CVII. ON THE PHYSIOLOGICAL ACTION OF ASCORBIC ACID AND SOME RELATED COMPOUNDS.

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THE biochemical action of *l*-ascorbic acid has hitherto been studied principally on the scorbutic guinea-pig. Only recently Harris *et al.* [1933] investigated the excretion of ascorbic acid in the human subject and found that with a normal intake of vitamin C about 30 mg. of ascorbic acid can be determined by dichlorophenolindophenol titration in the daily output of urine. The excretion is temporarily increased when large doses of vitamin C (150 ml. lemon juice corresponding to about 150 mg. of ascorbic acid are given). It was also found by van Eekelen *et al.* [1933] that with a normal diet about 25 mg. of ascorbic acid are excreted daily in the urine.

In order to determine if, as in the case of vitamins A and D, symptoms of hypervitaminosis occur after the administration of large quantities of ascorbic acid, the present author has investigated the tolerance of various laboratory animals (cold and warm blooded). The vitamin was administered *per os*, subcutaneously, intraperitoneally and intravenously, using an aqueous solution of ascorbic acid which was neutralised with sodium hydroxide shortly before use. In the following table the maximum single doses given to the various animals are summarised.

		Dosage in g./kg.			
Animal	Weight g.	Oral	Subcutaneous	Intraperitoneal	Intravenous
Axolotl	15–30	—	5	5	—
Frog	30–40	—	5	5	—
Mouse	12–20	5	5	2	1
Rat	120	5	2·5	—	1
Guinea-pig	250	5	1	—	0·5
Rabbit	2500	2	1	1	1
Cat	2500	1	0·5	—	0·5
Dog	8000	0·5	0·2	—	0·2

The doses were well tolerated in every case and no symptoms of hypervitaminosis were observed. Only in the mouse after oral administration was a brief laxative effect observed, this being probably caused by the relatively large quantities of salt administered. The body temperature of dogs and rabbits was not influenced for 24 hours after the injection. The Ca and P of the blood were not altered.

Ascorbic acid was also very well tolerated when administered over a long period. Guinea-pigs maintained on a normal diet received orally, subcutaneously or intravenously for 6 days 0·4–2·5 g. ascorbic acid per kg. daily (total dose 2·4–12 g./kg.). Under similar conditions mice were given 0·5–1 g./kg. for 7 days (a total of 3·5–7 g./kg.). The animals remained completely normal during the experiment and for 14 days thereafter. They showed no difference in appetite,

increase of weight and general behaviour from controls receiving the same amounts of the biologically inactive galacturonic acid. Histological investigation of various organs (kidney, pancreas, liver, heart and lungs) showed no definite changes.

As the minimum curative and prophylactic dose of ascorbic acid for the guinea-pig is 0·5–1 mg. daily and in the above experiments 2·5 g./kg. daily were administered, it is evident that the animals tolerate 500–1000 times the therapeutic dose of ascorbic acid daily without ill effects; the average weight of the guinea-pig being taken as 250 g. Experiments with a still higher dosage seem unnecessary as such is unlikely to occur in practice.

The reducing capacity of the urine for Fehling's solution or iodine in potassium iodide was definitely increased after administration of ascorbic acid. After intravenous or subcutaneous injection of 1–2 g./kg. of ascorbic acid to mice or rabbits, or after oral administration of 3–5 g./kg. to guinea-pigs, the urine obtained an hour later gave with Fehling's solution a pronounced precipitate of cuprous oxide. After 2–4 hours the reducing capacity reached a maximum and subsequently decreased fairly rapidly. After 20–24 hours the excretion was practically ended.

Method.

A dog weighing 9·6 kg. received 1 g. ascorbic acid, corresponding to approximately 0·1 g./kg. The urine was removed by catheter at intervals and the reducing capacity of the various fractions determined iodimetrically. The first fraction of urine (11 ml.), taken after 1 hour, showed a reducing capacity corresponding to a total amount of 0·153 g. ascorbic acid. In the second fraction (27 ml.), taken 3 hours after the injection, the reducing capacity corresponded to 0·377 g. ascorbic acid. The third fraction (27 ml.), taken after 5 hours, contained only 0·11 g. of ascorbic acid. The fourth fraction (100 ml.), taken after 8 hours, contained a total of only 0·057 g. of ascorbic acid. The reducing capacity of later fractions of urine showed normal values. The total excretion of ascorbic acid estimated iodimetrically was 0·701 g., *i.e.* about 75 % of the amount administered. The highest concentration of ascorbic acid (1·4 %) occurred in the second fraction of urine (1–3 hours after the injection). When still larger amounts of ascorbic acid are injected subcutaneously the concentration of ascorbic acid in the urine is increased still further and the excretion is continued for over 20 hours. The observed increase in the reducing capacity of the urine is undoubtedly due to an excretion of ascorbic acid because scorbutic guinea-pigs are cured by a daily dose of 0·2–1 ml. of this urine in 4–8 days.

The ascorbic acid in the circulation also increases the reducing capacity of the blood. After intravenous or subcutaneous injection of 0·4–0·5 g./kg. ascorbic acid to rabbits the apparent values for blood-sugar correspond to 140–160 mg./100 ml., the increase being only temporary. After one or two hours the blood-sugar values have again reached the normal of 110–120 mg./100 ml.

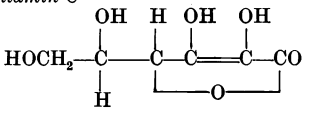
That this increased reducing capacity is not due to a mobilisation of glucose in the blood is proved by the fact that it is impossible to cure the convulsions of a hypoglycaemic rabbit by repeated injections of 0·1 g./kg. ascorbic acid intravenously or subcutaneously.

The action of related compounds.

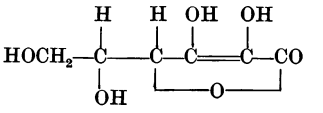
The researches of Reichstein *et al.* [1933, 1, 2; 1934] made it possible to investigate the antiscorbutic action of synthetic *l*-ascorbic acid and some of its isomerides and homologues. The complete identity in biological action of

synthetic *l*-ascorbic acid with the natural product from paprica was established, both when the synthesis was made from xylose and from sorbose as starting material. The isomerides and homologues investigated are shown below (in the case of the heptonic acids the lactone formulae are not given owing to the uncertainty as to whether a five- or six-ring is present):

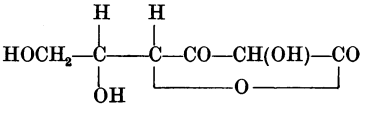
l-Ascorbic acid
Vitamin C



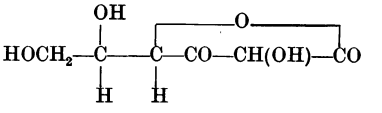
d-Ascorbic acid



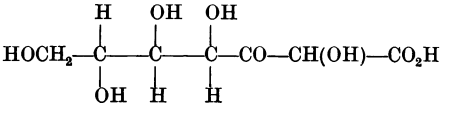
d-Erythro-3-ketohexonic acid



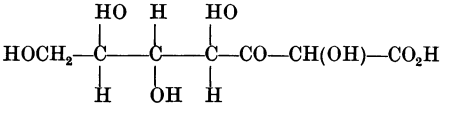
l-Erythro-3-ketohexonic acid



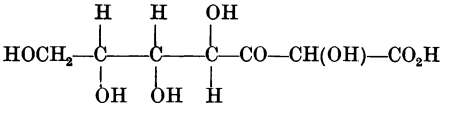
d-Lyxo-3-ketoheptonic acid



l-Xylo-3-ketoheptonic acid



d-Arabo-3-ketohexonic acid



The *d*-erythro-3-ketohexonic acid was found to have about 1/20 of the activity of *l*-ascorbic acid, in agreement with the result of Dalmer and Moll [1933]. The *d*-ascorbic acid was tested on the scorbutic guinea-pig and found to be completely inactive in doses 40 times as high (20 mg. daily) as were required for cure with *l*-ascorbic acid. The other homologues tested were also inactive in doses of 4–20 mg. daily.

Summary.

1. Large doses of *l*-ascorbic acid (up to 5 g./kg. in one dose or up to 12 g./kg. total amount in the chronic test) were tolerated without symptoms by our animals. Histological examination of the organs showed no change in the kidney, liver, heart and lung. There was no evidence of hypervitaminosis C.

2. After administration of 0·1 g./kg. ascorbic acid subcutaneously to a dog 75 % was excreted in the urine during the next 9 hours.

3. The urine of animals receiving large doses of ascorbic acid is capable of curing scorbutic guinea-pigs in daily doses of 0·2–1 ml. daily.

4. Synthetic *l*-ascorbic acid behaved biologically identically with *l*-ascorbic acid from paprica.

5. *d*-Ascorbic acid is inactive in doses of 20 mg. daily. Of the various isomerides and homologues examined only *d*-erythro-3-ketohexonic acid showed a definite antiscorbutic action which was about 1/20 of that of *l*-ascorbic acid.

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