

are fungicidal under conditions of the study, the process affected is evidently concerned with the growth alone, and not with the survival of the resting organism. That this property of histamine is specific toward the antihistaminics is evident from the fact that the fungistatic action of 10% undecylenic acid (as Desenex) is not affected. Additional work with other strains as well as on the underlying mechanism of action is now being pursued and will be reported at a future date. From the practical standpoint not only may the allergic component in host tissue response to dermatophytoses be controlled by the use of antihistaminics (in conjunction with conventional fungicidal preparations), but also some fungistatic action by the antihistaminics them-

selves might be expected. Finally, our results throw light on the mechanism of fungistatic action of the antihistaminics; thereby histamine (or chemically related substances), liberated by infected host tissue, could not be utilized for growth by the organism.

Summary. Several antihistaminics are fungistatic, preventing the growth of *T. mentagrophytes*. The effect is prevented by suitable amounts of histamine, but not by histidine.

While this report was in preparation, Reiss *et al.* published similar results with Trimeton Maleate (Exp. Med. and Surg., 1950, v8, 330) with reference to histamine.

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Protection by Flavonoids against Histamine Shock. (18549)

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Wilson, Mortarotti and DeEds(1) reported that pre-treatment with rutin resulted in slight, though definite, protection of guinea pigs given LD₅₀ doses of histamine. There was a previous report by Hiramatsu(2) that hesperidin afforded protection of guinea pigs against anaphylactic shock, and Parrot and Richet(3) had found that the increased sensitivity of scorbutic guinea pigs to histamine was returned to normal after administration of D-catechin isomers. Since publication of the paper by Wilson *et al.*, there have been a number of other reports on the subject. Clark (4) stated that rutin and quercetin afforded protection against histamine; others(5-11)

were unable to confirm this. Three papers (5,9,10) described a protection against anaphylactic shock, while three others(4,7,8) reported no protection. In addition to the above direct evidence, there are reports of beneficial effect of flavonoids in peptone shock (12), in the Shwartzman phenomenon(13), in an anaphylactoid reaction(14), in drug sensitization(15), and in allergic conditions in man(16,17). Shanno(18) stated that ru-

1. Wilson, R. H., Mortarotti, T. G., and DeEds, F., *J. Pharm. Exp. Therap.*, 1947, v90, 120.

2. Hiramatsu, N., *Japanese J. Dermat. Urol.*, 1941, v49, 304.

3. Parrot, J.-L., and Richet, G., *Compt. rend. soc. biol.*, 1945, v139, 1072.

4. Clark, W. G., *Am. J. Physiol.*, 1949, v159, 564.

5. Raiman, R. J., Later, E. R., and Necheles, H., *Science*, 1947, v106, 368.

6. Levitan, B. A., *Proc. Soc. Exp. Biol. and Med.*, 1948, v68, 569.

7. Roth, L. W., and Shepperd, I. M., *Science*, 1948, v108, 410.

8. Arjona, E., Lorente, L., Aguirre, M., and Perianes, J., *Bull. Inst. Med. Res. Univ. Madrid*, 1949, v2, 139.

9. Hepding, L., *Deutsch. med. Wochenschr.*, 1949, v74, 1575.

10. Moss, J. N., Beiler, J. M., and Martin, G. J., *Science*, 1950, v112, 16.

11. Semenza, F., *Acta Vitaminologica*, 1949, v3, 257; Abstracted in *Excerpta Med.*, 1950, v3, Sec. II, 1112, Item 4574.

tin was of no benefit in 3 cases of drug sensitivity. Peña(19) found that histamine wheals in man were smaller in size and disappeared more rapidly when rutin was administered.

The conflicting findings mentioned above indicate that the experiment is not as simple as it appears at first glance. In this paper we present data obtained in an attempt to repeat our earlier work with rutin, and in addition we have studied 3 other flavonoids for possible protection of guinea pigs injected with histamine.

Materials and method. Guinea pigs were purchased from local animal supply firms, and represented a heterozygous stock. After becoming acclimated to the conditions of the laboratory, the animals were placed on a scorbutic diet supplemented with ascorbic acid, as previously described(1), for a week or two, in order to deplete them of flavonoid materials. Body weights at time of test varied from 250 to 540 g, with 75% of the animals weighing from 310 to 465 g. Body weight, within the above limits, and sex did not appear to influence either the reaction to a given dosage of histamine, or the effect of flavonoids on histamine shock. What did affect susceptibility to histamine was the particular batch of animals. It was necessary to order 4 or more dozen animals at a time in order to have a sufficient number of experimental pigs available after standardizing the group against histamine. The dosage of histamine was critical and varied from batch to batch of animals. The variation was not seasonal. Histamine was given as the hydro-

chloride, with the approximate LD_{50} dose ranging from 0.20 to 0.25 mg of histamine base/kg. Injection was into the jugular vein after the vein had been exposed following procaine anesthesia. A 5% change in dosage was sufficient to change an LD_{50} to an LD_0 or an LD_{100} . To demonstrate a protective effect of flavonoids on histamine shock, an LD_0 was useless, of course, and the action of flavonoids is so slight that a clear demonstration of protection was not obtained if a dosage of histamine near the LD_{100} level were used. A brief discussion of this point has been published(20). All of the flavonoids* were given intraperitoneally, 40 mg/animal, 30 minutes before histamine. At this high level, which was chosen deliberately so as to get maximum intraperitoneal absorption, there was occasional precipitation within the peritoneal cavity, especially in the case of quercetin. Eight per cent solutions of rutin and quercitrin were made in 33% propylene glycol with the aid of heat and a trace of alkali ($NaHCO_3$). A 20% solution of quercetin in straight propylene glycol permitted injection of a small enough volume (0.20 ml) to cause minimal peritoneal irritation. The hesperidin methyl chalcone was dissolved in saline to give an 8% solution. All control animals received intraperitoneal injections of the appropriate solvents.

Results. The results are presented in Table I. The data on rutin-treated animals is divided into 2 parts. The earlier data were obtained from small groups of inadequately standardized animals, and indicate no protection against histamine. Later tests with more carefully standardized animals show that rutin gives some, though not marked, protection.

The other flavonoids were given to somewhat fewer animals, but the protection is more apparent. The animals pre-treated with

12. Lavollay, J., and Neumann, J., *Compt. rend. Acad. Sci.*, 1941, v212, 251.

13. Maratka, Z., and Ivy, A. C., *Gastroenterology*, 1948, v11, 357.

14. Goldstein, D. H., Stolman, A., and Goldfarb, A. E., *Science*, 1943, v98, 245.

15. Hüllstrung, H., and Hack, K., *Z. Immunitäts.*, 1941, v100, 393.

16. Isaacs, R., *Proc. Central Soc. Clin. Res.*, 1944, v17, 66.

17. Saylor, B. W., *Arch. Otolar.*, 1949, v50, 813.

18. Shanno, R. L., *Am. J. Med. Sci.*, 1946, v211, 539.

19. Peña Regidor, P. de la, Caverio, F., and Segarra, F., *Rev. Clin. Española*, 1950, v37, 118.

* The rutin was furnished by the Eastern Regional Research Laboratory, of this Bureau; the methyl hesperidin chalcone was kindly supplied by the California Fruit Growers' Exchange. The quercetin and quercitrin were isolated from lemon flavine by a recently-reported procedure(21).

20. Wilson, R. H., and DeEds, F., *Science*, 1948, v107, 369.

21. Booth, A. N., and DeEds, F., in press.

TABLE I. Survival of Histamine-Treated Guinea Pigs After Pre-Treatment with Flavonoid Compounds.

	Controls		Experimental	
	No. surviving	% surviving	No. surviving	% surviving
	No. injected		No. injected	
Rutin (early tests)	9/15	60	10/15	67
" (later tests)	8/15	53	16/20	80
Quercitrin	4/7	57	5/5	100
Quercetin	3/10	30	8/10	80
Methyl hesperidin chalcone	2/7	29	6/11	55

quercetin were especially noteworthy, in that survivors had no, or exceptionally slight, symptoms of histamine shock.

Discussion. There are two points at which modification of technic can change positive to negative results: the time between flavonoid administration and histamine injection (1), and histamine dosage, which may vary with each batch of animals. A critical consideration of the various papers concerned with the effect of flavonoids on histamine shock shows that the main variable was dosage of histamine. In most cases where negative results were reported, the amount of histamine was great enough to kill all, or nearly all, of the control animals. Since the protection by rutin is slight, it is much less apt to be apparent at high histamine levels. With a sufficiently large group of animals carefully standardized against histamine, we found protection. When less care was taken to standardize the animals, and when amounts of histamine approaching an LD₁₀₀ were used, we were unable to show any effect from the flavonoids. Incidentally, it has been our experience that the slope of the histamine curve in any one group of animals is very steep, so that small changes in dosage have a profound effect on mortality.

The three other flavonoids likewise afforded protection, with quercetin appearing to be the best of the substances tested. The exact degree of protection cannot be judged by these smaller groups. Clark(4) reported that he found quercetin sulfonate more efficient against histamine than was rutin.

It should be emphasized that the flavonoids are not antihistaminics. Not only is their protective action very slight, but there is evidence(1,8,9,19) that there is no direct antagonism between rutin and histamine. The reaction is indirect and is important only in suggesting the mode of action of the flavonoids.

Summary. A slight protection of guinea pigs treated with rutin and subjected to an LD₅₀ dose of histamine has been confirmed. The failure of certain other investigators to substantiate this finding may have been due to excessive histamine dosage or the use of inadequately standardized animals. Quercitrin, quercetin and methyl hesperidin chalcone also were found to afford protection against histamine. The protection is slight and is of theoretical rather than clinical interest.

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