THE EFFECT OF RUTIN PREPARATIONS ON THE EXCRETION OF PHENOLIC SUBSTANCES IN THE URINE

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The metabolism and urinary excretion of substances related to vitamin P and, in particular, of rutin have received insufficient study and the results obtained in this field are very contradictory. It has been reported that phenols may be excreted in the urine as products of rutin metabolism [6, 7, 8]. Various workers have found 3,4-dihydroxyphenylacetic and homovanillic acids in the urine under these circumstances, and also an increase in the concentration of methoxyphenylacetic acid [3, 4, 8, 9]. In general, other workers have observed no appreciable changes in the phenol content of the urine after administration of rutin and its derivatives [5].

The discrepancy between the results obtained by different workers may be explained by variations in the experimental conditions, namely differences in the choice of experimental animals, in the mode of administration of rutin and the size of the dose, in the times of collection of the urine, methods of analysis, and so on.

We set out to study the effect of rutin preparations on the excretion of phenols in the urine, using one species of animal (guinea pigs) and one method of administration (by mouth).

Most tests used for phenols are not specific for any one substance. In our investigations, besides the reaction with diazonium salt which is frequently used for the determination of phenols, we also used the reaction with $AlCl_3$ as used in the determination of flavones.

EXPERIMENTAL METHOD

Two groups of guinea pigs were used for the experiments, differing in age and in the composition of their vitamin P-free diet. The first group consisted of adult guinea pigs with an initial weight of 900-1100 g. The animals received an artificially made up diet (casein diet), consisting of starch (68%), casein (18%), yeast (5%), mixed salts (4%) and bran (5%) with the addition of 3% of filter paper. In addition each guinea pig received 30 mg ascorbic acid, 100 i. u. vitamin A and 10 i. u. vitamin D daily.

The second group consisted of young guinea pigs with an initial weight of 310-400 g. The animals received Lecocq's diet, consisting of wheat flour (50%), wheat bran (40%), brewers' yeast (2.5%), calcium lactate (5%), NaCl (1.5%), and sunflower oil (1%). In addition each guinea pig received 25 mg ascorbic acid, 20 mg chlorine chloride, and 80 μg folic acid daily. Vitamins A and D were given once a week in sunflower oil in a dose of 120 i. u. vitamin A and 10 i. u. vitamin D daily. Once a week the animals also received 1 mg vitamin E.

As a preliminary measure the guinea pigs were accustomed to each diet as the various ingredients of the vivarium diet were gradually withdrawn.

For a few days before the beginning of the experiment the animals were transferred to double-bottomed cages and received a diet free from vitamin P ad lib.

During the experiment the guinea pigs were kept in metabolism cages for collection of their urine. For the first 5 hours after the beginning of the experiment the animals of both groups received additional water to the amount of 8-10 ml (2 ml each hour) and they were fed once, for which purpose they were transferred for 30 minutes to an ordinary cage. The total amount of water obtained by the animal in the 24 hours was not brought to the normal level in the first group, but in some animals of the second group it was brought to normal to ensure a more or less equal

diuresis, its volume in most experiments being 22 ml. The animals' urine was collected in metabolism cages for 18 hours. The urine in the receivers was acidified with hydrochloric acid to prevent oxidation of the phenols.

The urine was collected several times from each animal at the beginning of the experiments before vitamin P loading (control investigations), and on the days after administration of the rutin preparation. During the experiment urine was collected from each animal from 14 to 18 times, and as a rule not less than 3 estimations were made for each dose of rutin.

Animals of the first group received a 5% solution of urutin* (100 mg calculated as rutin) or rutin as a suspension in water (50 and 100 mg). The animals of the second group received rutin only, in the same doses as the first group.

Vitamin P was given to the animals from a pipet by mouth in 2 doses of 2-3 ml each, a total of 4-6 ml, at an interval of 1 hour, great care being taken over the feeding.

The volume of urine collected over a period of 18 hours was measured and its content of phenols and substances reacting with $AlCl_3$ was determined. The total and free phenols in the urine were determined by a method based on the reaction with p-nitrophenyldiazonium, as recommended by Yu. I. Shillinger and N. V. Orlova [2]. The measurement was made on a photometer with an M-50 (No. 6) light filter. The calculations were made from calibration curves constructed for pure phenol. From 92 to 96% of the phenol added to urine was determined by this method.

Because rutin also gives a reaction with p-nitrophenyldiazonium, a preliminary determination was made of the correlation between rutin (dissolved in water and alcohol) and phenol. This showed that 10 mg rutin corresponds to 1.2 mg free phenol.

Rutin and the substances reacting with aluminum chloride were determined by our modification of the chemical method of Porter and co-workers [10]. The measurements were made with an SF-4 spectrophotometer at 415 mμ. A calibration curve drawn up for rutin was used for the estimations. From 94 to 112% of rutin added to urine was determined by this method.

For the qualitative identification of rutin we used the method of radial paper chromatography, which we developed earlier [1].

EXPERIMENTAL RESULTS

The volume of urine excreted by the animals of the first group, kept on a casein diet, during the experimental period (18 hours) varied from 23 to 40 ml. The results of the estimation of free phenols in the urine of 2 guinea pigs are shown in Fig. 1. It will be clear from Fig. 1 that the content of phenolic substances in the urine of the animals was unchanged after administration of the 5% urutin solution, the free phenols amounting to 1.5-3.0 mg in the course of 18 hours**, and the difference between the total and free phenol content was insignificant, indicating the absence of combined phenols in the urine. Substances detected by Porter's method were not found in the urine of the animals not receiving rutin preparations. The reaction with aluminum chloride was also negative after administration of urutin. Thus urutin, when added to the casein diet of guinea pigs, caused no change in the free phenol content of the animals' urine and did not lead to the appearance of substances in the urine reacting with aluminum chloride.

In contrast to urutin, the administration of rutin to the same animals led to a marked increase in the content of free phenols in the urine, which rose with increasing dosages to reach 5.5-9.5 mg for a period of 18 hours; no combined phenols were found in these experiments. Qualitative investigations of the urine revealed substances detectable by Porter's method. These were not estimated quantitatively or separated by chromatography.

The volume of urine excreted by the animals of the second group, kept on Lecocq's diet, varied sharply between 9 and 27 ml in spite of the administration of a standard volume of water (22 ml on the day of the experiment). No regular pattern could be detected in the changes in diuresis after administration of rutin. The results of the determination of phenols in 5 experimental animals of this group are shown in Fig. 2. The free phenol content of the urine of these animals when kept on a diet without addition of rutin was 1.2-4.8 mg in 18 hours, i.e., approximately the same as that found in the urine of animals kept on a casein diet. After the addition of rutin to Lecocq's diet the content of phenols in the urine rose appreciably. For instance, when 50 mg rutin was given, from 2.9 to 6.5 mg free phenols was found in the urine of 4 guinea pigs in 18 hours of the experiment, and in one guinea pig (No. 4) the free phenol

*Urutin is a soluble preparation of rutin with urotropin.

**In preliminary experiments no obvious changes in the excretion of phenols in the urine were observed 3-5 hours after administration of urutin.

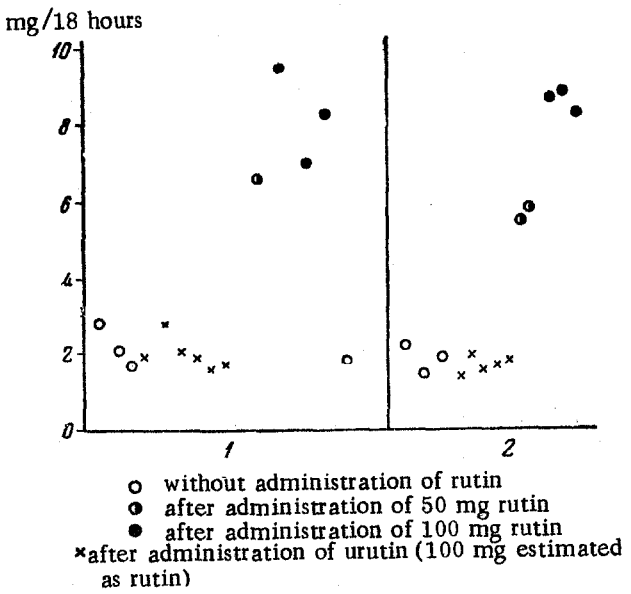

Fig. 1. Content of free phenols in the urine of guinea pigs kept on a casein diet before and after administration of rutin preparations. The figures denote the identification No. of the guinea pigs.

excretion was 9.5 and 11.5 mg. When 100 mg rutin was given the excretion of free phenols varied between 3.3 and 10.3 mg. The total phenol content was close to the free phenol content, indicating the absence of combined phenols in the urine in this case too.

No experiments were conducted with urutin because in the first series of experiments this preparation had no effect on the excretion of phenols in the urine.

In our investigations of the urine of animals not receiving rutin, we found no substances giving a qualitative reaction with $AlCl_3$, which would indirectly indicate the presence of flavones. In contrast to this, after administration of rutin (100 mg) to the animals the urine turned yellow on the addition of $AlCl_3$, indicating that some new substances had appeared. As determined by the reaction with $AlCl_3$, they amounted to between 0.3 and 4.2 mg in the total volume of urine, estimated as rutin.

In order to find out to what extent the appearance of the new substances in the urine was associated with the presence of rutin in the urine, we used the method of radial paper chromatography. However, during the chromatographic separation of the urine collected after administration of rutin to the animals, no rutin was found. This

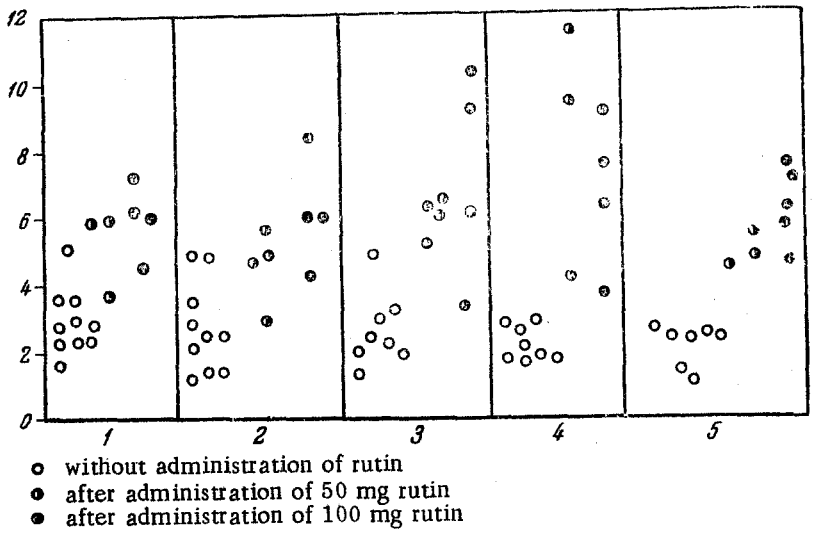

Fig. 2. Content of free phenols in the urine of guinea pigs kept on Lecocq's diet before and after administration of rutin. The figures denote the identification No. of the guinea pigs.

could have been due either to the complete absence of rutin from the urine, or to its presence in a concentration outside the limits of sensitivity of the method used (less than 4 μg rutin in 1 ml urine). Meanwhile the chromatograms of the urine from the animals receiving rutin clearly showed the presence of certain new substances giving bluish-green spots of fluorescence when developed with $AlCl_3$ in ultraviolet light. The object of our future research will be to establish the nature of these substances.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
