XL. VITAMIN A AND CAROTENE.

XI. THE DISTRIBUTION OF VITAMIN A IN THE ORGANS OF THE NORMAL AND HYPERVITAMINOTIC RAT.

By ALAN WILLIAM DAVIES AND THOMAS MOORE.

*From the Nutritional Laboratory, University of Cambridge
and Medical Research Council.*

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IN a previous paper Moore [1931] described the distribution of vitamin A and carotene, as determined by colorimetric methods, in the organs of rats which had received large amounts of carotene as red palm oil or carrot-fat. It was found on the one hand that unchanged carotene persisted throughout the alimentary tract, while on the other a high concentration of vitamin A accumulated in the liver. Outside the liver only small amounts of vitamin were detected. Thus the concentration in the storage fat was only about 1/100,000 of that found in the liver-fat, while in most other tissues, which of course contained only small percentages of fat, the amounts of vitamin present were too small to be detected by colorimetric means. In agreement with previous biological experiments by Sherman and Boynton [1925] however and with the subsequent work of Simmonet and Busson [1932] the kidney and lungs gave faintly positive reactions in some cases. It was concluded that the liver must not only be able to store vitamin A to a much greater extent than any other organ, but must also in a complementary sense play a large part in keeping the concentration of vitamin A throughout the rest of the body within prescribed limits. This question was discussed further in the case of the cow [Moore, 1932, 1].

The experiments described below were carried out as an extension of the previous work in order to keep pace with recent developments in our knowledge of the physiological behaviour and significance of vitamin A as studied from other angles. In the first place work on the vitamin A of human livers obtained at autopsy has shown that chronic nephritis is one of the most noteworthy of those diseases which are characterised by the occurrence of a high proportion of cases having subnormal vitamin A reserves [Wolff, 1932; Moore, 1932, 2], while the same tendency, although to a much less well marked extent, is noticed in bronchitis and pneumonia [Moore, 1932, 2; Fox, 1933]. It is therefore of interest to decide whether measurable amounts of vitamin A are normally present in the kidneys and lungs, and how far these organs are to be differentiated from others in this respect. Secondly it seemed desirable to extend the previous work to include rats which had received large amounts of vitamin A in place of carotene. Finally the ready availability of comparatively pure preparations of vitamin A has led to the confirmation by many workers of Takahashi's original finding that excess of vitamin A has a toxic action [Takahashi *et al.*, 1925; Harris and Moore, 1928; 1929; Drigalski, 1933; Moll *et al.*, 1933; Bomskov and Seemann, 1933;

Collazo and Rodriguez, 1933]. Knowledge of the distribution of vitamin A in the organs of rats suffering from hypervitaminosis must obviously be of interest as a step towards the elucidation of the cause of the toxic action.

EXPERIMENTAL.

Vitamin A was assayed colorimetrically, using the alkali digestion technique [Davies, 1933], in the organs of albino rats which had been given varying amounts of carotene or vitamin A for varying periods. In the case of small organs, or organs containing small amounts of vitamin, the fat obtained after digesting the tissues was made up in 1 or 2 ml. of chloroform instead of in the 5 ml. usually employed in routine work.

The presence of vitamin A in the kidneys of rats having moderate liver reserves.

Five rats which had been used in vitamin A tests, and which presumably possessed negligible vitamin A reserves at the end of the test period, were given for several weeks a diet containing carotene as 15 % of red palm oil, with the addition of carrots in some cases. Vitamin A was assayed in the liver, kidneys, lungs and (in 3 cases) the intraperitoneal fat. The results are given in Table I.

Table I. *Rats having moderate liver reserves.*

	Rat 1 ♂		Rat 2 ♂		Rat 3 ♂		Rat 4 ♂		Rat 5 ♂	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	900	90	1200	150	1500	150	1000	100	500	50
Kidneys	10	5	6	3	4	3	2	1	8	3
Lungs	6	3	>3	>1.5	0	0	0	0	0	0
Intraperi- toneal fat	—	—	—	—	—	0	—	0	—	2

It will be seen that the dietary treatment resulted in the accumulation of moderate vitamin A reserves in the liver¹. Under these circumstances small amounts of the vitamin were detected in the kidneys in all cases. Only in two out of five was a positive reaction given by the lungs, and in one case out of three by the intraperitoneal fat.

The distribution of vitamin A in the organs of rats given large, but non-toxic, doses of vitamin A concentrate.

Three adult rats were given about 3.5 mg. of B.D.H. vitamin A distillate (3000 B.U. per mg.) each daily for 38 days. They were anaesthetised and killed by bleeding. In order to facilitate the detection of vitamin A in tissues expected to contain only small amounts, the various organs from all three rats were combined after dissection. Results are shown in Table II.

In these rats the liver reserve was extremely high, being 160 times greater than the normal human reserve. The concentration in the kidneys was about 10 times greater than in the previous experiment, but even greater concentrations were found in the lungs and suprarenals, in both of which values were observed

¹ For purposes of comparison it may be mentioned that the "normal" human liver reserve in this country, taken as the median value over a wide range of cases dying by accident, is about 250 B.U. per g. A similar value was obtained in the case of a single wild rat shot in the vicinity of this laboratory.

Table II. *Rats given large, but non-toxic, amounts of vitamin A concentrate.*

Combined tissues from 3 adult ♀ rats, Nos. 6, 7, 8.

	Total B.U. per rat	B.U. per g.
Liver	300,000	40,000
Kidneys	70	50
Lungs	500	450
Suprarenals	100	2,500
Heart	1	1
Spleen	1	2
Pancreas	15	25
Thymus	4	12
Brain	0.5	0.3
Muscles	—	0.5
Blood	—	2
Intraperitoneal fat	—	80

which would be considered quite high for the liver in normally fed rats. In the brain, heart, spleen, muscles and blood only small traces of vitamin were detected, but slightly larger amounts seemed to be present in the pancreas, thymus and intraperitoneal fat. The value in the case of the pancreas may have been made unduly high through the inadvertent inclusion of fat deposits.

To obtain further evidence on the points of interest raised in this experiment further assays were carried out on the liver, kidneys, lungs, pancreas and suprarenals of four adult rats which had received large amounts of vitamin A concentrate. These animals differed from those just described in so far that the feeding of concentrate was discontinued some weeks before killing, an ordinary stock diet of bread and milk, cereals and vegetables being given in the meantime. The organs from each rat were kept separate. Results are given in Table III.

Table III. *Rats given large, but non-toxic, doses of vitamin A concentrate. Concentrate feeding discontinued some time before killing.*

	Rat 9 ♀		Rat 10 ♂		Rat 11 ♀		Rat 12 ♂	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	75,000	8,000	150,000	15,000	125,000	15,000	125,000	10,000
Kidneys	10	5	10	5	15	10	5	2
Lungs	250	150	60	35	75	50	125	60
Pancreas	0	0	5	5	0	0	0	0
Suprarenals	0	0	2.5	25	5	70	1.5	15

In most cases the concentration of vitamin A in the liver was about half that observed in the preceding experiment. The concentration in the kidneys was remarkably low, being little in excess of that found in the case of the rats having moderate reserves. In the lungs the concentration was again greater than in the kidneys but was much lower than the value found in the previous experiment. The amounts of vitamin present in the suprarenals were much smaller than in the preceding experiment, while in the pancreas a negative result was obtained in three rats, a trace being found in the case of one animal.

The distribution of vitamin A in the organs of rats given toxic doses of vitamin A.

Two young albino rats were given the following diet. "Light white casein" 20 %; rice starch 35 %; coconut oil 40 %; salt mixture 5 %; dried yeast 10 % (additional); radiostol (vitamin D) 1 drop per week. Vitamin A concentrate was given dissolved in the coconut oil at such a concentration that 40 mg. were

included in every 10 g. of diet. To avoid undue loss of the vitamin before ingestion by the rat the solution of the vitamin in the oil was stored separately and admixed with the remaining components of the diet daily immediately before feeding. The history of the rats was as follows.

Rat 13 ♂. Initial weight 95 g. Declined in weight from commencement of the experiment with the development of a hunched attitude, loss of hair round the mouth¹ and haemorrhagic rhinitis. Died after 12 days, final weight 60 g. Average daily food consumption 3.1 g.; consumption of concentrate 12.5 mg. per day. Total concentrate ingested, 150 mg. *Post mortem* examination revealed extreme emaciation and severe lung disease. Parts of the lungs were red, wet and soft, other parts were filled with putty-like hard nodules which were present in all sizes up to about 7 mm. in diameter.

Rat 14 ♀. Initial weight 81 g. Declined in weight from commencement with superficial appearances similar to those observed in the previous rat. Died after 33 days, the final weight being 55 g. Average daily consumption of food 3.6 g., of concentrate 14.3 mg. Total concentrate ingested 471 mg. At autopsy the lungs appeared slightly reddened but did not exhibit the gross lesions observed in the preceding rat. The immediate cause of death appeared to be extreme emaciation. The most striking feature, however, was the extreme softness of the bones. X-ray examination revealed that several of the larger leg bones had sustained spontaneous fractures, the broken ends of the bones being healed together in irregular shapes with the formation of calluses.

Table IV. *Rats given toxic amounts of vitamin A concentrate.*

	Rat 13 ♂		Rat 14 ♀	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	50,000	15,000	200,000	60,000
Kidneys	40	60	500	600
Lungs	2,500	1,250	1,000	1,000
Suprarenals	0	0	2.5	50
Heart	60	100	15	25
Spleen	0	0	2.5	15
Pancreas	0	0	1	10
Brain	25	15	20	15
Eyes	0	0	0	0
Testicles	0	0	—	—
Muscles	—	0	—	25
Bone	—	0	—	2
Total B.U. ingested	450,000	—	1,400,000	—
Found in faeces	1,500	—	6,400	—
Found in urine	0	—	0	—

The results of vitamin A assays carried out on the organs of these rats are given in Table IV. In the case of the rat dying after 12 days the liver reserve was considerably below the values obtained in some other cases. The lungs contained a very high concentration of vitamin, which in view of the diseased and unduly weighty condition is best judged in this case from the total vitamin present rather than the value expressed per g. of tissue. Considerable amounts of vitamin were present in the kidney and heart. A small amount was found in the brain. Other organs gave negative results.

¹ The loss of hair round the mouth would appear to be due to the irritant action of traces of diet remaining on the skin after feeding. It is noteworthy, however, that the concentrate exerts no immediate irritant action. Our solution of the vitamin A concentrate in coconut oil could be eaten by ourselves in small amounts without irritation or discomfort.

In the case of the rat dying after 33 days the concentration of vitamin A in the liver was four times greater than in the preceding rat. The total amount of vitamin present in the lungs was less, but in this case a high value was found for the kidneys. Significant amounts of vitamin were also found in all organs except the eyes¹.

In order to determine the efficiency of the absorption of vitamin A in these rats the faeces and urine were collected daily and stored in 5 % KOH until the end of the experiment, when vitamin A assays were carried out on aliquot portions by the usual technique. In each case the urine gave a negative result. Positive results were found for the faeces, but the amounts present were insignificantly small when compared with the difference between the amount of vitamin ingested and that found in the body. In each case only about $\frac{1}{7}$ of the ingested vitamin could be accounted for, the remainder presumably being lost by oxidation at some stage as yet unknown.

The distribution of vitamin A in the organs of rats given large doses of carotene.

Two albino rats from the same litter as those used in the preceding experiment were given the same diet except for the substitution of carotene (B.D.H.) in place of vitamin A. Owing to the limited solubility of the pigment it was found impossible to incorporate more than 10 mg. per 10 g. of diet (*i.e.* $\frac{1}{4}$ of the concentration of vitamin A in the preceding experiment), but since the food consumption was much greater the actual daily consumption of carotene was about half that of vitamin A. The animals grew rapidly and when killed after 34 days were found at autopsy to be perfectly normal. The storage fat was plentiful and quite colourless. Particulars were as follows.

Rat 15 ♂. Initial weight 95 g. Final weight 156 g. Average daily food consumption 8.3 g.; carotene consumption 8.3 mg. daily; total carotene ingested 281 mg.

Rat 16 ♀. Initial weight 83 g. Final weight 129 g. Average daily food consumption 7.9 g.; carotene consumption 7.9 mg. daily; total carotene consumption 268 mg. The distribution of vitamin A in the organs of these rats is shown in Table V.

Table V. *Rats given large amounts of carotene.*

	Rat 15 ♂		Rat 16 ♀	
	Total B.U.	B.U. per g.	Total B.U.	B.U. per g.
Liver	12,500	1,250	12,500	1,700
Kidneys	5	4	5	3
Lungs	30	30	20	12
Suprarenals	0	0	0	0
Heart	0	0	0	0
Spleen	1.5	3	0	0
Pancreas	2.5	10	0	0
Brain	0	0	0	0
Eyes	0	0	0	0
Testicles	0	0	—	—
Muscles	—	0	—	0
Bone	—	0	—	0
Intraperitoneal fat	—	5	—	2
y.U. carotene ingested	560,000	—	530,000	—
Found in faeces	55,000	—	25,000	—

¹ In view of the fact that the retina has been reported to contain considerable amounts of vitamin A [Smith *et al.*, 1931; Wald, 1933] this negative result should not be taken to imply that the vitamin was entirely absent, but it is evident that the concentration of the vitamin, if present, was not sufficiently high to compensate for the small amounts of tissue available for assay.

The vitamin A reserves found in the liver would represent high values for ordinary conditions of nutrition but are some 30 times lower than the maximum value obtained by giving vitamin A. In the kidney the concentration of vitamin was no greater than in the rats having only moderate liver reserves, but in the lungs the amounts present were of a slightly higher order. In each case a faintly positive reaction was given by the intraperitoneal fat, while in rat 15 traces of chromogen were also detected in the spleen and pancreas. In the case of the spleen the yellow/blue ratio indicated that the reaction was due to carotene.

In order to determine the efficiency of absorption of the carotene the faeces and urine were examined as in the preceding experiments, the amounts found representing about 10 % and 5 % of the ingested pigment in rats 15 and 16 respectively. Since the amount of vitamin A present in the body must in each case have represented only some 3–6 mg. of carotene, or about 1–2 % of the ingested carotene, it is obvious that the utilisation of carotene at such high levels must be very inefficient, and that the greater part of the pigment has not been accounted for.

DISCUSSION.

The distribution of vitamin A in the organs of the rat.

The above experiments indicate that small amounts of vitamin A (*ca.* 5 B.U. per g.) are consistently present in the kidneys even when the liver reserves are of a moderate order (*ca.* 100 B.U. per g.). Traces of the vitamin are sometimes present in the lungs under these conditions.

When the liver reserve is raised by the inclusion in the diet of large amounts of carotene or vitamin A the order of the kidneys and the lungs is reversed, since the lungs now invariably show a greater concentration of vitamin A. As far as can be judged both from the present experiments and from previous work [Moore, 1931] carotene feeding does not result in the accumulation of vitamin A in high concentration in any organ except the liver, the highest value observed being 30 B.U. per g. for the lungs of rats Nos. 15 and 16 in the present work. The feeding of vitamin A concentrate on the other hand may raise the concentration in the kidneys and lungs to values that would be high even for the liver in the case of animals receiving ordinary diets. At the same time appreciable amounts of vitamin may appear throughout the whole body. In the case of the suprarenal glands very inconsistent results have been obtained. For rats 6, 7 and 8 a very high average value (2500 B.U. per g.) was observed, but in other experiments the results were much lower and in some cases negative. It may be noted that in view of the small size of these organs the actual amount of vitamin involved was relatively small.

Hypervitaminosis A.

The production of toxic symptoms in the present work may be considered to provide further evidence as to the reality of hypervitaminosis A in so far as the preparation used was a distillate, as opposed to the non-distilled concentrates used, as far as we are aware, by other workers in this field. Drigalski [1933] has already shown that the toxic power of the concentrates is destroyed with the vitamin on ultra-violet irradiation, it is now evident that the vitamin potency and toxic power are stable to distillation and are not separated by this means. Although only two rats were used in the present work we were fortunate in observing almost all the symptoms described by previous workers. The lung lesions observed in rat 13 exactly resembled those described in several cases by Takahashi *et al.* [1925]. It is perhaps noteworthy that these lesions should occur

in the organ in which the greatest concentration of vitamin A outside the liver was located. In rat 14 the bone lesions were exactly similar to those described by Bomskov and Seemann [1933] and also Collazo and Rodriguez [1933], our own observations representing the third independent discovery of the phenomenon.

Speculation as to the mechanism of the production of hypervitaminosis A would be premature at the present stage, but two clearly established points may be worth mention. In the first place it is obvious that the liver possesses a very high toleration for the vitamin, since concentrations of the order observed in the hypervitaminotic rats (Nos. 13 and 14) were also observed in healthy animals (Nos. 6, 7 and 8). At the maximum level observed the vitamin must have constituted about 5 % of the dry weight of the liver and have represented a supply sufficient to last the rat for about 200 years if economically used. Secondly it is plain that in the case of rat 13 death through hypervitaminosis intervened at a point when the liver still contained less than half the maximum observed concentration of vitamin. This finding shows that death may occur while there is still room for a considerably greater accumulation of vitamin A in the liver and suggests that the production of hypervitaminosis does not depend on the absolute amount of vitamin A present in the organism but on the ingestion of the vitamin at a greater rate than that at which it can be stored by the liver or eliminated by the organism.

The absence of toxic symptoms after feeding excess of carotene.

The absence of any symptoms of hypervitaminosis in the rats (Nos. 15 and 16) given about 8 mg. of carotene daily suggests that it is virtually impossible to induce hypervitaminosis A by carotene feeding. This conclusion has already been reached by Kuhn and Brockmann [1933], but since these workers administered carotene at the rate of only about 1 mg. per day, which is little greater than the dosage of 0.75 mg. used by Moore [1930] in earlier experiments, it is obvious that further evidence on this point was desirable.

It is still possible, of course, that large masses of solid carotene, as opposed to solutions of carotene in oil, might possess toxic action. Even if this proved to be the case, however, the phenomenon observed would almost certainly be quite different from the hypervitaminosis induced by giving vitamin A concentrate, since a comparison of the vitamin A contents of the livers of rats 13 and 14, which were given large amounts of vitamin A concentrate, with those of rats 15 and 16, which were given large amounts of carotene, indicates that the rate at which the liver reserve can be raised by the conversion of carotene (50 B.U. per g. per day) is much slower than the rate of increase obtained by giving vitamin A (1200–1700 B.U. per g. per day). The superior rate and efficiency of absorption of vitamin A suggest that in intensive vitamin A therapy preformed vitamin A should be more efficacious than carotene. At levels approaching the minimum dose, as previously shown [Moore, 1933], vitamin A and carotene appear to be equally efficiently utilised.

SUMMARY.

1. Vitamin A was assayed by the antimony trichloride method in the organs of rats which had received varying amounts of vitamin A or carotene.
2. Even when the liver reserves were quite low (100 B.U. per g.) small amounts of vitamin A (5 B.U. per g.) were found in the kidneys and sometimes in the lungs.
3. When large, but non-toxic, doses (3.5 mg. daily) of vitamin A distillate were given to adult rats (*a*) the concentration of vitamin in the liver rose to

extremely high levels (40,000 B.U. per g.), (b) the concentration in the lungs was of the order found in the liver under normal conditions of nutrition; (c) the concentration in the kidney remained fairly low (50 B.U. per g.), (d) the concentration in other organs was extremely low, except that surprisingly high values were observed in the suprarenals (up to 2500 B.U. per g.).

4. Even more massive doses of the distillate (12–14 mg. per day) given to young rats proved toxic, causing emaciation, haemorrhagic rhinitis, loss of hair round the mouth and (a) in one rat, dying after 12 days, gross lung lesions, (b) in another rat, dying after 33 days, a peculiar softening and hollowing of the bones which had resulted in spontaneous fractures of the legs. The distribution of vitamin A resembled that found in rats given non-toxic doses except that the concentration in the lungs was even higher (1250 B.U. per g.), while in the rat dying after 33 days a high kidney value (625 B.U. per g.) was observed.

5. Attempts to induce hypervitaminosis A by feeding carotene failed. On account of its limited solubility in fats only 8 mg. of the pigment could be given daily. Rats receiving this amount grew rapidly and remained in perfect health. On killing after 34 days the liver reserve was found to be only moderately high (1250 B.U. per g.) pointing to only about 1 % absorption, as opposed to 15 % in the case of vitamin A. Only very small amounts of vitamin were present in the lungs and kidneys, negative results being obtained in all other organs.

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