CXXXVIII. MANNOSE AS A POSSIBLE PRECURSOR OF ASCORBIC ACID IN THE TISSUES OF THE RAT

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CERTAIN animals, for example rats, are apparently able to synthesize ascorbic acid in their tissues and do not therefore require vitamin C in their diet. Some time ago we attempted unsuccessfully to find the precursor of this synthesized ascorbic acid by feeding likely substances such as sugars and their derivatives, and estimating ascorbic acid by titration with dichlorophenolindophenol. While these experiments were in progress, Guha & Ghosh [1934, 1; 1935, 1] published work indicating that certain tissues of the rat, notably the liver, when incubated *in vitro* with mannose produced small amounts of ascorbic acid, as determined by titration. In a later publication [1935, 2], they stated that injection of mannose into the living rat also led to an increase in ascorbic acid as shown by titration of extracts of certain tissues from the freshly killed animal. Euler *et al.* [1935] were unable to confirm the production of ascorbic acid by rat tissues *in vitro*, but Guha & Ghosh [1936] claimed recently that this was because Euler *et al.* carried out the incubation of the minced tissues in nitrogen, and that the synthesis occurred only in oxygen.

The conversion of mannose into ascorbic acid by animal tissues might, if substantiated, be of practical interest in nutrition in view of the claim of Rohmer *et al.* [1935] that young infants do not require vitamin C in the diet, and of Banerjee [1934] that giving mannose to infants increases the amount of ascorbic acid in the urine, as determined by titration.

We attempted therefore to repeat the experiments of Guha & Ghosh using the conditions specified by them, but our results do not support the view that synthesis of ascorbic acid from mannose takes place in presence of minced rat liver, nor have we found any evidence of synthesis of the acid from mannose after injection of this sugar into the intact animal.

In the majority of experiments *in vitro* we used adult rats. The animals were killed by a blow on the head and were allowed to bleed, and the liver was then quickly removed and minced finely with a Latapie mincer. 0.5 g. of tissue was weighed into a number of 25 ml. conical flasks and shaken up with a mixture of 3 ml. Ringer-Locke solution (without glucose) [Liddell & Sherrington, 1929] and 2 ml. *M*/20 phosphate buffer (*pH* 7.4) together with 1 ml. mannose (20 mg.). In the controls 1 ml. water replaced the mannose solution. The flasks (containing air) were closed with rubber stoppers and incubated in a water-bath for 3 hr. at 37°. Following the procedure of Guha & Ghosh, the flasks were not usually shaken during incubation. Trichloroacetic acid (6 ml. of 10% solution) was then added to each flask and, after shaking, the protein was filtered off and the filtrate titrated with 2:6-dichlorophenolindophenol, using the method of Birch *et al.* [1933]. In some cases duplicate titrations were carried out using more dilute trichloroacetic acid followed by glacial acetic acid to bring the *pH* to 2.5 [Guha & Ghosh, 1934, 2], though this procedure did not appear to give any increase in the accuracy of the titration. In most cases the ascorbic acid

was also determined on samples of minced tissue (with and without mannose) before incubation, as shown in columns 1 and 3 of Table I. A number of different samples of mannose were used in our experiments.

The oxidation of a considerable proportion of the initial ascorbic acid occurs during incubation, but comparing the figures in columns 2 and 4 it is clear that there is no evidence of formation of ascorbic acid from mannose *in vitro*.

Table I

mg. ascorbic acid per g. liver

Exp.	Without mannose		With mannose		Remarks
	Initial	After 3 hr.	Initial	After 3 hr.	
1	0.21	0.15	0.22	0.14	—
2	0.24	0.11	0.23	0.10	Ringer with 0.1 % glucose
	—	0.15	—	0.15	Incubated in nitrogen
3	0.24	0.14	0.25	0.15	In PO ₄ buffer only
4	—	0.13	—	0.13	—
5	0.24	0.11	0.23	0.13	—
	—	0.09	—	0.09	Shaken during incubation
6	—	0.12	—	0.12	—
	—	0.09	—	0.09	Shaken during incubation
Av.	0.23	0.12	0.23	0.12	

In most of the experiments on the intact animal we used young albino rats, pairs of animals of the same litter being used for comparison with and without mannose. Most of the rats had received a mixed diet containing cabbage, but a few experiments were done on adult rats which had been maintained for a long period on a diet low in vitamin C. The intravenous injections were made into the tail vein, 0.5 ml. of a mannose solution containing 20 mg. (200 mg. in one experiment) being given 5 hr. before killing the rat. The control rats received an equal volume of 0.9 % NaCl. In the experiments in which the mannose was injected subcutaneously, injections of 20 mg. of the sugar were made on 3 consecutive days as described by Guha & Ghosh, the animals being killed 24 hr. after the last injection. (In one experiment three injections of 200 mg. mannose were given.) Control animals received injections of equal volumes of saline. After killing the animals a weighed amount of the liver (approximately 0.5 g.) was well ground with a little acid-washed sand and 10 ml. of 5 % trichloroacetic acid. After filtering, the extract was titrated with dichlorophenolindophenol. The results are shown in Table II. The bracketed pairs of figures represent duplicate experiments.

With the possible exception of Exp. 1 there is no evidence from these experiments of any synthesis of ascorbic acid from mannose in the intact animal, either after injections of 20 mg. mannose as given by Guha & Ghosh, or after injections ten times as large. Considering that different animals were necessarily used for the saline and mannose injections the two groups show remarkably close values for ascorbic acid. The higher average value for the ascorbic acid of liver in Table II as compared with the initial value in Table I is possibly due to the fact that young growing rats were used in most of the experiments in Table II.

In further work Guha & Ghosh [1934, 3] described the preparation of an extract from acetone liver which was capable of converting mannose into

Table II

mg. ascorbic acid per g. liver			
Exp.	Without mannose	With mannose	Remarks
1	0.36	0.45	Intravenous
2	{ 0.40 0.43	{ 0.37 0.38	,,
3	0.36	0.37	,,
4	{ 0.31 0.32	{ 0.29 0.29	,,
5	0.21	0.20	,, (200 mg. mannose)
6	0.34	0.34	Subcutaneous
7	{ 0.30 0.31	{ 0.30 0.31	,,
8	0.34	0.32	,,
9	0.22	0.23	,, (200 mg. mannose)
Av.	0.32	0.32	

ascorbic acid. They attributed this change to the action of a mannose dehydrogenase in the cell-free extract. In an attempt to repeat this experiment we prepared acetone rat liver by the method used in the preparation of glucose dehydrogenase [Harrison, 1931], and extracted 3 g. of the dry powder by grinding with 15 ml. water for 3 hr. in the ice-chest, centrifuging and re-extracting with 5 ml. water. Portions of the combined extracts were incubated for 3 hr. at 37° and pH 7.0 with 20 mg. mannose and without mannose. The total volume of the solution was 6 ml., containing extract equivalent to 3.5 g. of fresh liver. Indophenol titration of the solution containing mannose gave 0.26 mg. ascorbic acid while that without mannose gave 0.23 mg., the difference corresponding to less than 0.01 mg. ascorbic acid per g. fresh liver and probably lying within the limits of experimental error.

Though we have been unable to obtain evidence of a synthesis of ascorbic acid from mannose, we have, using the Thunberg methylene blue technique, confirmed the presence of an active mannose dehydrogenase in rat brain, as shown by Euler *et al.* [1935], and have further found the presence of mannose dehydrogenase in rat stomach tissue and in ox brain, though owing to the difficulty of obtaining stable preparations of the enzyme we have not so far been able to identify the oxidation product of mannose. In view of the fact that glucose is oxidized by glucose dehydrogenase to gluconic acid [Harrison, 1932], it seems possible that mannonic acid may be formed. The glucose and mannose enzymes appear to be distinct however, for we have obtained stable enzyme preparations from ox liver and pig kidney which oxidize glucose but not mannose.

Our experiments give no support therefore to the claim that ascorbic acid is synthesized from mannose by rat liver, either *in vivo* or *in vitro*. We are unable to suggest an explanation for the differences between our results and those of Guha & Ghosh, though we would point out that whereas in their earlier papers these authors claim an average synthesis *in vitro* of 0.3 mg. of ascorbic acid per g. of liver, their last published results [1936] apparently give an average figure only one-quarter as large.

SUMMARY

No evidence could be obtained of synthesis of ascorbic acid from mannose by minced rat liver when incubated in Ringer-phosphate buffer solution in presence of oxygen. Intravenous or subcutaneous injection of mannose into rats produced no increase in the ascorbic acid content of the liver. These results do not substantiate the claim of Guha & Ghosh that mannose is converted into ascorbic acid by rat liver both *in vivo* and *in vitro*.

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Note added 25 June 1937. In a recent paper, Ammon & Grave [(1936), *Z. Vitaminforsch.* **5**, 185], working under experimental conditions slightly different from those of Guha & Ghosh, have been unable to obtain any evidence of synthesis of ascorbic acid from mannose by rat liver *in vitro*.