91. The Physiological Properties of Vitamin A

1. A Specific Effect upon Body Weight and Body Composition in the Albino Rat

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(Received 3 September 1942)

In the case of most of the vitamins a deficiency causes a restriction in food consumption. The question has frequently been raised as to whether observed results in vitamin experiments are due directly to a deficiency of a vitamin or to the lowered food intake. The use of isocaloric feeding has given significant results in the case of several of the vitamins but has seldom been reported for vitamin A. Sampson & Korenchevsky [1932] found that pair-fed rats receiving vitamin A were heavier and had more body fat than animals similar in food intake but not receiving the vitamin. Green [1934] reported that the loss in weight in rats deprived of vitamin A was not significantly greater than in animals with a restricted food supply and which received the vitamin. In view of the conflicting evidence we decided to study the possible specific effect of vitamin A upon body weight. Six series of observations in two laboratories, in different seasons, have given results in complete agreement. Results of only one will be presented, and data on body weight and composition will be included.

Experimental methods

Albino rats of the Wistar strain, reared in the Connaught Laboratories' colony or in the Laboratory of Hygiene, were used at the age of 3-4 weeks with an initial weight of about 50 g. They were housed in individual screen-bottom cages at room temperature of 72-74° F. Water was supplied *ad lib.* The basal diet was that recommended by Coward [1938] for the biological assay of vitamin A; it had the following composition:

Casein, Labco vitamin-free	...	...	...	15%
Dextrinized rice starch	...	...	...	73%
Brewers' yeast, Mead Johnson	...	...	...	8%
Salt mixture, Steenbock	...	...	...	4%

As a source of vitamin A, a solution of carotene in corn oil was used in some experiments and in others a cod liver oil concentrate. Results from both sources of vitamin A activity were similar. In each case all rats received the same amount of vitamin D (10 i.u. per week), sufficient irradiated ergosterol being added for that purpose.

In each series of experiments 3 groups of animals were employed, the corresponding numbers of the groups being of the same sex and approximately the same weight. Each group contained at least 10 animals. In each series the 'normal' group received the basal diet *ad lib.* plus a daily supplement of 5 i.u. vitamin A. The 'basal' group was given the basal diet *ad lib.*, and the 'paired' group received the daily supplement of 5 i.u. vitamin A and an amount of food equal to that consumed by the corresponding animal in the basal group on the previous day. The animals were weighed 3 times weekly during the first 4 weeks and daily thereafter. Daily records of food consumption were kept.

In the series reported, determinations of body composition and analyses of food and faeces were made. During the first 4 weeks on the deficient diet, animals in the basal group continued to gain in weight and no changes in appetite were observed. After 4 weeks it was likely that body stores of the vitamin were considerably depleted, since the

basal animals then showed diminishing appetite and a failure to increase in weight. For this reason analyses of body composition were made at the end of the first 4 weeks. From an initial number of 13 rats in each group, 6 were sacrificed for analysis. The remaining rats were continued on the same regimen as previously for 2 more weeks. During this time intakes of food and water were measured daily. Faeces from each group were collected and weighed daily during the 5th and 6th weeks, and analyses for moisture, nitrogen, fat and ash were done on half-weekly accumulations. At the end of 6 weeks the basal rats all exhibited severe xerophthalmia. Since previous work had shown that animals on this basal diet will not survive more than 7 weeks, the series was terminated at the end of the 6th week. Analyses of body composition were carried out on the 7 residual rats in each group.

The bodies were prepared for analysis by freezing with CO₂ ice. The frozen bodies from each group were finely minced and then thoroughly mixed. Aliquots, for moisture determinations, were withdrawn from the mixed material and dried to constant weight in a vacuum oven at 50°. The dried aliquots were reground and mixed and fresh aliquots were used for determination of fat, N and ash. Fat was estimated by ether extraction in a Soxhlet apparatus, N by the Kjeldahl method (the factor 6.25 being used to convert N to protein) and ash was determined by ignition to constant weight in an electric furnace.

Results

Changes in body weight are shown in Fig. 1. Data on nitrogen and fat intake and excretion are given in Table 1. Tables 2 and 3 record results of analyses for the determination of body composition.

Fig. 1. Average food consumption and average weight curves for 3 groups of 7 rats each.

Table 1. *Intake and faecal excretion of N and fat (g./rat/day) for last 14 days*

	Normal	Basal	Paired
N intake	2.68	1.92	1.92
N in faeces	0.27	0.22	0.22
N absorbed	2.41 (90%)	1.70 (88%)	1.70 (88%)
Fat intake	0.15	0.11	0.11
Fat in faeces	0.04	0.03	0.05
Fat absorbed	0.11 (73%)	0.08 (73%)	0.06 (56%)

Table 2. *Average body composition of 6 animals from each group sacrificed at end of 4 weeks*

	Normal		Basal		Paired	
	%	g.	%	g.	%	g.
Water	61.1	94	66.0	87	63.6	91
Protein	17.8	27	19.7	26	18.4	26
Fat	18.5	28	9.9	13	13.8	20
Ash	2.6	4	3.0	4	3.0	4
Total	100.0	153	98.6	130	98.8	141
Body weight		154		132		143

Table 3. *Average body composition of 7 animals from each group sacrificed at end of 6 weeks*

	Normal		Basal		Paired	
	%	g.	%	g.	%	g.
Water	62.6	95	66.5	82	60.5	90
Protein	18.9	29	20.5	25	19.3	29
Fat	16.1	24	8.3	10	15.8	23
Ash	2.4	4	3.9	5	3.3	5
Total	100.0	152	99.2	122	98.9	147
Body weight		152		123		148

Discussion

Since the method of paired feeding was employed, any difference in the observed results between the basal and the paired group is due to the specific effect of vitamin A on metabolism or absorption.

Vitamin A was responsible for a 17% difference in weight between the basal and the paired groups. Results in agreement with this figure were obtained with the other 5 series, these being respectively 16, 20, 15, 20 and 25%.

The effect of food upon body weight is shown by the difference in weight between the paired group and the normal group. This effect varies considerably, depending upon the appetite of the animals in the basal group, from an insignificant value of 3% in this series to 20, 25, 8 and 23% in other series.

In the series reported 32% of the difference in body weight between the basal and paired groups was due to water. There was no appreciable difference between the water intake of the 3 groups and yet the basal group contained a higher percentage of water. This is in agreement with the results of Cameron [1938]. 52% of the weight difference between the basal and the paired groups was due to a favourable effect of vitamin A upon fat retention. The paired group absorbed less fat and yet had more fat in their bodies than the basal group. The remainder of the difference in weight (16%) between the paired and basal groups was due to a difference in the amount of protein.

The effect of vitamin A on body weight does not appear to be due to alterations in absorption of food but to utilization after absorption. According to Green [1934] fat can be absorbed normally in vitamin A deficiency.

SUMMARY

Rats which were pair-fed and received vitamin A were significantly heavier than control animals deprived of the vitamin. The difference in weight was due to a loss of protein from the deficient animals and to a greater retention of water, fat and protein in the animals supplied with the vitamin. The difference was apparently not due to alterations in absorption of food.

The portion of this investigation carried out in the University of Toronto was made possible by a grant from the Division of Natural Sciences of the Rockefeller Foundation.

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