

## INVESTIGATION OF THE VITAMIN P ACTIVITY OF A SOLUBLE PREPARATION OF RUTIN

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(Received August 19, 1957. Submitted by Active Member Acad. Med. Sci. USSR B.A. Lavrov)

Difficulties are encountered during investigations with pure rutin on account of its low solubility in water. This requires the use of such solvents as, for example, propylene glycol or a hot 5% solution of ethyl alcohol [11, etc.]; these authors point out, however, that on cooling for only a few minutes the rutin begins to be precipitated.

In the literature the use of rutin is described in the form of its soluble compound with urotropin [10, etc.]; the preparation and properties of this compound were recently described in detail in articles by T.P. Litvinova [5, 6]. Little has been written about the biological action of this soluble preparation. Accordingly, we performed this present investigation in order to study the biological action of this soluble compound of rutin with urotropin.

### EXPERIMENTAL METHOD

The preparation used in the experiments was obtained in ampoules from the Moscow Pharmaceutical Institute, and we also used a rutin preparation made in our own laboratory by the following formula [5]: to 2.5 g rutin and 5 g urotropin was added 100 ml of distilled water, and the mixture was kept at boiling point for 2-3 minutes, then after cooling the solution was made up to its original volume. A transparent solution was obtained, dark yellow in color and with a sweet taste and neutral reaction. Weaker solutions of rutin were prepared by suitable dilution of the 2.5% rutin solution in water.

In order to determine the action of rutin a biological prophylactic method was used. We employed a formula used in the Institute of Biochemistry of the AN SSSR [4], introducing certain changes (a different diet, different type of cage, for measurement of capillary resistance we selected another part of the animal's body, and so on). The vitamin P action of the test preparation was determined by its effect on the change in capillary resistance of the skin of the hind part of the back of a rat by applying vacuum suction at a negative pressure of 200 mm of mercury below atmospheric. The capillary reaction was determined while a shaved (2 to 3 hours before the determination) area of the animal's skin was kept under the vacuum until the appearance of the first petechiae (the number of petechiae must not be less than three). The surface of this area of the animal's skin was smeared with vaseline before measurement of the permeability.

Experiments were made on young, male white rats weighing 80 g. Before the experiments the animals were kept on a general diet consisting of oats, bread, meal, meat, vegetables, milk and sunflower seeds.

In the period immediately before the experiment preliminary measurements were made of the time of appearance of petechiae in the rats. In each group rats were selected with close capillary reactions. Animals whose capillary reactions were very different were not included in the experiments.

The experimental rats were kept on a diet lacking in vitamin P, consisting of corn starch - 65%, casein -

18%, bakers' yeast - 8%, lard - 5% and mixed salt - 4%. According to the findings of our Institute, the yeast contained vitamin B<sub>1</sub> - 2 mg % and vitamin B<sub>2</sub> - 4.8 mg %. From the constituents listed cakes were baked every day and these were given in equal weight to all the animals in such quantities that surplus food was left: on the 5th to 10th days each rat was given from 20-25 g and on subsequent days from 30-40 g. The rats' rations also included water. In addition, each rat received every day 10 I.U. of vitamin A and 5 I.U. of vitamin D, dissolved in sunflower oil, by mouth.

Altogether in the experiments there were three groups of animals, each of twenty-five rats. The rats were distinguished by the quantity of the rutin preparation given.

The rats in the first group (control) received every day 4 mg of urotropin (0.08 mg in 5% solution). The rats of the second group received every day a preparation equivalent to 0.1 mg of rutin (0.08 ml of 1.25% solution of rutin with urotropin). The rats of the third group received daily the preparation equivalent to 2 mg of rutin (0.08 ml of 2.5% solution of rutin with urotropin). The experiments were performed in May and June 1954.

During the experiment each cage contained 4 rats, with the exception of 5 animals from each group which were kept in isolation so that their dietary intake could be calculated.

In order to determine the dietary intake, the residual food from all the rats was weighed daily. To determine the effect of the test doses of the preparation of rutin on growth, the animals were weighed once every five days.

The experiment lasted one month. At the end of the experimental period the capillary reactions of the animals were again tested at the same pressure and using the same areas of skin. On the day they were removed from the experiment the rats received the appropriate amount of rutin preparation in the morning; the measurements of the capillary reaction were made about 3 hours after administration of the rutin.

In the animals of the first and third groups the ascorbic acid content of the organs (liver, brain, spleen) was estimated by means of a method used routinely at the Institute of Vitaminology.

#### EXPERIMENTAL RESULTS

The rate of growth of the animals kept on a diet including the rutin preparation and on the same diet without vitamin P was almost the same (see table).

Average Weight of Rats Before and After Experiment

No. of group	Dose of preparation (in mg of rutin)	Average weight of rats (in g)		Average increase in weight in %
		at the start of the experiment	at the end of the experiment	
First	0	80.1	217.5	171
Second	0.1	81.7	193.5	136.8
Third	2.0	80.2	215.6	168.8
Fourth	General diet*	79.8	170.8	114.0

\*For comparison observations were made on animals (19 rats) kept on the general diet for the same period.

The average increase in weight of the animals at the end of the experiment in the control group and in the third group was the same (about 170%); in the second group the increase was rather less (about 140%). In animals kept on the general diet the increase was smaller (114-117%) than in the remaining groups. Thus animals kept on an artificially blended diet grew more rapidly in the same period of time than rats kept on the general diet of the vivarium.

The absence of any influence of rutin on the rate of growth of male white rats with an initial weight of 90 g, with a considerably larger content of rutin in the diet (0.25, 0.5 and 1% of rutin in the diet) was observed [11] in an experiment of long duration (140 days with 6 animals in a group). These authors also point out that rutin had no effect on the weight of the organs nor on their histological picture. Mescherskaia-Shteinberg and

co-workers [7] also showed that the daily administration of 2 mg rutin per 1 g weight to growing rats and mice did not affect their growth.

The appetite of the various groups of animals kept on the artificial diet with or without the rutin preparation was practically the same, as confirmed by the fact that the quantity of food eaten by the animals during the experiment was almost the same.

The results of the measurements of the time of appearance of the capillary reaction in the animals at the commencement of the experiment and 30 days afterwards are shown in Figure 1.

In the animals of the second and third groups, at the commencement of the experiment the capillary reaction appeared quite quickly, mostly during 10 to 30 seconds (in 83 or 92% of cases) while in the animals of the control group the reaction appeared more slowly (in 76% of cases during 30 seconds or more).

At the end of the experiment the animals of each group showed considerable variation between the times of appearance of the reaction. In rats of the control group, not receiving the preparation (see Figure 1a), the time of appearance of the capillary reaction was the same in 60% of cases, and amounted to less than 20 seconds. This shows that the capillary permeability of the animals was increased in the absence of vitamin P<sub>1</sub>.

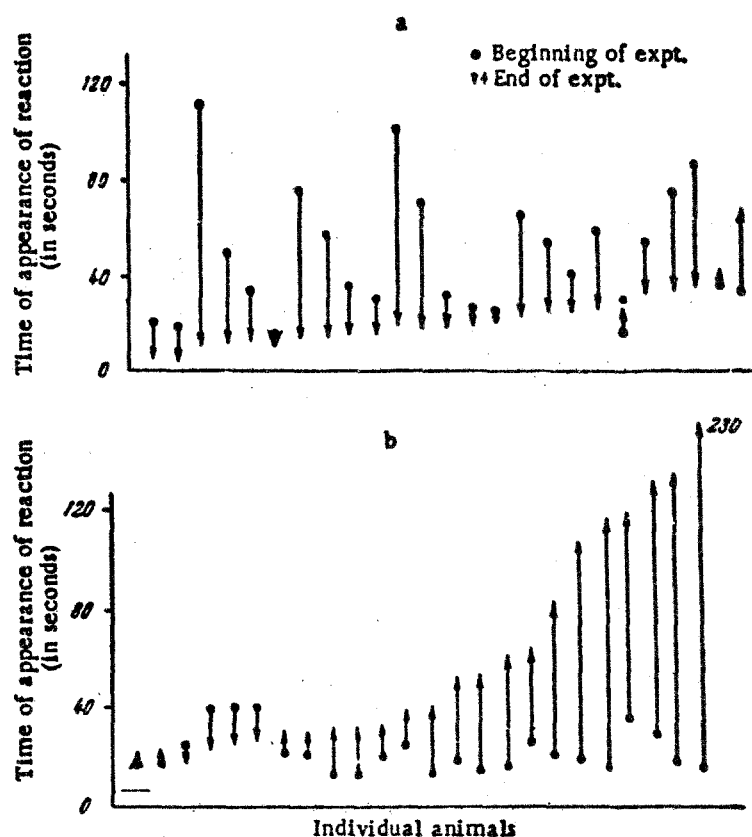


Fig. 1. The capillary reaction in animals: a) not receiving a rutin preparation (first or control group) and b) receiving the preparation in a dose of 0.1 mg per day calculated as rutin (second group).

In rats receiving preparation equivalent to 0.1 mg of rutin (second group, Figure 1b), in the majority of cases (71%) the time of appearance of the capillary reaction was over 30 seconds. In animals receiving a large dose of the preparation - equivalent to 2 mg of rutin (third group), in 52% of cases the time of appearance of

the reaction was also over 30 seconds. Comparison of these findings with the results of observations on animals of the control group shows that the capillary permeability of the animals of the two experimental groups at the end of the experiment was lower (time of appearance of the reaction over 30 seconds) than in the control animals (time of appearance of the reaction under 20 seconds).

The results obtained show that the preparation of rutin with urotropin\* reduces the capillary permeability. However, we were unable to establish a connection between the physiological activity and the selected doses of the preparation. V.N. Bukin and N.N. Erofeeva [2] also report on the action of this preparation in strengthening the capillaries.

Values of the ascorbic acid content of the organs of the rats (Figure 2) kept on the vitamin P-deficient diet and on a diet containing the preparation (equivalent to 2 mg of rutin daily) show that the ascorbic acid content of the liver, brain and spleen of these two groups of rats was almost identical. Consequently the vitamin P preparation has no effect on the concentration of ascorbic acid in the organs of rats.

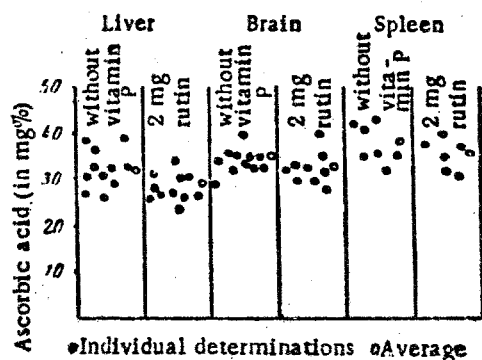


Fig. 2. The ascorbic acid content of the organs of the rats.

It must be mentioned that our values of the ascorbic acid content of the liver of the rats (30-33 mg %) are considerably higher than those found by other workers in experiments on animals kept on ordinary diet [9, 3] and are closer to the results obtained in experiments using the same artificially blended diet [8, etc.]. In the brain of the experimental rats we found from 33-35 mg % of ascorbic acid, which is somewhat higher than the findings of other workers [3].

Our findings of the ascorbic acid content of the spleen (39-37 mg %) are close to the experimental results of other workers [9, 3, 8]. Since the increased content of ascorbic acid, especially in the liver, was observed in both animals not receiving vitamin P and in rats receiving the rutin preparation, the increased synthesis of ascorbic acid may be attributed to the effect of the diet.

## SUMMARY

A soluble preparation of rutin with urotropin has a P-vitaminic activity which is manifested by increased stability of capillaries. This preparation has no effect on the growth and appetite of the animals or on the concentration of ascorbic acid in the liver, brain or spleen of rats.

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\*Any influence of urotropin on the growth and appetite of the animals and also on the permeability of capillaries was excluded by comparison of our results obtained from observations on animals of the control group, receiving urotropin, with the findings obtained at the same time by N.N. Berezovskaia [1] from observations on animals receiving no additions to the diet and kept under the same experimental conditions.

\*\*Original Russian pagination. See C.B. translation.

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