suggesting the persistence of unchanged pigment. The usual high concentration of vitamin A together with a small amount of unconverted pigment was found in the liver. The lung fat gave only a faint reaction,

[1] Solution was effected quite easily without even mincing by warming with 5 % aqueous KOH in the usual way, although heating had naturally to be continued for a somewhat longer period than was necessary in the case of smaller amounts of tissues.

which, from the absence of accompanying yellow pigmentation, may probably be ascribed to the vitamin.

Biological tests. As a check on the genuine character of the extremely high colour values given by the liver oils throughout the present experiments it was decided in this case to carry out parallel rat-growth tests, the results of which are shown in Fig. 1. The liver oil was effective at 0·005 mg. (the lowest dosage examined), and thus possessed a biological activity in good agreement with its colour value.

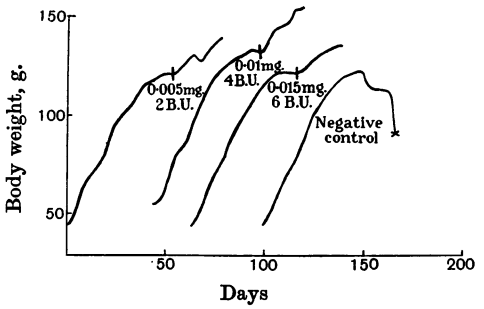

Fig. 1. Confirmation of the vitamin A activity of a representative rat-liver oil (that of rat 3, Table I) by rat-growth tests. The basal diet contained Glaxo caseinogen 20 %, rice starch 60 %, palm kernel oil 15 %, salt mixture 15 %, supplemented by 10 % of dried yeast and one drop of radiostol daily. The test doses were diluted in arachis oil and administered at the points indicated.

Rat 4, ♀ (208 g). Red palm oil diet (Table I). Preparatory feeding was again similar, the red palm oil diet being given for 278 days. The liver, kidneys, lungs, intraperitoneal fat and blood were examined, positive $SbCl_3$ reactions being given by all except the blood. The liver showed the usual high concentration of vitamin A, with small amounts of unconverted pigment. The kidneys, lungs and intraperitoneal fats all gave faintly positive reactions suggestive, in the absence of corresponding intense yellow coloration, of small amounts of vitamin A. The blood, which was obtained *post mortem* by cutting the blood vessels in the thoracic cavity just above the diaphragm, gave no indication of containing either carotene or vitamin A, although much of the sample taken must have flowed directly from the liver[1].

The distribution of carotene and vitamin A in rats restricted to a diet deficient in vitamin A after the preliminary feeding of excess of carotene.

Rat 5, ♂ (340 g.). Red palm oil diet, followed by vitamin A-free diet (Table II). Preliminary feeding was similar to that adopted in the preceding cases, the red palm oil diet being given for 176 days. Before killing, a diet deficient in vitamin A was given for a further period of 10 days.

On *post mortem* examination a faintly positive reaction probably due to

[1] The result would indicate great efficiency on the part of the liver in removing carotene from the general circulation of the rat, which is in good agreement with its power of regulating the vitamin A concentration (see discussion).

the incomplete removal of traces of red palm oil was given by the fat of the small intestine. The fats from the stomach and large intestine gave reddish reactions, which can probably be ascribed to the presence of arachis oil in the vitamin A-free diet. In the liver a high concentration of vitamin A persisted together with small amounts of unconverted pigment. Of the remaining tissues the muscle and peritoneal fats gave faintly positive reactions suggesting the presence of vitamin A at a low concentration, while in all other cases negative results were obtained.

Table II.

Organ	Wet weight (g.)	Weight of fat (g.)	Yellow units	Blue units	Inference
Rat 5, ♂ (340 g.). Red palm oil diet, followed by vitamin A-free diet.					
Stomach and contents	2·2	0·0508	0	Reddish	?
Small intestine and contents	5·7	0·145	20	12·5	Carotene?
Large intestine and contents	3·0	0·15	1	Reddish	?
Liver	15·5	0·208	150	50,000	Vitamin A
Brain	2·3	0·019	0	0	—
Heart	1·1	0·0075	0	0	—
Kidneys	3·0	0·032	0	0	—
Lungs	2·4	0·025	0	0	—
Pancreas	0·78	0·015	0	0	—
Spleen	0·55	0·004	0	0	—
Suprarenals	0·04	0·004	0	0	—
Testes and seminal vesicles	1·93	0·0669	0	0	—
Thymus	0·85	0·007	0	0	—
Thyroids and parathyroids	0·6	0·0064	0	0	—
Muscles	117	3·9	10	40	Vitamin A
Intraperitoneal fat	23	12·2	18	120	Vitamin A
Bones	55	1·6	5	Reddish	?
Skin	76	13·0	40	Reddish	?
Rat 6, ♂ (300 g.). Red palm oil diet, followed by vitamin A-free diet.					
Small intestine and contents	5·0	0·5127	4	Reddish	?
Liver	14·2	0·1978	15	38,000	Vitamin A
Intraperitoneal fat	13·0	10·7	8	80	Vitamin A?

Rat 6, ♂ (300 g.). Red palm oil diet, followed by vitamin A-free diet (Table II). Preliminary feeding treatment was exactly as in the preceding case, but the final period on the vitamin A-free diet was now increased to 68 days. Only the small intestine and contents, liver, and intraperitoneal fat were examined. In the case of the small intestine a negative result was obtained. In the liver the concentration of vitamin A still remained at a high level, but no appreciable amounts of unconverted pigment were present. The intraperitoneal fat still gave a reaction suggesting the presence of vitamin A in relatively low concentration.

The excretion of carotene in the faeces of rats receiving excess of carotene.

Two samples of faeces were collected from the combined droppings of the rats receiving red palm oil, after the animals had received this diet for 131 and 234 days respectively.

The results obtained in both samples were very similar (Table III). It should be noted that in each case the yellow/blue ratio was about 6, pointing

to the presence of unconverted pigment. The concentration of pigment attained actually rose to quite a high level, representing, from calculations based on the $SbCl_3$ colour value, some 4 % of the faeces fat, or a 12-fold concentration over that of the ingested palm oil.

Table III.

Wet weight (g.)	Weight of fat (g.)	Yellow units	Blue units	Inference
Sample of faeces collected after 131 days of red palm oil feeding.				
1·54	0·097	4,500	750	Carotene
Sample after 234 days.				
7·3	0·458	23,000	3500	Carotene

The second sample of faeces fat was tested biologically as a source of vitamin A to rats, and was found active at a level of 0·25 mg. daily, which corresponds, on a basis of the $SbCl_3$ value, to a dosage of 0·01 mg. of actual carotene. The growth curves are shown in Fig. 2.

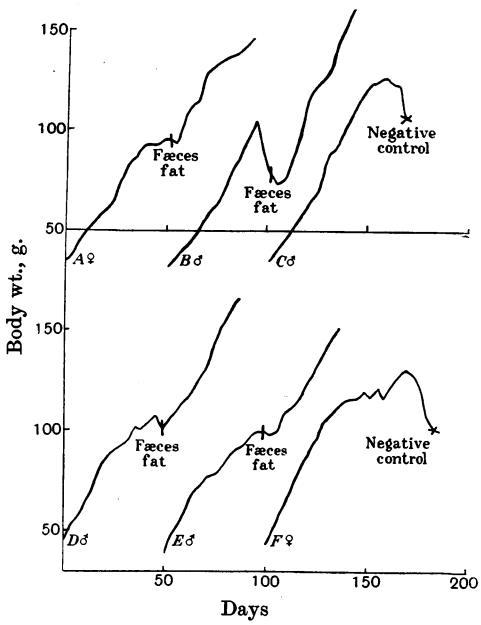

Fig. 2. The vitamin A activity of the faeces fat of rats receiving red palm oil. Rats *A*, *B* and *C* received a basal diet containing Glaxo caseinogen 20 %, rice starch 60 %, palm kernel oil 15 %, salt mixture 5 %, supplemented by 10 % of dried yeast and one drop of radiostol daily. Rats *D*, *E*, *F* received a diet differing only in the replacement of the palm kernel oil by additional rice starch. Doses of 0·25 mg. of faeces fat, equivalent to 0·01 mg. of carotene (1·8 B.U.) were administered in small amounts of arachis oil at the points indicated.

Discussion.

The site of the conversion of carotene. A most consistent feature of the above results is the persistence of carotene, apparently unchanged, within the alimentary tracts of those animals which had received diets rich in carotene

up to the time of killing. It is obvious, therefore, that conversion to the vitamin is not effected in the course of digestion, but at some period subsequent to absorption into the blood stream. Since the liver is unique not only in its ability to hold large stores of vitamin A, but also in containing noteworthy amounts of unchanged pigment it would seem unnecessary to look beyond this organ for the site of the conversion of carotene. Further indirect evidence by other workers may be quoted in support of this view. Rydbom [1930] has carried out experiments on the fate of carotene when injected intramuscularly into the legs of rats, and has recovered *post mortem* substantial amounts of the pigment in apparently unchanged state from the tissues surrounding the site of injection. This result would at least suggest that the power to convert carotene to vitamin A is not possessed indiscriminately by all animal tissues. The special importance of the liver, however, is most strikingly illustrated by the work of Buckley *et al.* [1930], who have encountered cases of parenchymatous degeneration of the liver in cattle, presumably produced by the eating of poisonous plants, in which this organ becomes so overcharged with carotene that it appears deep yellow in colour, and is capable of yielding large amounts of crystalline carotene on suitable treatment. Since in the present writer's experience normal ox-liver oils have invariably been found to contain large amounts of vitamin A, accompanied by relatively small amounts of carotene (so small as to render their isolation almost impossible by means at present available), the above condition presents a pathological picture of the failure of carotene conversion coincident with a degeneration of the liver tissues.

The capacity of the liver in vitamin A storage. Apart from suggesting the probable site of the conversion of carotene, the present experiments are of interest in demonstrating the extremely high levels to which the vitamin A reserves of the liver may rise when lavish amounts of carotene are included in the food supply. In the case of the rat these levels may attain values quite out of proportion to immediate or future requirements. Thus in rat No. 2 (Table I) the liver was found to contain vitamin A equivalent to 70,000 blue units in the $SbCl_3$ test. If we assume that 2 B.U. per day would have represented the minimal requirements of this animal, then this store would have been adequate to have lasted for about a century, as compared with the natural life period of about 3 years.

Vitamin A in the remainder of the body. In contrast with the high level attained in the liver the amounts of vitamin A distributed throughout the remainder of the body are relatively small, although in some cases representing a supply sufficient for two or three months. In confirmation of Sherman and Boynton [1925] indications of the presence of vitamin A were observed in the fats obtained from the lungs and kidneys of certain animals (Nos. 3 and 4, Table I). The colour values obtained agree well with the minimal rat doses of the fresh tissues as determined by these workers, although, of course, the conditions of vitamin A excess were quite different. It must remain doubtful, however, whether these results should be taken to imply

that the organs concerned play any outstanding part in the metabolism of the vitamin. Not only were negative results given by the same organs in two other rats (No. 1, Table I and No. 5, Table II) but even in the cases under review the superiority of the vitamin A content over the general level in the stored fat of the body was so small as to be of very doubtful significance. Indeed from the aspect of quantity as opposed to concentration the stored fat (*e.g.* intraperitoneal fat) certainly contained the great bulk (at least 90 %) of the total vitamin A reserves found outside the liver. The consistently negative results given by the remaining organs of rats Nos. 1 and 5, while not necessarily implying that vitamin A was completely absent, must similarly suggest that the concentration of vitamin did not rise above that of the stored fat, which would not have given appreciably positive results if only minute quantities similar to those obtained from the various organs, had been available for testing.

The rôle of the liver in the regulation of the distribution of vitamin A. When an attempt is made to co-ordinate the conclusions reached in the two preceding paragraphs it appears that the liver must play a large part in the regulation of the concentration of vitamin A throughout the remainder of the body. This rôle, of course, is linked up with that of storage, but whereas the function of the liver in receiving and concentrating superfluous amounts of vitamin A from the diet has always been fully realised, its complementary function in facilitating the maintenance of a low level of vitamin A concentration in the body tissues has not been sufficiently appreciated. The case of rat No. 2 (Table I) provides a good illustration of the importance of this function. The concentration of carotene in the red palm oil included in the diet of this rat was equivalent to about 0·6 B.U. per mg., while in the liver oil the concentration of vitamin A was equivalent to about 250 B.U. per mg. If, for the sake of simplicity in argument, we compare these values directly, without making any allowance for the fact that different chromogens are involved, then the concentration of vitamin A in the liver oil may be considered to correspond roughly to an activity some 400 times greater than that of the ingested red palm oil. When on the other hand we turn to the fat derived from the remainder of the body (carcase fat) we find that the concentration of vitamin A is equivalent to not more than 0·002 B.U. per mg., which corresponds to only 1/300 of the activity in the ingested fat, or less than 1/100,000 of the activity of the liver oil. From these results it may be inferred that, while the vitamin A concentration in the liver may vary over an enormously wide range without producing any obvious effect, the concentration in the remainder of the body does not rise above a prescribed limit.

The efficiency of the conversion of carotene. In the previous communication [Moore, 1930] a point of difficulty arose in explaining how cod-liver oil concentrates, supposed by Drummond and Baker [1929] to contain only a minute proportion of actual vitamin A, could approach pure carotene so closely in

vitamin A activity. It was suggested either that the estimate of Drummond and Baker might be unduly low, or alternatively that the conversion of carotene might be of an inefficient character, thus necessitating its administration at a dosage greatly exceeding the amount actually converted into the vitamin.

It may be recalled that carotene gave a value in the $SbCl_3$ reaction of about 180 B.U. (at 590 $\mu\mu$), and was effective in growth tests at levels down to about 0·004 mg. A typical cod-liver oil concentrate, on the other hand, gave a colour value of about 270 B.U. (at 610–630 $\mu\mu$) and was found in one instance to be biologically active at 0·0033 mg., although 0·01 mg. was necessary to ensure regular results. These figures indicated that vitamin A must at least have a slightly higher colour value than carotene, but since it was by no means necessary to suppose that the different blue colorations given by carotene and vitamin A bore exactly the same relation to biological activity, this evidence did not seem incompatible with the simple assumption that the conversion of carotene to the vitamin might be almost complete, and that vitamin A might after all represent the main constituent of the cod-liver oil concentrates.

The data obtained in the present experiments, however, must now lead to a revision of this view, since in several cases the rat-liver oils themselves (not their unsaponifiable fractions) gave colour values much higher than those previously determined for cod-liver oil concentrates[1]. Thus the liver oil of rats Nos. 3 and 4 (Table I) gave colour values of about 430 and 600 B.U. per mg. respectively. Preliminary experiments have indicated that the proportion of unsaponifiable matter in these oils is much greater than in cod-liver oils, but it is safe to assume that by saponification and the removal of sterols, etc. the colour values could be easily raised to a much higher level. Since there is every reason to believe that these colour values bear a genuine relation to the biological values of the oils (see Fig. 1) it must be inferred that preformed vitamin A not only possesses a much higher colour value than carotene, but also, under the usual conditions of administration, is biologically effective in much smaller doses.

Two alternative explanations of these findings might be advanced. In the first case it might be suggested that carotene is heterogeneous, containing in small amounts a component responsible for its biological and chromogenic activity, which is concentrated and converted to vitamin A in the liver. Secondly it might be supposed that the absorption and conversion of carotene is normally inefficient, but that actual conversion, when attained, is accompanied by a great increase in chromogenic value. The converted portion of the pigment, as vitamin A, now becomes available for utilisation by a second animal without a similar heavy loss, and the minimal dosage is therefore correspondingly reduced. This latter alternative is supported by the appearance

[1] The writer has been privileged to examine chicken-liver oils of approximately equal activity prepared by Mr N. S. Capper.

of excess of unchanged carotene in the faeces of the rats used in the present experiments, and for the present is perhaps to be preferred.

The effect of dietary vitamin A deficiency upon pre-existing reserves of vitamin A and carotene. The results obtained in the cases of rats Nos. 5 and 6 (Table II) are of interest in showing the effect of exposure to a diet deficient in vitamin A upon the reserves of vitamin A and carotene stored up during the period of excess. After receiving the deficient diet for periods of 10 and 68 days the vitamin A content of the liver oils remained extremely high. Similarly the intraperitoneal fats gave colour values differing but little from those shown by rats receiving carotene up to the time of killing. The most noteworthy change observed was the virtual disappearance of unchanged pigment from the liver oil of rat No. 6. The extremely low yellow/blue ratio (0·0004) shown by this oil supports almost to the degree of certainty the current view that pure vitamin A is completely colourless.

Summary.

1. Albino rats were given diets containing lavish amounts of carotene either as red palm oil or carrot-fat for prolonged periods. The animals were then killed, and estimations of vitamin A and carotene were carried out on the dissected tissues by colorimetric methods.

2. Excess of carotene was found to persist apparently unchanged throughout the alimentary tract. The pigmented fat derived from the faeces was found to be biologically active at a level based upon its apparent carotene content.

3. The liver oils invariably contained vitamin A at extremely high concentrations, the oils themselves in several cases giving higher colour values than typical cod-liver oil concentrates. Small amounts of unconverted pigment were also present in the liver oils of all rats which had received carotene up to the time of killing.

4. Indications of the presence of vitamin A were usually shown by the "storage" fats of the body, the concentration per unit of fat, however, being not more than about 1/100,000 of the concentration found in the liver oils. Similar indications were also given by the lung and kidney oils of certain rats, but in general negative results were obtained in all organs other than the liver. A single test upon blood also gave a negative result.

5. Exposure of the rat to dietary vitamin A deficiency subsequent to carotene feeding led to no dramatic departure from the above distribution, except that after 68 days of such treatment unchanged pigment had virtually disappeared from the liver oil.

6. From the above evidence it is deduced that the conversion of carotene to vitamin A probably takes place in the liver, that the efficiency of the conversion is by no means quantitative and that the liver plays an important rôle in the regulation of the concentration of the vitamin throughout the remainder of the body.

My thanks are due to Dr L. J. Harris for his valuable criticism, and to Mr K. MacLennan of Lever Brothers, Ltd. for supplies of red palm oil. The care of the experimental animals was in the reliable hands of Mr A. Ward.

REFERENCES.

Ahmad and Drummond (1930). *Biochem. J.* **24**, 27.
Buckley, Joss, Creech and Couch (1930). *J. Agric. Res.* **40**, 991.
Drummond and Baker (1929). *Biochem. J.* **23**, 275.
Kerppola (1930). *Skand. Arch. Physiol.* **56**, 181.
Moore (1929). *Lancet*, ii, 219.
—— (1930). *Biochem. J.* **24**, 692.
Palmer and Kennedy (1920). *J. Biol. Chem.* **46**, 559.
Rosenheim and Webster (1927). *Biochem. J.* **21**, 111.
Rydbom (1930). *Biochem. Z.* **227**, 482.
Sherman and Boynton (1925). *J. Amer. Chem. Soc.* **47**, 1646.