CII. THE EFFECT OF VITAMIN B DEFICIENCY UPON THE VITAMIN A RESERVES OF THE RAT.

BY WILLIAM JOHN DANN AND THOMAS MOORE¹.

From the Nutritional Laboratory, Cambridge.

(Received May 2nd, 1931.)

IN a previous communication [Moore, 1931] it was shown that when rats are given diets rich in carotene, vitamin A may accumulate in the liver in amounts that seem fantastically great when compared with the minimal daily requirements of the animal. Further, if a rat which had been fed on carotene in this way were then placed upon a diet free from vitamin A the reserves of that factor did not materially decrease after a lapse of 60 days. These findings confirmed the current view that the liver possesses the power of concentrating vitamin A and storing it over prolonged periods without deterioration. In distinct contrast the fats of the other organs, together with the general storage fat of the body, continued to contain only small traces of the vitamin.

This one-sided distribution of the vitamin A reserves suggested the problem which forms the subject of the present communication. It is well known that a rat subsisting upon a diet which is deficient in the vitamin B complex loses weight progressively, and that this loss of weight is accompanied by disappearance of the body fat. In so far as the writers are aware, however, no observation has been placed on record to show whether this loss of fat is accompanied by a corresponding loss of fat-soluble vitamins. It was considered of interest, therefore, to examine the vitamin A reserves of the liver under these conditions, especially since two distinct possibilities seemed open. On the one hand, it might be supposed that in the severe emaciation resulting through vitamin B deficiency the great superfluity of vitamin A, serving no obvious purpose, would be dispersed. On the other hand, the vitamin A reserves of the liver, being concentrated there by means of some mechanism quite distinct from that regulating the decomposition and resorption of storage fat, might be expected to show no change, at least so long as the liver itself remained undamaged. This second hypothesis received *a priori* support from the observation of Drummond and Hilditch [1930] that the vitamin content of cod-liver² is not influenced by the variations in fat content associated with the sexual cycle, and in the present paper it is shown to be correct.

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

² It may be pointed out that the liver of the cod may contain almost 40 % of fat, whereas in mammalian livers the fat content does not exceed about 2 %. The liver in the cod must therefore play a much more important part in the economy of stored fat than is the case with mammalian liver.

EXPERIMENTAL.

The experimental animals used were adult albino rats which had been reared to maturity upon a complete synthetic diet and subsequently maintained for a prolonged period (about 200 days) upon a diet rich in carotene ("light white casein" 20 %, rice starch 60 %, red palm oil 15 %, salt mixture 8 %, dried yeast 15 %, (additional) radiostol 1 drop per rat daily). Under these conditions the vitamin A reserves of the liver, as previously reported [Moore, 1931]¹, had been found to be remarkably constant between individuals, the usual value being about 70,000 blue units. The experimental procedure consisted of the exposure of the rats to diets deficient in the vitamin B complex until severe emaciation had resulted. The animals were then examined *post mortem* both in regard to the extent of the remaining body fat and the amount of vitamin A still contained in the liver oils.

Vitamin A reserves of the rat when fed upon a complete diet with excess of carotene. Rat 1 ♀. This animal, weight 220 g., served as a control. It was killed during the present experiment, without changing from the complete red palm oil diet, in order to ensure that the vitamin A reserves of the batch of rats used should not depart materially from the value expected through previous experience. The liver only was examined.

	Weight of fat mg.	Total natural yellow units	Total SbCl ₃ blue units
Liver	154	150	70,000

Persistence of the vitamin A reserves during continued feeding of carotene accompanied by vitamin B deficiency. Rat 2 ♂. From the complete diet containing red palm oil this rat was transferred to a diet deficient in the vitamin B complex which still contained red palm oil (Glaxo caseinogen 20 %, cane sugar 60 %, salts 5 %, red palm oil 15 %). On this diet it declined rapidly and died after 48 days, having decreased in weight from 378 g. to 171 g. *Post mortem* examination showed complete absence of fat depôts and general emaciation. Only the liver was analysed for fat and vitamin A.

	Weight g.	Weight of fat mg.	Total natural yellow units	Total SbCl ₃ blue units
Liver	6.7	114	130	110,000

Persistence of vitamin A reserves on a diet lacking both vitamin A and vitamin B. Rat 3 ♀. From the complete diet containing red palm oil this rat was transferred to a diet similar to that given to rat 2 with the exception that arachis oil now replaced the red palm oil as the fat component. The diet was therefore completely free from vitamins. The body weight fell from 266 g.

¹ The method employed in the extraction of tissues, although essentially the same as that previously described [Moore, 1930], has now been modified to permit more rapid estimations. By the addition of 50 % of alcohol to the alkaline solution of the tissue it has been found possible to remove nearly all the fat by one extraction with about 3 volumes of ether. After washing, the ethereal extract is dried by filtering through a layer of anhydrous sodium sulphate contained in a funnel provided with a sintered glass diaphragm.

to 168 g. in 35 days when the animal, being extremely emaciated, was killed. *Post mortem* examination revealed that the remains of the fat depôts were very scanty. The liver was dissected out and examined in the usual way. In addition the remainder of the carcass, including the pelt and intestinal tract and contents, was extracted with a view to finding the actual amount of fat present.

	Weight g.	Weight of fat mg.	Total SbCl ₃ blue units
Liver	6.8	120 mg.	68,000
Carcass	161	9.0 g.	Very low

Rat 4 ♀. This animal received the same diet as the preceding one but was killed after the slightly longer period of 41 days, the body weight falling from 202 g. to 126 g. At the end of this time it appeared extremely emaciated but was still active. The autopsy revealed a complete absence of intraperitoneal fat. Analyses were made separately of the liver, the intestinal tract and contents, the pelt and the remainder of the carcass.

	Weight g.	Weight of fat mg.	SbCl ₃ blue units
Liver	5.2	78	70,000
Intestinal tract	13.2	181	200*
Pelt	19.6	398	0
Carcass	85.1	114	0

* The meaning of this positive result is not clear, but faulty dissection may be suspected. This value could be accounted for by the unintentional inclusion of about 20 mg. of liver tissue.

Spectroscopic data. In the case of the liver oils examined above, the blue colour produced in the SbCl₃ reaction was characterised by the prominent band at 610–630 μ which is associated with vitamin A. The natural yellow colour, when not stated, was not more than a trace. The chromogen present must therefore have consisted almost exclusively of vitamin A.

DISCUSSION.

It is at once evident from the data given that the remarkably high reserves of vitamin A which the rat is able to store up when fed on a diet rich in carotene are not depleted when it is concurrently or subsequently suffering from a lack of the vitamin B complex. The figures for rats 2, 3 and 4 show that in the state of extreme emaciation following on a deficiency of the vitamin B complex the liver oils still contained the characteristically very high vitamin A content observed by Moore [1931]. At the same time, the observation that the liver oil of rat 2 was of a normal pale colour indicates that vitamin B deficiency in no wise diminishes the power of the animal to transform carotene to vitamin A when the pigment is present in the diet in very great excess of the animal's requirements.

The data for rat 4 are particularly instructive in showing how far the loss of body fat can go without materially affecting the large vitamin A store of the liver. The total amount of fat which could be extracted from the four parts into which the body was divided was less than 1 g. The liver fat may

have been depleted (it is not quite certain whether the low figure of 78 mg. is sufficiently below the normal one of approximately 150 mg. to allow of this conclusion being drawn) but there is no doubt that the vitamin A store of the liver remained unaffected, since the figure of 70,000 blue units obtained is representative of those found for rats still receiving the complete red palm oil diet.

These results confirm the view that the vitamin A reserves of the liver constitute a system quite apart from the general fat reserves of the body. To complete the picture mention may be made of a complementary condition which is sometimes encountered in rats suffering from avitaminosis A. It is certainly true that rats surviving during long periods of decline in this condition become no less emaciated than those suffering from avitaminosis B, but when death is more sudden quite large fat deposits may remain even though no traces of vitamin A can be detected colorimetrically either in the liver or in the body fat. Thus the body of a young male rat, typical of several others, which died rather suddenly at a weight of 174 g. after 61 days' exposure to a diet deficient in vitamin A, showed almost normal fat deposits, amounting to 16.2 g., while the liver yielded 144 mg. of oil. Nevertheless, both the body fat and liver oil gave completely negative results when tested for vitamin A.

SUMMARY.

Adult albino rats, previously fed upon diets containing lavish amounts of carotene, were exposed to diets deficient in the vitamin B complex until severe emaciation had resulted. In spite of the disappearance of the fat reserves throughout the body this treatment did not lead to any material diminution in the vitamin A reserves of the liver, which remained at an extremely high level.

Our thanks are due to Dr L. J. Harris for his valuable criticism. The care of the experimental animals was in the capable hands of Mr A. Ward.

REFERENCES.

- Drummond and Hilditch (1930). The relative values of cod-liver oils from various sources (H.M. Stationery Office).
Moore (1930). *Biochem. J.* **24**, 692.
— (1931). *Biochem. J.* **25**, 275.