XLIH. THE PHYSIOLOGICAL PROPERTIES OF ASCORBIC ACID

II. THE INCREASE IN METABOLISM OF GUINEA-PIGS ON A DIET DEFICIENT IN ASCORBIC ACID

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It was reported [McHenry *et al.* 1938] that, when two similar groups of guinea-pigs were pair-fed, one group receiving ascorbic acid while the other did not, there was a significant loss in weight in the deficient group at the end of three weeks. In seeking an explanation for this loss in weight, the metabolic rates of the two groups were investigated.

Although the basal metabolism of guinea-pigs suffering from scurvy has been determined by several investigators, conflicting results have been obtained. Söderström & Törnblom [1933], Soneda & Kato [1934], Törnblom [1934], Ardy & Bellini [1936], Fujino [1936] and Scoz *et al.* [1937] all reported that the O₂ consumption in experimental scurvy decreased until death. On the other hand, Mosonyi & Rigo [1933] and Calcinai & Galigani [1934] found a markedly increased O₂ consumption in scorbutic animals.

Methods

In order that the animals in our experiments might be similarly fed, the technique of paired feeding was employed [McHenry *et al.* 1938]. It consists, briefly, in fixing the quantity of food of the ascorbic acid group by the amount of the basal diet consumed by the scorbutic group. In this manner an isocaloric intake was maintained in the two groups. The minimal survival time of the animals on the deficient diet was 21 days, but the metabolic rate was taken on the 18th day. The composition of the basal diet and the care of the animals were similar to those previously reported. Guinea-pigs receiving ascorbic acid were given 5 mg. per day by mouth, a dose which is adequate for growth.

For the determination of the gaseous exchange the respiration apparatus used was that of Macleod *et al.* [1929] with some recent modifications. Although originally planned for dogs it could be used for a group of small animals such as rats and guinea-pigs where the combined volume of the gaseous exchange was sufficiently great to minimize the error. There was no method for recording the activity of the small animals, but in some earlier work with rats it had been found that in a run of about 6 hr. following a 16-hr fast, an average of the two lowest hourly rates gave a figure suitable for comparative purposes even though no record of activity was obtained. It was not always convenient to have the temperatures at that of "thermal neutrality" for the small animals, but when groups were to be compared the average temperature of the cabinet was within a fraction of a degree of the same temperature.

Experimental results

Results were obtained in three series of experiments, the first with two groups of 6 and 8 animals each, the second with two groups of 10 animals each and the third with two groups of 10 animals each together with a normal control group of 10 receiving basal diet *ad lib.* plus ascorbic acid. The O_2 consumption is given on a body-weight basis and the heat output calculated on a body-surface basis in the hope of correcting, to some extent, the errors due to weight differences. The formula used for calculating body surface was $S = 8.9 \times W^{\frac{1}{2}}$ where W = weight after the metabolism was measured. For the determination of heat production the caloric equivalent of the O_2 consumption was derived from the R.Q. by the usual table. The highest R.Q. was 0.81, the lowest 0.74, but in the majority of the hourly periods the range was between 0.75 and 0.78. There were no significant differences between groups.

Metabolic rates obtained on the three series are given in Table I. Dates of the experiments are given because of the fact that there is possibly an alteration in guinea-pigs from season to season; at least this applies to resistance to infection or to bacterial toxins.

Table I

Date	Group	No. of animals	Sex	Diet	O_2 /kg./hr. ml.	Diff. %	Cal./m ² / 24 hr.	Diff. %
26-27 Apr.	Paired	6	2M., 4F.	Basal + ascorbic acid	925	—	755	—
	Scorbutic	8	4M., 4F.	Basal <i>ad lib.</i>	1206	+23	941	+19
31 May- 1 June	Paired	10	—	Basal + ascorbic acid	979	—	793	—
	Scorbutic	10	—	Basal <i>ad lib.</i>	1282	+30	988	+19
	After 40 mg. of ascorbic acid				1192	—	916	—
18-20 Oct.	Normal control	10	7M., 3F.	Basal <i>ad lib.</i> + ascorbic acid	1093	—	925	—
	Paired	10	7M., 3F.	Basal + ascorbic acid	1036	—	872	—
	Scorbutic	10	7M., 3F.	Basal <i>ad lib.</i>	1366	+24	1096	+20

There is no record of the sex of animals in the second series but, as in the other two, they were mixed, the two groups being identical in this respect. In the second series an effort was made to demonstrate that the rise in metabolic rate was due specifically to a deficiency of ascorbic acid by giving the deficient animals a total of 40 mg. ascorbic acid and repeating the determination. The metabolism was lowered somewhat and, in all probability, had the treatment been continued, would have returned to normal.

In the third series the normal control group shows that the pair-fed animals receiving ascorbic acid have a metabolism slightly below normal but close enough to be taken as normal in comparison with scorbutic ones.

DISCUSSION

These three sets of experiments carried out in two seasons of the year have shown that guinea-pigs on a diet which though deficient in ascorbic acid is otherwise adequate, exhibit a definite increase in metabolism. Since paired feeding was employed, the results are due to a single variable, namely, the absence of ascorbic acid.

The fact must not be ignored, however, that the calculation of a metabolic rate after a loss in weight can, under certain conditions, be quite misleading.

Should the loss in weight be due to decrease of inert substances such as water and fat while active tissue remains the same in all respects, then the metabolic rate, if based on unit of weight, will appear to rise as the weight falls, although the animal is consuming the same amount of O_2 throughout. A rate in terms of surface area tends to reduce this error, and although some workers maintain that there is no causal relationship between surface area and heat production, this method of expression continues to be employed. Nevertheless, since the formulae ordinarily used for obtaining the surface area of laboratory animals are based entirely on weight, the objection remains that there is no distinction between inert material and active tissue, and therefore increases in metabolic rate associated with weight-loss or decrease in rate accompanying a weight-gain should be carefully considered.

If it be assumed, though quite unjustifiably, that the changes in weight are due solely to fluctuations of inert material, then the weights at the beginning of the experiment might be used as the basis of calculation. Even on this assumption the scorbutic groups show increases in metabolic rate of 13, 13 and 12% respectively in the three series of experiments. Also, the ratios of O_2 consumed to the amounts of dry material found in the bodies of the animals at the end of the experiment confirmed the conclusion that the rise in metabolic rate was not due to loss of water. Under these circumstances there is no need to depart from the conventional form of expression of the metabolic rate as calories per square metre of body surface. Comparisons can then be made with other work.

A number of investigators have suggested that there is a relation between the intake of ascorbic acid and the activity of the thyroid gland. For example, Mosonyi [1936] states that the increase in metabolism observed by him is due to hyperthyroidism, but as yet we have no experimental evidence on which to base such a conjecture.

SUMMARY

Guinea-pigs on a basal scorbutic diet show, on the 18th day of this regimen, a greater metabolic rate than do animals with the same food intake but receiving ascorbic acid.

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