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Urinary Excretion of Ascorbic Acid by the Rat as Influenced by Ingestion of Certain Carbohydrates.*

CLAIRE A. FREDERICK, N. B. GUERRANT, R. ADAMS DUTCHER AND C. A. KNIGHT.

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

It is now recognized that the rat is not only able to subsist indefinitely on a diet that is markedly scorbutogenic to the guinea pig, but while doing so, stores in its tissues and excretes in its urine measurable amounts of ascorbic acid. Workers in this field are not in complete agreement regarding the relationship of the composition of the ingested diet to the amounts of ascorbic acid stored and excreted by the rat. Some investigators[1,2] have contended that the composition of the diet is an influencing factor, while other investigators[3-6] have failed to demonstrate that ascorbic acid output is affected by changes in dietary ingredients. Other investigators[7-10] have contended that the ordinary constituents of the diet, such as sugar, fats and proteins, have no effect on the urinary excretion of ascorbic acid by the rat, but that high rates of excretion can be induced by feeding oats, oat oil, the unsaponifiable portion of oat oil, halibut liver oil and certain cyclic compounds of the terpene and sesqui-terpene series. Early reports by this group of investigators postulated the existence of a precursor from which the ascorbic acid was formed by the rat. In the later reports, however, these authors

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abandon this view and postulate that the ascorbic acid is formed through intermediary metabolism, a view previously expressed by other investigators.[6]

Because of the conflicting reports concerning the possible origin of ascorbic acid in the body of the rat, as well as those concerning factors influencing its elimination, it seemed desirable that our previous studies be repeated, especially those relating to the carbohydrate portion of the diet. The present report contains some of the data obtained in the course of the latter investigation.

Experimental. In our studies we have used half-grown rats as the experimental subjects. In order to collect the urine quantitatively, the animals were maintained in individual, cylindrical, galvanized wire cages, each of which was suspended above a 10-inch glass funnel. Beneath the funnel was placed a small glass vessel containing, as a preservative, 4 ml of metaphosphoric acid solution (10%) to which had been added a trace of 8-hydroxy-quinolin and a one-fourth-inch layer of paraffin oil. The cages were provided with galvanized wire bottoms with mesh of sufficient size to allow all fecal particles to pass through. Under each cage was placed a finer galvanized wire screen to prevent the fecal matter from entering the funnel. In order to further minimize contaminants, which might enter the funnel, the cages were provided with special food cups and drinking fountains. Each cage and its supplementary equipment were cleaned thoroughly at weekly intervals and, when necessary, the funnels were changed daily.

The collections of urinary samples were begun as soon as the animals were transferred to the metabolism cages and fed the experimental diet. The 24-hour collections of urine were removed at a definite time each day, their volumes recorded and aliquots titrated with Na 2,6-dichlorobenzenone indophenol solution (175 mg of the dye in 500 ml of hot water).

The diets used in these studies were composed of fat-free casein 18, salt mixture 3, Cell U flour 2, fat-free yeast 8 and fat-free carbohydrate 77 parts. In the majority of experiments, the fat-soluble vitamins were furnished as beta carotene and calciferol. In a few instances these vitamins were supplied by adding 2 parts of cod liver oil to the basal diet. The carbohydrates used were: dextrinized corn starch, raw corn starch, sucrose, glucose, and in a limited number of feeding periods of short duration, mannose, sorbose, fructose and lactose. The amount of food consumed daily by each animal was recorded and the amount fed was only slightly in excess of that consumed during the previous 24 hours.

In the instance of the first series of animals, all animals were fed the diet containing the dextrinized corn starch during the first period. This was followed by the sucrose-containing diet, the glucose-containing diet and the starch-containing diet, respectively. At the termination of the studies with these diets, the animals were again fed the dextrinized corn starch diet for a period of several weeks.

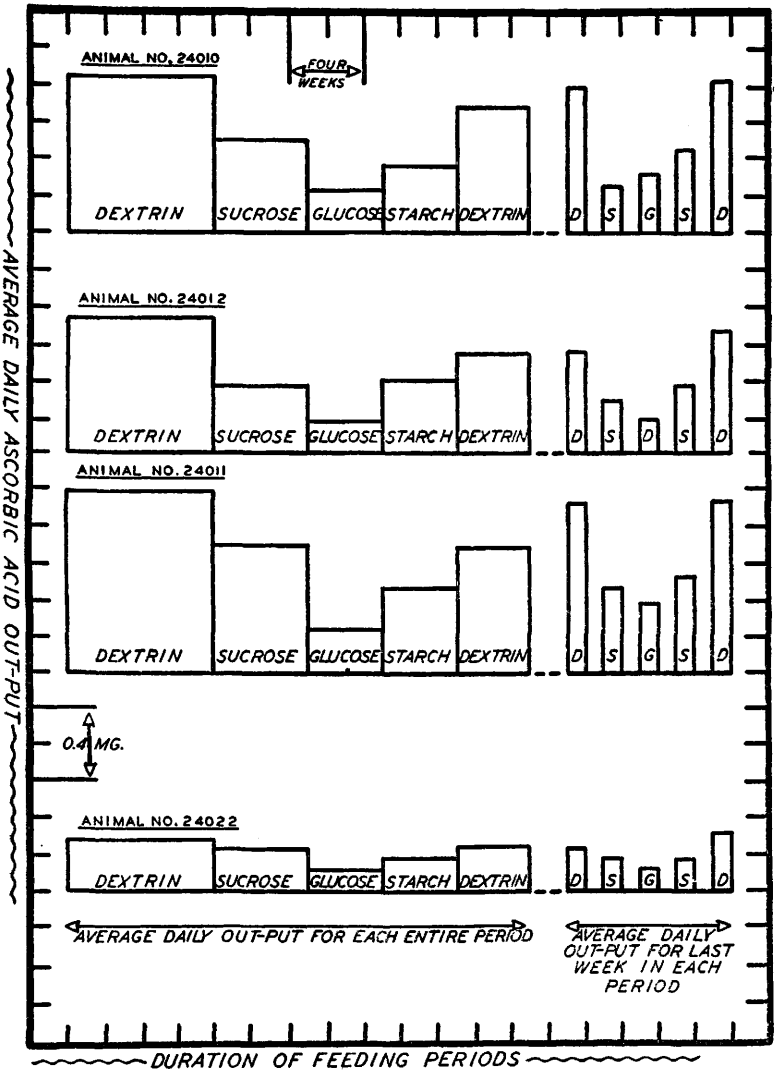

FIG. 1.

Amounts of ascorbic acid eliminated in the urine of rats while receiving diets similar in composition, the only difference being in the type of carbohydrate which they contained.

With subsequent series of animals, diets of similar composition were used, the experimental difference being in the sequence in which they were fed to the test animals.

Since space does not permit the presentation of all the data at this time, only the condensed data relating to the urinary ascorbic acid output of 4 typical animals from the first series are given

The duration of the various feeding periods were 8, 5, 4, 4 and 4 weeks, respectively. The urinary ascorbic acid elimination has been expressed as the average daily output for the entire period during which each of the respective diets was fed. In order to indicate the probable carry-over effect of one diet on the succeeding diet, the average daily urinary ascorbic acid output for the last week of each feeding period is given on the right of the graph (Fig. 1).

Discussion. With all of the animals used, the urinary ascorbic acid output was greatest while the animals were consuming the diet containing the dextrinized corn starch. This was found to be true irrespective of the sequence in which the diets were fed. On the other hand, in most instances, these same animals eliminated the smallest quantity of ascorbic acid while consuming the glucose-containing diet. However, the frequency of the latter observation seemed to depend somewhat on the sequence in which the glucose diet was fed.

The greater ascorbic acid elimination resulting from the consumption of the diet containing the dextrinized corn starch as compared to the other diets, does not appear to be readily explainable from the data at hand. While the average daily consumption of the dextrinized corn starch was slightly greater than the consumption of the other diets and while there seemed to be a slight correlation between daily food intake and the amount of urinary ascorbic acid, this observation is sufficient only to explain a small portion of the increase in ascorbic acid elimination. The inadequacy of such an explanation can be readily observed from the fact that animal No. 24022 consumed as much of the respective diets as did animal No. 24011 and, while doing so, eliminated only about one-third as much urinary ascorbic acid as did the latter animal. However, it may be stated that the relative amounts of ascorbic acid eliminated by the various animals while receiving the sequence of diets were of the same order of magnitude for the respective diets.

The question as to why greater amounts of ascorbic acid were excreted, while the rats were consuming the dextrinized corn starch diet, than when the raw corn starch diet was consumed, cannot be

answered at this time. The possibility of the existence of different amounts of an ascorbic acid precursor in the two diets does not seem tenable, at least in this instance. To verify this point, some of the rats, after having been returned to the dextrinized corn starch diet for several weeks, were given weighed amounts (25, 50 or 100 mg) of carvone (Eastman No. 1094) to determine the effect of this substance on ascorbic acid output. Since it was found impossible to determine, quantitatively, the amount of this volatile substance actually consumed by the rats when it was mixed with the diet, the carvone was diluted with olive oil and given by stomach tube. However, with this procedure, the tests proved unsatisfactory in that the animals began to lose weight after the administration of the first or the second dose of carvone and the majority of the test animals died within 6 or 7 days. In no case was there more than a twofold increase in the amount of ascorbic acid eliminated in any one day and such increases did not remain consistent from day to day.

Conclusions. It is apparent that the amount of ascorbic acid eliminated in the urine of the rat depends upon at least 2 major factors, namely, the type of carbohydrate ingested by the rat, and the physiological variations within the rat itself. Since it was frequently found that ascorbic acid elimination varied as much with different animals as it did with different diets, it is evident that the latter factor must be given due consideration. The data submitted, however, do not explain the origin of the ascorbic acid nor do they explain the differences in the amounts of ascorbic acid excreted in the urine of different animals while receiving comparable amounts of the same diet. A number of theoretical possibilities suggest themselves but these offer no immediate solution to the problem. It would serve no useful purpose to say that the ascorbic acid is probably of endogenous origin or that it has its origin in intermediate metabolites. Such suggestions fall far short of explaining the origin of the ascorbic acid in the body of the animal and would be equally ineffective in explaining why different animals excreted different amounts of this substance while consuming comparable portions of the same diet.