INFLUENCE OF VITAMIN P UPON TOXIC EFFECTS CAUSED BY POISONS OF THE ACCOMPANYING PHOSPHORYLATION

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Jeney and co-workers [3-5] in a series of papers have established that quercetin (a preparation of the vitamin P-group) in a dose of 12 γ/g lowers the body temperatures of rats, and – which is especially important – that it prevents hyperthermia caused by dinitrophenol. In the opinion of the authors, the influence of quercetin facilitates the synthesis of glycogen from lactic acids even under the condition of dinitrophenol poisoning.

In the present work we studied the influence of compounds of the vitamin P group (quercetin and rutin) upon the contractile effects of dinitrophenol and other poisons connected with phosphorylation. The object of our studies was the tonic muscle (straight abdominal muscle of the frog), in which such a contracture occurs under the influence of 50-100 times lower concentration than in the atonic (sartorius) muscle.

EXPERIMENTAL METHOD

The experiments were performed upon frogs which were caught in the spring and in the fall of 1954. In a portion of the experiments we used half the muscle (before isolating the muscle, we severed with the scissors along the midline so that one side served as a control for the other side), in other experiments we used two whole muscles from different animals of the same sex and of approximately the same size. These muscles were kept for one hour in aerated Ringer's solution and then for a period of 15-30-45 minutes one of them was subjected to the influence of solutions of quercetin and rutin in concentrations of 1:7500 to 1:2000 (prepared from a 0.2% solution of quercetin and rutin kept in a 0.1% solution of sodium bicarbonate). After that both to the control and the test solutions in which the muscles were being kept was added one of the poisons of of the accompanying phosphorylation (methylene blue, dinitrophenol, gramicidin S, and monobromoacetate of sodium).

The concentrations of the muscles were recorded on the smoked paper of kymograph drums. The length of the recording moving arms (12 and 3.5 cm) and the load (0.9 and 0.85 g) were kept constant in all experiments.

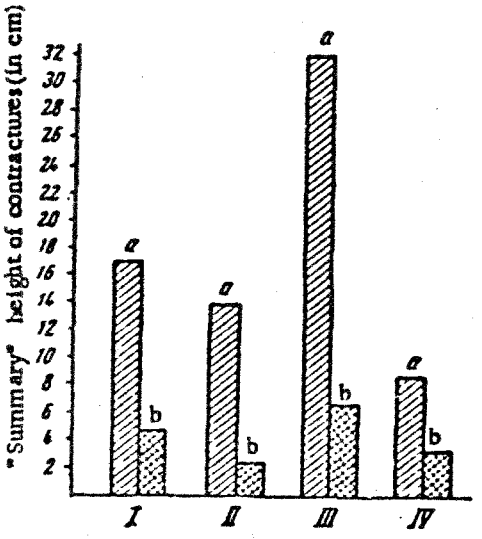
EXPERIMENTAL RESULTS

Contractures, caused by methylene blue (15 expts). Methylene blue (1:4000) by the 20th minute caused a regular contracture, which grew by the end of an hour of observation to a height of 1-3 cm. With the 5 muscle preparations previously treated with quercetin or rutin (1:5000), methylene blue caused in the same time interval no muscle contractures whatever. After rinsing out the same five muscle preparations, methylene blue (1:4000) caused the writing arm to rise 2.0-2.5 cm within 15-20 minutes. In the other 10 experiments contractures began to arise only after 35-40 minutes, and even after an hour did not exceed 0.5-0.7 cm. The

results of experiments on the muscles of winter and spring frogs were practically indistinguishable and are shown on the illustration.

The substitution in the immersion bath for rutin and quercetin of 0.1% soda solution in the same volume did not alter the contracture effect of methylene blue.

Contractures caused by dinitrophenol (43 experiments). Muscle contractures in spring frogs appear under the influence of weaker concentrations of dinitrophenol (1:200,000) than in winter frogs (1:100,000-1:75,000). In the spring frogs, muscle contractures begin 10-12 minutes after the addition of the poison to the bath, and with winter frogs – after 25-35 minutes. Out of 23 experiments conducted with dinitrophenol, in ten the prior use of rutin and quercetin (1:5000) prevented this shortening entirely. After repeated rinsing out with Ringer's solution of the quercetin and rutin, dinitrophenol in the original concentration quickly produced the contractures in all 10 experimented muscles. In the other experiments quercetin and rutin delayed both the appearance and growth of the muscle contractures. In the muscles of the winter frogs the latent period of the appearance of contracture was multiplied 3-4 times, and the increase was delayed 5-6 times. Accordingly, it is assumed that in the muscles of spring frogs, which react less readily to dinitrophenol, the influence of quercetin and rutin is manifested more strongly.



Influence of rutin and quercetin on the development of contractures of the straight muscle from the abdomen of the frog, caused by poisons of the accompanying phosphorylation. Duration of action of the poisons – 60 minutes: columns a) contractures of control muscles, columns b) contractures of treated muscles. I) Methylene blue (1:4000) – 15 experiments; II) monobromoacetate of sodium (1:25,000) – 10 experiments; III) dinitrophenol (1:200,000-1:100,000) – 12 experiments, Spring, 1954; IV) dinitrophenol (1:100,000-1:75,000) – 11 experiments, Fall, 1954.

In connection with the experiments of N. V. Golyakhov [1], who has shown that the toxic effects of dinitrophenol are greater in an acid than in an alkaline medium, special work was done. In 3 out of 7 cases when soda solutions were added in concentrations similar to that of quercetin and rutin (1:15,000-1:10,000), the dinitrophenol contracture effect was delayed. In the other 4 cases the addition of sodium solution did not alter the development of the dinitrophenol contractures.

In 20 experiments muscles were subjected to the action of quercetin (1:5000-1:1000) without addition of soda solution (0.1% solution can be used for 3-5 hours before the quercetin flocculates). In 6 of the 20 experiments, in the presence of quercetin no contractures developed; in 12 they appeared later and were weaker than in the controls. In 2 cases, both when a 1:10000 solution of quercetin were used, there was a more rapid development of muscle contracture.

Contractures caused by gramicidin S (12 experiments). In the muscles of spring frogs under the influence of gramicidin S (1: 200,000-1:100,000) contractures began within 2-3 minutes under the influence of the poison, reaching a maximum after thirty to forty minutes.

In 2 experiments in the presence of quercetin (1:5,000) muscle contracture did not occur. In the other 10 experiments muscle contracture took place later and for the first 10-15 minutes developed more slowly, with the maximum however, occurring as in the controls, within 30-40 minutes.

In the fall frogs neither rutin nor quercetin altered the time of the appearance of contractures caused by gramicidin S in concentrations of 1:100,000-1:75,000.

Contractures caused by the monobromoacetate of sodium (10 experiments). Parallel with the poisons suppressing respiratory phosphorylation, there was used a typical glycolytic poison – the monobromoacetate of sodium. In control muscles, the monobromoacetate in concentrations of 1:50,000-1:25,000 caused muscle contractures within 25-30 minutes of addition (7 to 10 experiments). At the end of the first hour, all muscles were in a state of contracture, the elevation of the writing needle varying from 0.5 to 3.5 cm. In muscles previously subjected to the influence of rutin (1:5000; 4 experiments), or quercetin (1:5000; 6 experiments), the contractures appeared only after an hour, and then only in 3 out of 10 cases. By the end of the 2nd and beginning of the 3rd hour both the experimental and control muscles showed contractures reaching 3-5 cm.

It must be noted here that in neither control nor experimental preparations was there any evidence of rutin or quercetin influence when acetylcholine or potassium were tested.

SUMMARY

In the presence of quercetin or rutin there is delay, diminution, or even prevention of contractures caused by poisoning associated with phosphorylation. The protecting action is more pronounced against muscle poisoning due to methylene blue and monobromoacetate of sodium, and is weaker against poisoning with dinitrophenol and gramicidin S. So, the more toxic the substance [2], the more difficult it is for vitamin P to protect against its effect.

The influence of quercetin and rutin upon the development of a shielding from the effect of contractures caused by poisoning associated with phosphorylation is selective, since contractures caused by acetylcholine and potassium are not affected.

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