CLXXIV. THE PHYSIOLOGICAL PROPERTIES OF ASCORBIC ACID

I. AN EFFECT UPON THE WEIGHTS OF GUINEA-PIGS

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THE technique of paired feeding has been extensively used in nutritional investigations, particularly in those dealing with amino-acids and with vitamin B₁. The advantage of this procedure has been clearly indicated by Mitchell & Beadles [1930]. Its chief merit is that the otherwise variable factor of food consumption may be controlled. It has been known for some years that guinea-pigs, maintained on diets deficient in ascorbic acid, lose appetite, consume less food and diminish in weight. As in the case of vitamin B₁ it seemed reasonable that some of the symptoms manifest in these deficient animals might be caused by inanition. An extensive search of the literature has shown that only on one reported occasion has paired feeding been undertaken in studies on ascorbic acid. Anderson & Smith [1924], using a small number of animals and employing foods as sources of ascorbic acid, found that guinea-pigs fed a basal diet with supplements, the total amount being isocaloric with the consumption of scorbutic animals, were able to maintain body wt. at a higher level. The present investigation deals with an effect of *l*-ascorbic acid upon body wt. in paired feeding.

METHODS

The basal diet, which was designed to be deficient only in ascorbic acid, was a modification of that used by Coward & Kassner [1936]. Its composition was as follows:

	%
Wheat bran	45
Crushed oats	25
Dried skim milk	25
Brewers' yeast	2
Cod liver oil	1
CaCO ₃	1
NaCl	1

The dried milk was heated in open trays at 110° for 2 hr. with frequent stirring to destroy its content of ascorbic acid. This diet without supplement produced normal growth in young rats and, when supplemented with ascorbic acid, in young guinea-pigs.

Guinea-pigs reared in the Connaught Laboratories' colony, weighing 200–230 g., were used. They were housed in individual screen-bottom cages. Water was supplied *ad lib.* and food was given in detachable metal boxes deep enough to prevent spilling. The room temp. was regulated at 72–74° F.

For the first week of experimental feeding the basal diet was supplied *ad lib.* and each animal received daily oral doses of 5–10 mg. *l*-ascorbic acid in freshly prepared aqueous solution. Daily wt. records were kept. It was assumed that,

at the end of this preliminary week, all animals were saturated with ascorbic acid since daily doses of 2 mg. have been found to give maximal weight increase in this strain of guinea-pigs. This preliminary period also served to accustom the animals to changes in housing and diet. By the end of the week most animals showed a normal weight response; a few which failed to do so were discarded.

The animals were then divided into 3 groups, each individual of which corresponded as closely as possible to individuals in the other 2 groups. The division was made on the basis of wt., sex and rate of wt. increase during the preliminary week. The first group, designated as "normal", received the basal diet *ad lib.* and 2 mg. ascorbic acid daily. The second or "control" group was given the basal diet only. The third or "paired" group received 2 mg. ascorbic acid daily and an amount of food equivalent to that which had been consumed by the control group on the previous day. Animals in this group retained a good appetite throughout experimental feeding and ate all the food furnished. The animals were weighed daily after ascorbic acid was given to the normal and paired groups and before being fed. Daily records were kept of the amounts of food given to and consumed by each animal.

The duration of the experiment was 21 days after the preliminary week, the average survival time of the paired and control animals. If for any reason an animal in either the control or paired group died before this time, its wt. and those of the corresponding animals were not included in the averages which are reported.

EXPERIMENTAL RESULTS

Observations have been made on 8 series. In some of these there was no normal group. Concordant results have been secured throughout. Since results

Fig. 1. Body weights and food consumption. 1, normal group; 2, control group; 3, paired group.

in good agreement have been given by all of 94 pairs in control and paired groups, data on one series only will be given. The accompanying figure shows

the curves of average wt. for three groups of 10 animals each and also shows the average food consumption for each group.

DISCUSSION

Following a period of 10–12 days, during which body stores of ascorbic acid are presumably exhausted, control animals lose appetite and diminish in wt. until death occurs. When food is furnished *ad lib.* a continuous supply of ascorbic acid causes an increase in wt. This response has been used for the quantitative estimation of ascorbic acid by Bracewell *et al.* [1930], Harris & Ray [1932], Hou [1936] and by Coward & Kassner [1936]. Ascorbic acid prevents a loss in wt. in animals paired with the control group. At the end of 3 weeks there has been in every case a significant difference in wt. between animals receiving isocaloric intakes.

A loss of appetite in guinea-pigs deprived of ascorbic acid has been commented upon by other observers. Anorexia is as characteristic of a deficiency of ascorbic acid as it is of vitamin B₁ deficiency. The supply of ascorbic acid to the paired and normal groups prevented the loss of appetite. Appetite can be maintained by as little as 0.1 mg. ascorbic acid per guinea-pig per day.

There are several likely explanations of the difference in wt. between two animals given isocaloric intakes, one of which received ascorbic acid while the other did not. The difference might be due to alterations in water balance, in absorption of food, or in metabolism. Observations on these three factors are being conducted and will be reported later.

SUMMARY

After 21 days of paired feeding young guinea-pigs receiving a scorbutic diet plus ascorbic acid are significantly heavier than animals of the same initial weight given only the basal diet. Since the difference in weight cannot be due to difference in food intake, it is believed to be due to alterations in metabolism, waterbalance and food absorption caused by a lack of ascorbic acid.

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