CLVII. THE ABSORPTION OF NITROGEN AND OF FAT FROM THE ALIMENTARY CANAL OF RATS KEPT ON A VITAMIN A-DEFICIENT DIET.

BY MYRA MELISSA SAMPSON, MARJORIE DENNISON
AND VLADIMIR KORENCHEVSKY.

From the Lister Institute, London.

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THE factors responsible for the pathological condition leading to failure of normal growth in rats kept on a vitamin A-deficient diet are not exactly known. Since the appetite of such rats is less than that of normal rats, the most obvious explanation of the decreased rate of growth would be an insufficient food intake. A second possible cause of the disturbance of rate of growth might be a decreased absorption of food from the alimentary canal. This latter is suggested by the fact that frequently gastro-intestinal disturbances are associated with this dietetic defect. In this paper a preliminary investigation of these two suggestions is described.

Technique.

A litter of four male rats was used for the experiment. Two of the rats were placed on a vitamin A-deficient paste diet and two on the same paste supplemented by cod-liver oil, given by pipette. In addition to the paste, the vitamin A-deficient rats were given as many milligrams of cotton-seed oil as the control rats received of cod-liver oil.

The rats were placed on the experimental diet at the age of 33 days and kept in the usual cages for 24 days, by which time the rate of growth of the rats on the deficient diet had begun to decrease. At the age of 58 days, on the 25th day of feeding with the experimental diet, the rats were placed in metabolism cages in a special room kept at a constant temperature, and all the necessary precautions for accurate metabolism estimations were taken, as described in a previous paper [Korenchevsky and Dennison, 1932]. The paired feeding method was used in these experiments. The rats on the vitamin A-deficient diet, Nos. 1756 and 1757, however, were allowed to consume the paste *ad libitum* since the main object of the experiment was an estimation of the appetite and the absorption of the food. The control rats, Nos. 1758 and 1759, received the same quantity of food, the amount given being equal to the amount eaten on the previous day by rats 1756 and 1757 respectively.

Although the daily food intake was not kept constant some conclusions on nitrogen metabolism can be made owing to the fact that the rats were paired and that the food intake per 200 g. of body weight of the controls was little less than that of the rats on the vitamin A-deficient diet.

In order to avoid decreasing the amount of food given to the control rats on complete diet to such an extent as to be injurious to their condition, each pair was killed on the day that the vitamin A-deficient rat of the pair showed considerable loss of appetite. As can be seen from the composition of the paste, given below, the content of vitamin B in the form of dried yeast was very high. On such a vitamin A-deficient diet a considerable loss of appetite occurs only a few days before death. By observing these precautions the control rats were kept in a healthy condition throughout the experiment, in spite of the adjustment of the consumption of the diet to that eaten by the vitamin A-deficient rats.

The paste, as in previous metabolism experiments, was given in a semi-liquid form in order to prevent the rats from dropping the food inside the cage, and so into the urine and faeces containers. The composition of the paste was as follows:

						Parts by wt.
Heated caseinogen	...	...	...	...	...	18
Wheat starch	...	...	...	...	...	50
Cotton-seed oil (irradiated $\frac{1}{2}$ hr.)	...	...	...	...	...	15
Salt mixture	...	...	...	...	...	5
Dried yeast	...	...	...	...	...	18.3
Lemon juice	...	...	...	...	...	5
Water	...	...	...	...	...	181.7

The cotton-seed oil in the amount used in the diet was considered to be a sufficient source of vitamin E [Evans and Burr, 1927]. The control rats always ate their ration completely.

For the estimation of the general absorption from the alimentary canal, the absorption of nitrogenous substances (*i.e.* mainly proteins) and of fat was studied, since the digestion and absorption of these substances are more complicated processes than in the case of carbohydrates or salts and are, therefore, a clearer indication of the state of absorption in general. The estimation of nitrogen in the food, food residues, urine and faeces was made by the Kjeldahl method, whilst for the estimation of fat the Holt, Courtney and Fales [1919] method was used. In this method the soaps of the dried material are first hydrolysed by aqueous HCl, and to this acid mixture is added an equal volume of 96 % alcohol. This is then extracted with ethyl ether and light petroleum in the cold in a special apparatus. In our experience this method of fat estimation gives satisfactory results and we wish to express our thanks to Dr G. A. Harrison for being so kind as to demonstrate the method to us.

Four day periods were chosen for nitrogen and fat estimations, such periods

being sufficiently long to overcome the difficulty of accurate separation of the urine and faeces of each period [Korenchevsky and Dennison, 1932]. However, in order to economise space, the results of the experiments (summarised in Tables I, II and III) are given as averages for 8 day periods, except in the last period, in which, because of the considerable loss of appetite, the periods for the two pairs of rats were 2 and 3 days respectively. At the end of this period the rats were killed, the pair, Nos. 1756 and 1758 on the 84th day of age and the pair, Nos. 1757 and 1759, on the 85th day of age, having been on the metabolism experiment 26 and 27 days respectively.

*The influence of a vitamin A-deficient diet on the appetite
and weight of the rats.*

In Table I the figures in the columns "N intake" and "fat intake" show a slow decrease in the appetite of rats 1756 and 1757 kept on a vitamin A-deficient diet. A very considerable reduction was noted on the last day of the last period (IV), thus decreasing the figure for this period, which, although it lasted only 2 or 3 days, was calculated, for the sake of comparison, for a week. In the first three periods the control rats, Nos. 1758 and 1759, kept on a complete diet, received per 200 g. of body weight less food than those rats with which they were paired, *viz.* 1756 and 1757 respectively, as shown by the nitrogen and fat intake (Table I).

Table I. *The absorption of nitrogen and of fat from the alimentary canal of the paired rats 1756 and 1758, 1757 and 1759 per week, per 200 g. of body weight.*

No. of rat	No. of period	Average wt. of rat g.	Intake		Urine N g.	Faeces			N balance g.	Fat absorbed g.
			N g.	Fat g.		Wt. g.	N g.	Fat g.		
1756 on - A diet	I	140	3.69	15.17	2.51	5.02	0.33	0.81	+0.85	14.36
	II	150	3.33	13.66	2.42	5.19	0.34	0.83	+0.57	12.83
	III	157	3.00	12.31	2.44	4.42	0.30	0.73	+0.26	11.57
	IV	150	1.36	5.59	1.73	2.88	0.20	0.56	-0.57	5.03
1758 on complete diet	I	170	3.34	13.71	2.12	4.85	0.30	0.73	+0.92	12.98
	II	191	2.79	11.47	1.81	4.01	0.27	0.70	+0.71	10.77
	III	212	2.54	10.44	1.80	3.33	0.23	0.53	+0.51	9.91
	IV	216	1.75	7.20	1.41	2.98	0.19	0.53	+0.15	6.67
1757 on - A diet	I	152	3.88	15.93	2.54	5.72	0.37	0.79	+0.97	15.14
	II	162	3.03	12.45	2.36	4.49	0.29	0.74	+0.38	11.71
	III	161	2.90	11.90	2.33	3.85	0.27	0.64	+0.33	11.26
	IV	157	2.19	9.00	2.14	4.06	0.28	0.74	-0.23	8.26
1759 on complete diet	I	163	3.84	15.79	2.22	6.18	0.40	0.87	+1.22	14.92
	II	185	2.89	11.85	2.01	4.34	0.31	0.71	+0.57	11.14
	III	195	2.63	10.79	1.89	3.35	0.24	0.59	+0.50	10.20
	IV	204	2.49	10.23	1.87	4.08	0.28	0.72	+0.34	9.51

Table III shows the changes in weight compared with the food intake from the beginning of the experiment to the end of the period III, *i.e.* during the first 24 days of the metabolism experiment when the appetite, although less, was still satisfactory. The figures for the last period IV are not included in this Table since in this period the difference in the amounts of food consumed by the deficient and normal rats was considerable. The figures in Table III

are the actual figures, and not the figures calculated per 200 g. of body weight as in Tables I and II.

Table II. *Percentage of the nitrogen intake excreted in the urine, deposited in, or lost from, the body and non-absorbed from the alimentary canal; also percentage of the fat intake non-absorbed.*

No. of rat	No. of period	% N faeces to N intake	% N urine to N intake	% N balance to N intake	% fat non-absorbed to fat intake
1756 on -A diet	I	8.94	67.9	+23.0	5.33
	II	10.21	72.7	+17.1	6.07
	III	10.00	81.3	+ 8.7	5.93
	IV	14.70	127.2	-41.9	10.02
1758 on complete diet	I	8.98	63.5	+27.5	5.32
	II	9.67	64.8	+25.4	6.10
	III	9.05	70.9	+20.1	5.08
	IV	10.85	80.6	+ 8.6	7.36
1757 on -A diet	I	9.53	65.4	+25.0	4.96
	II	9.56	77.9	+12.5	5.94
	III	9.30	80.3	+10.3	5.37
	IV	12.78	97.7	-10.5	8.22
1759 on complete diet	I	10.41	57.8	+31.8	5.50
	II	10.72	69.5	+19.7	5.90
	III	9.12	71.8	+19.0	5.47
	IV	11.24	75.1	+13.6	7.13

Since the control rats were growing more quickly than the vitamin A-deficient rats the amount of food per 200 g. of body weight given to and completely consumed by the rats on a complete diet was less than that consumed by the rats on the vitamin A-deficient diet.

It should also be noted that it was only possible to make an approximate, immediate, estimation of the food eaten on the previous day by the vitamin A-deficient rats, since this was calculated by subtracting the weight of the residue of the food left by the rat from the weight of food given. There is, however, always during the 24 hours, some evaporation of water from the paste left unconsumed.

In later experiments, which will be described in the next paper, the error due to this evaporation is, as far as possible, corrected. For our conclusions, however, it was fortunate that the control rats on a complete diet were allowed less food per 200 g. of body weight than was consumed by the rats on the vitamin A-deficient diet, and in spite of this grew much better. This is especially evident with the pair of rats Nos. 1757 and 1759, for while the rat on deficient diet stopped growing during the 16 days of periods II and III (Table I) and finally lost weight, rat 1759 continued to grow until it was killed. Certainly there is little doubt that the control rats would gain in weight more if the amount of food given were increased.

The rats kept on the vitamin A-deficient diet, however, do not seem to be able to increase in weight in normal correlation to the amount of food ingested. If, from the actual increase in body weight and the actual nitrogen intake of the four experimental rats the increase in body weight per g. of nitrogen intake is calculated, the above statement would seem to be corro-

borated (Table III). Thus, whilst 1 unit of vitamin A-deficient food, as measured by 1 g. of nitrogen, produced an increase in body weight of only about 2.5 g., the same amount of complete diet produced an increase in the weight of the two control rats of 6.54 and 5.55 g. respectively, *i.e.* the increase in weight in the control rats was on an average 143 % greater than in the deficient rats.

Table III. *Gain in body weight per g. of nitrogen intake during the experimental period of the first 24 days.*

No. of rat	Total gain in weight (actual) g.	N intake (actual) g.	Gain in weight per g. of N intake g.
1756 - A	21	8.47	2.48
1758 Nor.	61	9.31	6.54
1757 - A	22	8.80	2.50
1769 Nor.	53	9.54	5.55

From the above experiments it is obvious that:

(1) the body weight of rats kept on vitamin A-deficient diet did not increase in normal correlation to the amount of food ingested;

(2) during the periods when the growth of the rats is checked or remains stationary the decrease in the appetite, with the consequent decrease in food intake, of rats kept on a vitamin A-deficient diet could only partly explain the decrease in rate of growth, and as a chief cause is probably only applicable to the last stages of the deficiency.

Nitrogen and fat absorption.

The comparison of the figures for nitrogen and fat excreted in the faeces, *i.e.* non-absorbed, shows that absorption during all the periods except the last was slightly less in only one of the rats on the vitamin A-deficient diet, No. 1756, as compared with its paired control, No. 1758 (Table I). Only in the last period was the absorption considerably affected in both deficient rats. When absorption of nitrogen and fat is expressed as the percentage of the intake (Table II) it is evident that such a small decrease in the absorption cannot explain the difference in growth.

This conclusion is seen to be justified in view of the results obtained with the second pair of rats Nos. 1757 and 1759, there being no constant appreciable difference in absorption in these two rats during the first three periods (Tables I and II), whilst the difference in growth still occurred.

It is true that during the last (fourth) period (just before the rats were killed) the percentage of non-absorbed nitrogen and fat (Tables I and II) was more pronounced in the deficient than in the control rats. It must be mentioned, however, that in rat 1756 there was an inclination to diarrhoea, and only in this rat was the non-absorbed nitrogen raised to 14.7 % and the non-absorbed fat to 10.02 %. During this last period, close to death, the absorption of nitrogen and fat were decreased more noticeably but still not very greatly.

Taking all the above data into consideration the following statements can be made.

(1) Except for a period of a few days before being killed, the absorption of nitrogenous and fatty substances from the alimentary canal was very slightly, if at all, decreased in rats kept on the vitamin A-deficient diet.

(2) Therefore the checking or cessation of growth of rats on a vitamin A-deficient diet cannot be explained by a decrease in the function of absorption of food from the alimentary canal.

(3) Only during the last period before death, when the vitamin A-deficient rats have begun to lose weight, and also to have an inclination to diarrhoea, does any impairment of the function of absorption become apparent and even then not to a very great extent.

Nitrogen metabolism.

Since the rats were young, nitrogen deposition for growth should be considerable. In both the vitamin A-deficient rats, as compared with their paired controls, although the nitrogen intake per 200 g. of body weight was greater, the deposition of nitrogen in the body was less (Table I). The reduction in food intake also produced a greater reduction in the nitrogen deposition in the deficient rats than in the rats on the complete diet. Table II shows also that in the vitamin A-deficient rats the percentage of the nitrogen intake excreted in the urine was greater, while that deposited in the body was less than in their respective controls.

These facts indicate that from the beginning of the metabolism experiment, namely, from the 25th day of feeding with the vitamin A-deficient diet to the end of the experiment (*i.e.* for about 4 weeks), the nitrogen metabolism of these rats was increased, during some periods to a marked degree (compare the periods II, III and IV in Tables Nos. I and II).

DISCUSSION.

From this observation on the loss of appetite and consequent decrease in food intake, and also in some instances a lessened absorption from the alimentary canal in rats kept on a vitamin A-deficient diet, it is possible to conclude that these factors alone cannot explain completely the checking of growth which was observed. In rats kept on a vitamin A-deficient diet, during the period preceding death, when the loss of appetite becomes considerable, the influence of the decreased food intake on the rate of growth seems to become the important, and probably, the chief factor. Even in this period, however, and more particularly in the previous periods, the increased nitrogenous metabolism (*i.e.* chiefly protein metabolism), may be one of the factors which check the growth of the vitamin A-deficient rats, since the deposition of nitrogen has already been decreased by a lessened food intake. Certainly with a normal appetite such a degree of increase of metabolism as was observed in the experiment would hardly alone be able to check growth. This patho-

logical condition of metabolism may be the direct result of deficiency of vitamin A, or the indirect result of the deficiency on the endocrine organs controlling metabolism. The data at present available give no indication as to which of these factors is responsible.

The chief factor in checking the growth seems to be that one unit of the vitamin A-deficient food consumed by the rats produces a much smaller increase in body weight than the same unit of complete diet. Perhaps this fact is correlated with the increased metabolism. If even a slight pathological condition of absorption is also present the total effect on growth will be greater.

SUMMARY.

1. The body weight, appetite, intake and absorption of fat and nitrogen, and the nitrogenous metabolism of two male rats kept on a vitamin A-deficient diet were compared with those of two control litter-mates kept on a complete diet.

2. Each control rat was paired as regards food intake and the other conditions of the experiment with a rat on a vitamin A-deficient diet.

3. During the period of checking or cessation of growth of the rats the decrease in food intake was only moderate and could offer only a partial explanation of the change in rate of growth, since the amount of food, if calculated per 200 g. of body weight, consumed by the rats on the deficient diet was even larger than that consumed by the rats on the complete diet.

4. During the same period the absorption of nitrogen and of fat was not appreciably altered and could not, therefore, be the factor responsible for this checking of growth.

5. In the last period, when the appetite was greatly decreased, the decrease in food intake was probably the main cause of the loss of weight, this effect being augmented by the definitely decreased absorption from the alimentary canal observed during the last period.

6. During the whole period of observation the nitrogenous metabolism increased.

7. This increase of metabolism occurring simultaneously with the decreased food intake may check the normal growth of rats kept on a vitamin A-deficient diet.

8. In such rats the increase in body weight was less than in rats kept on a complete diet when correlated with the same unit of food ingested. This fact would seem to be the chief factor in checking the growth of rats kept on a vitamin A-deficient diet.

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