XIX. VITAMIN A AND CAROTENE.

XII. THE ELIMINATION OF VITAMIN A FROM THE LIVERS OF RATS PREVIOUSLY GIVEN MASSIVE DOSES OF VITAMIN A CONCENTRATE.

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IN a previous paper [Davies and Moore, 1934] we presented data showing that very high concentrations of vitamin A may be attained in the liver of the rat when large amounts of vitamin A concentrate are included in the diet. It was calculated that the amount of vitamin A stored might represent as much as 5 % of the dry weight of the liver, and be sufficient, if used economically, to satisfy the normal requirements of the animal for a theoretical period of about 200 years. The experiment to be described below was carried out to decide whether this theoretical economy would hold good in practice, or whether the reserve would be dissipated more rapidly. Under the particular experimental conditions adopted, the second alternative has been found to be correct.

EXPERIMENTAL.

32 female piebald rats were used. Until 10 months old they had been employed for breeding purposes, receiving a mixed dietary which included milk, liver (once weekly), green vegetables and carrots in sufficient amounts to permit the accumulation of a very high concentration of vitamin A in the liver even without further treatment. After retirement from breeding they were given the same mixed diet until 18 months old, when they were taken for use in the present experiment.

Preliminary "saturation" of the liver with vitamin A. With the exception of 3 animals, which were used in determining the level of the vitamin A reserve attained on the previous mixed diet, all the rats were now given a diet containing large amounts of vitamin A concentrate ("light white casein" 20 %, sugar 50 %, coconut oil containing vitamin A 25 %, salt mixture 5 %, dried yeast 10 %, wheat germ 5 %). The amount of vitamin A was adjusted so that 10 g. of diet contained 2 mg. of vitamin A concentrate (=6000 B.U.). Groups of three rats were killed at intervals, and the vitamin A reserves per g. of liver were determined by our usual method [Davies, 1933]. Although the feeding of this diet was continued for 12 weeks the liver reserves, as indicated by the mean values of the groups of three animals, rose only slightly (about 25 %) above the level attained on the mixed dietary. At this level a maximum value for these particular conditions of feeding appeared to have been reached.

The mean vitamin A reserves for each group of rats during this period are shown in the ascending part of the graph in Fig. 1. Although the values obtained in the individual rats of each group (see Table I) showed considerable variation from the mean value (usually about 33.3 %), the means for the groups

gave a consistent series of values except for one group, which gave an anomalous mean indicated by point *A* in Fig. 1. One of the rats in this group gave an unexpectedly low result (1400 B.U. per g. as compared with the expected mean of 14,000 B.U. per g.).

Fig. 1. *B*, Actual rate of decline. *C*, Calculated rate of decline of liver reserve assuming expenditure to be in accordance with physiological requirements. ↑ Rats given vitamin A-free diet. ↓ Rats given large amounts of vitamin A concentrate.

Table I.

Weeks from commencement of experiment	Weeks on vitamin A-free diet	Individual vitamin reserves. B.U. per g.			Mean vitamin A reserve B.U. per g.	Remarks
0	—	7,400	16,000	19,000	14,000	As taken from breeding stock
1	—	11,000	19,000	11,000	14,000	Vitamin A concentrate given
2	—	1,400	11,000	8,000	6,700	do.
4	—	1,100	16,000	17,500	15,000	do.
7	—	10,500	24,000	19,000	18,000	do.
12	—	14,000	20,000	19,000	18,000	do.
16	4	3,500	3,500	1,100	2,700	Vitamin A-free diet
20	8	2,500	2,500	2,400	2,500	do.
24	12	460	250	475	400	do.
36	24	600	500	470	500	do.

Dissipation of the vitamin A reserve on a diet deficient in vitamin A. The surviving rats were next given a diet having a composition similar to that recently proposed for use in vitamin A tests by Morgan¹ [1934] (extracted meat meal 10 %, coconut meal 30 %, ground rice 48 %, dried yeast 8 %, salt mixture 4 %, radiostol 1 drop per rat per week). This diet was found to be virtually free from vitamin A by independent growth tests with young rats.

¹ Our thanks are due to Lever Brothers, Ltd. for the gift of the special materials used in this diet.

Groups of three rats each were killed after they had received the vitamin A-free diet for periods of 4, 8, 12 and 24 weeks. The rapid fall in vitamin A reserves is shown in the descending part (*B*) of the graph in Fig. 1. After 4 weeks the mean liver reserve was found to have fallen to about $\frac{1}{6}$ of its previous value (2700 B.U. per g. as compared with 18,000 B.U. per g.), after 8 weeks the mean value (2500 B.U. per g.) was much the same as after 4 weeks, but after 12 weeks there was a further fall to 400 B.U. per g. The value obtained after 24 weeks (500 B.U. per g.), however, was virtually the same as that observed after 12 weeks, suggesting that a level of more stable storage had now been reached.

DISCUSSION.

The above experiment proves that superfluous reserves of vitamin A held in the liver may sometimes be eliminated at a much greater rate than that demanded to satisfy the known physiological requirements of the organism. In Fig. 1 a broken line (*C*) has been drawn to represent the rate of decrease in the mean vitamin A reserve per g. of liver which would have been expected if the vitamin had been used up at a rate consistent with physiological requirements, on the assumption that 0.2 γ (or about 1 B.U.) of pure vitamin A per g. of liver would be metabolised daily¹. It will be seen that the rate of decrease of the mean reserve on this basis would be extremely slow, amounting to less than 1 % of the initial value during the 24 weeks under investigation. In contrast the mean reserve actually fell at the rate of about 100 γ (=500 B.U.) of pure vitamin A per g. of liver per day during the first 4 weeks after the adoption of the vitamin A-free diet, while after 12 weeks about 98 % of the initial reserve had been eliminated. At this point there was a definite check in the rate of expenditure of the vitamin, since no further decrease could be detected after a further period of 12 weeks. It may be significant that the level of storage was now of the same order as the value of 250 B.U. per g. which we would consider a typical value for the healthy human being².

The above results stand in contrast with those obtained by Dann and Moore [1931], who used rats whose vitamin A reserves had been raised to high levels (up to 7000 B.U. per g.) as the result of their receiving red palm oil, a rich source of carotene. The vitamin A reserves of these rats after they had been restricted for 5-6 weeks to a diet deficient both in vitamin A and B were found to be as high (within the limits of experimental error) as those of similar rats which received the diet containing red palm oil up to the time of killing. Moore [1931] also failed to observe any spectacular reduction below the reputed initial values in the vitamin A reserves of two rats kept for periods of 10 and 68 days on a vitamin A-free diet, although after the longer period it is possible that a deterioration of up to 40 % may have occurred. From Fig. 1 the reduction during the same period in the present experiments must have amounted to 90 %.

Two explanations of the divergence between the present results and those obtained in previous work are possible. The more rapid decline of the vitamin A reserve in the present experiments may be due either to the use of a different form of vitamin A-free diet, or to the fact that the reserves were built up from alimentary supplies of preformed vitamin A instead of carotene which was used

¹ This estimate is more generous than that previously assumed [Moore, 1931].

² Data kindly made available by Dr A. Leese indicate that the vitamin A reserves per g. of liver in wild rats, as determined for specimens caught in the Edinburgh Zoological Gardens, are of about the same order as in the human being.

in the previous work. Both these possibilities are now under investigation, but in view of the time-consuming nature of the work we have thought it advisable to publish an account of our results at their present stage of development.

As a point of secondary interest arising in the course of the present investigation, we may draw attention to the extremely high level which the vitamin A reserve of the liver of the rat may reach in rats receiving an ordinary dietary containing carrots, green vegetables and moderate amounts of liver. According to our observations the concentration of vitamin A produced under such conditions is only slightly lower than that which may be obtained by the feeding of vitamin A concentrate.

SUMMARY.

Adult rats, already possessing high liver reserves of vitamin A, were given a diet containing massive doses of vitamin A concentrate. The liver reserves under these conditions reached levels having a mean value of about 18,000 B.U. per g., representing a supply sufficient to satisfy the theoretical requirement of the rat for about a century, if used up at a rate corresponding to the minimum physiological requirements.

When the rats were subsequently restricted to a diet free of vitamin A, however, a rapid elimination of vitamin A from the liver took place, the mean vitamin A reserve falling to 400 B.U. per g. after 12 weeks. At this level a condition of stable storage of the vitamin appeared to have been attained, since no further fall in the mean reserve could be detected after a further period of 12 weeks of restriction to the vitamin A-free diet.

These results stand in contrast with those obtained in previous experiments carried out in this laboratory, in which a similar rapid elimination of vitamin A from the liver was not observed. The possible causes of the difference are discussed.

Our thanks are due to Dr L. J. Harris for his valuable criticism.

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