

LXXXI. THE PHYSIOLOGICAL PROPERTIES OF ASCORBIC ACID

III. EFFECTS UPON WATER BALANCE AND UPON BODY COMPOSITION OF GUINEA-PIGS

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IN the first paper of this series a weight difference caused by ascorbic acid in paired-fed guinea-pigs was reported [McHenry *et al.* 1938]. Several explanations regarding differences in metabolism, water balance and food absorption were suggested, and, in a later paper, observations on a difference in O₂ consumption were described [Fidlar *et al.* 1939]. The present communication deals with differences in water balance and in body composition under conditions similar to those previously used.

Comparatively few references dealing with the effects of ascorbic acid upon water balance and upon the amounts of protein, fat and ash in guinea-pigs have been found in the literature. Doi [1938] reported an increase in the water content of liver, kidney, lung and muscle in scurvy. There are various reports on changes in nitrogen metabolism or in plasma protein [Doi, 1938; Nagayama & Sato, 1928; Shipp & Zilva, 1928] but none have been found regarding changes in the total nitrogen content of the animal. Doi [1938] found that the ash content of scorbutic guinea-pigs was normal. Other reports deal only with bone ash. The content of fatty acids was reported by Nagayama & Tagaya [1929] to be lower in scorbutic animals than in normal ones.

Many of these investigators have pointed out that inanition may have been a factor in causing the observed results since there is a progressive loss of appetite as animals become deficient in ascorbic acid. We have endeavoured to eliminate the factor of inanition by using paired feeding, although *ad lib.* feeding has also been employed to study the effect of inanition.

METHODS

The technique of paired feeding employed by us, the care of animals and the composition of the basal diet have been described previously [McHenry *et al.* 1938]. As before, 3 groups of guinea-pigs were used, a "normal" group given basal diet *ad lib.* plus 5 mg. ascorbic acid daily, a "basal" group given basal diet *ad lib.* and a "paired" group receiving that amount of basal diet consumed by the basal group on the previous day, plus 5 mg. ascorbic acid daily. The animals were housed in individual metabolism cages and received water *ad lib.*; daily records were kept of the intake of water and excretion of urine.

At the end of 21 days the animals were killed and the bodies prepared for analysis by freezing on CO₂ ice. The frozen bodies from each group were finely minced and then thoroughly mixed. From the mixed material aliquots were withdrawn for moisture determinations, conducted by drying to a constant weight at 50°.

After drying, the aliquots were reground and mixed, after which fresh aliquots were used for determinations of fat, nitrogen and ash. Total nitrogen was estimated by the Kjeldahl procedure, fat by ether extraction in a Soxhlet apparatus and ash by ignition to constant weight in an electric furnace.

RESULTS

Observations were made on three series totalling 15 pairs of animals and on two series of 10 normal guinea-pigs. Results from one series only are given since those from all series were comparable.

Table I gives the observations on water balance. All figures are averages for groups of five animals.

Table I

Group	Total water intake ml.	Total urinary excretion ml.	Difference ml.	Body-water g.	Body-wt. g.
Normal	946	189	757	213	293
Basal	869	286	583	163	222
Paired	1260	542	718	186	248

Table II

	Normal		Basal		Paired	
	%	Wt. g.	%	Wt. g.	%	Wt. g.
Water	72.8	213.0	73.6	163.0	74.9	186.0
Fat	4.5	13.2	3.6	8.1	1.9	4.9
Protein	16.6	48.8	18.0	38.9	16.4	40.7
Ash	3.4	10.0	3.7	8.3	3.9	9.8
Total	97.4	285.0	98.9	218.3	97.3	241.4
Body-water		293		222		248

Table II shows the results of the nitrogen, fat and ash determinations. The body-water content is repeated for comparison. Nitrogen content is given as protein, calculated with the conventional factor of 6.25. Values are given in weight as well as percentages since there are definite differences in the body weights of the separate groups.

DISCUSSION

Paired feeding has been used by us, as it has been employed by many others, to restrict the effect of differences in food consumption. The basal and paired groups received isocaloric amounts of basal diet and, so far as other foodstuffs were concerned, differed from each other only in supplies of ascorbic acid and in the amount of water consumed. The normal group had available considerably more food. Records of food consumption show that the total food consumption of the basal and paired groups was 368 g. per animal, while that of the normal group was 443 g. The general interpretation of the experiment carried out in this way is that the basal and paired groups differed only in respect of the supply of ascorbic acid and that any observed results were due to this single variable.

Considerable differences in water intake and urinary excretion were observed among the three groups. Although both the intake of water and output of urine in the paired group were much greater than in either of the other groups the water retention of the paired animals was of the same order as that of the normal group. The increased water consumption of the paired-fed guinea-pigs may have been due to their unsatisfied hunger because of the restricted food intake.

Ascorbic acid is apparently concerned, directly or indirectly, with water retention. These results show that the weight differences observed in paired feeding experiments previously reported from this laboratory [McHenry *et al.* 1938] can be largely explained by variations in water retention. 71% of the difference in body-weight between the normal and basal groups is due to water, while 88 % of the difference between the weights of the basal and paired groups is due to water.

The decreased amounts of fat and protein found in the paired animals in contrast to those fed *ad lib.* would be expected because of the restricted supply of food but there is no difference in the total amount of ash. Animals in the paired group have not a normal body composition. While the amounts of protein and ash are less in the basal group than in the paired animals the differences are slight. There is one marked distinction between the basal and paired animals, namely, the amount of fat in the bodies. Guinea-pigs on a scorbutic diet have a greater O₂ consumption than paired animals given ascorbic acid, yet they retain almost double the amount of fat. We have no explanation at present for this difference in total body fat.

SUMMARY

Water intake, urinary excretion, and the amounts of water, fat, protein and ash have been studied in guinea-pigs receiving a basal scorbutic diet, in animals given isocaloric supplies of the diet plus ascorbic acid and in animals receiving the vitamin but allowed to eat *ad lib.* Under these conditions paired guinea-pigs drink more water and excrete more urine than do animals in the other groups. A lack of ascorbic acid causes a diminished retention of water which largely accounts for differences observed in body-weight. The deficient animals retain considerably more body fat than do the paired guinea-pigs despite the increased O₂ consumption which has been reported previously.

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