a greater percentage of the available cytochrome oxidase, it seems logical that lethality in the cold would occur when a proportionately smaller fraction of the total enzyme was inhibited.

Mice that were acclimatized to the cold were no more susceptible to DFP intoxication than were the controls treated at room temperature, while the same drug was 45 per cent more potent in mice that had been exposed to the cold for only 24 hours before injection. The anatomical and functional changes that occur in acclimatization to the cold are not entirely understood. However, several workers have reported significant increases in the kidney and liver weight as a function of long exposure to low ambient temperatures(11,12). These findings, confirmed by recent work done in this laboratory, indicate that the detoxification potential of these tissues in cold-acclimatized animals may be greater than in unacclimatized or normal animals. This phase of toxicology obviously requires more study. However, the generalized decreased resistance of the animals to drugs after short exposure to the cold indicates that these mice were essentially weaker and somewhat hypothermic animals under stress. This is borne out by the relatively high natural mortality evident during the first 24 hours at 4°C.

Summary. The toxicity of colchicine, DFP, Intocostrin and KCN was increased in mice that were exposed to 4°C for 24 hours and maintained in the cold after injection. Animals that were acclimatized to the cold were no more susceptible to DFP than were controls treated at room temperature. The possible factors implicated in these findings are discussed.

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Influence of Histamine upon Fungistatic Action of Antihistaminics. (18548)

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The work of Carson and Campbell(1), which reported the fungistatic action of 3 antihistaminics, prompted an investigation concerning which the following is a preliminary report. In addition to testing fungistasis by several antihistaminics, we were also interested in determining whether the compounds are fungicidal as well. Lastly, it was considered of fundamental biochemical interest to determine whether the antifungal action of antihistaminics is based on interference with utilization of histamine (or a compound or compounds of similar structure) by the fungus.

The compounds studied were as follows: Benadryl, (Diphenhydramine HCl) Parke-Davis; Thephorin, (Phenindamine Tartrate) Hoffmann-La Roche; Thenylene, (Methapyriline HCl) Abbott; Neo-Antergan, (Pyranisamine Maleate) Merck; Pyribenzamine, (Tripelenamine HCl) Ciba, and Diatrine, (Metaphenilene HCl) Wm. R. Warner.* The test culture employed was Trichophyton mentagrophytes (Emmons No. 640).

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* The authors gratefully acknowledge supplies of antihistaminics by Dr. Graham Chen, Parke-Davis & Co.; Dr. R. K. Richards, Abbott Laboratories; Dr. H. Molitor, Merck and Co.; Dr. F. F. Yonkman, Ciba Pharmaceutical Co., and Dr. J. A. Aeschlimann, Hoffmann-La Roche.

TABLE I. Activity of Antihistaminics Against *T. mentagrophytes.*

Compound	Highest active dilution (1:)	Highest active dilution (1:) With histamine mg/.5 ml vol. .05	.1	.25	.5	.75	1	2.5	5	With histidine mg/.5 ml vol. 2.5
Thenylene	6400	6400	6400	6400	3200	400	800	400	400	3200
Benadryl	12800	25600	12600	12800	3200	800	800	400	800	12800
Thephorin	51200	51200	51200	51200	3200	400	400	800	800	51200
Neo-Antergan	6400	3200	6400	3200	800	400	400	400	200	6400
Diatrine	25600	25600	25600	25600	800	800	800	400	400	25600
Pyribenzamine	6400	12800	6400	12800	6400	800	400	400	800	6400
Desenex	25600	51200	25600	25600		25600	25600		51200	25600

Methods. The method of testing for fungicidal action was that of Oster and Golden(2). The fungistatic test technic was a serial dilution method using Sabouraud's maltose broth medium in a series of 15 test tubes (75 x 12 mm with aluminum caps to facilitate pipetting) for each test preparation. In each tube except the first was placed 0.5 ml of the base medium. To tubes 1 and 2 were added 0.5 ml of a 1.0% solution of the test material dissolved in the medium. Beginning with tube No. 2, the contents were well mixed and 0.5 ml transferred to the next tube. The same procedure was continued through tube 14 from which 0.5 ml was discarded. Tubes 1 and 15 were controls. With a No. 2 cork borer, inoculum disks 2 mm in diameter were cut from an agar plate culture 12-15 days of age, and were introduced into each of the tubes. Incubation was at 25°C and at the end of 2-3 days initial estimates of growth were made.

Results and discussion. The results obtained indicate that Benadryl, Thephorin, Diatrine, Thenylene, and Pyribenzamine do not exert any fungicidal activity in concentrations of 1.0%, 2.5%, 5.0% and 10.0%. Diatrine and Thephorin, being water insoluble above 2.5%, were tested as suspensions above this concentration and the results, therefore, may not be a true indication of their fungicidal capacity in other solvents making possible higher concentrations. The fungistatic activity of Pyribenzamine appears to be of the order reported by Carson and Campbell. The fungistatic action of the other antihistaminics tested, as is evident in the left column of the table, may be listed in the following descending order of effectiveness: Thephorin, Diatrine, and Benadryl; Pyribenzamine, Thenylene, and Neo-Antergan. It is interesting to note that the action is completely prevented by certain amounts of histamine. By the addition of varying amounts ranging from 0.05 mg to 5.0 mg to each 0.5 ml volume, it was found that in most cases the prevention of fungal growth by the antihistaminic was moderately overcome in the presence of at least 0.25 mg, and completely overcome with 0.75 mg (/0.5 ml volume) of histamine. Concentrations below 0.25 mg were ineffective (See table). On the other hand, histidine had no such effect on our fungal strain with as much as 2.5 mg/0.5 ml, although Robbins and Ma (3) reported in 1945 that histidine augmented the growth of *Trichophyton mentagrophytes* in a casein hydrolysate medium. Whether or not the need for histidine noted by Robbins and Ma is to supply a source of histamine, our observation on histidine seems to indicate that in the presence of the antihistaminics the organism is unable to decarboxylate histidine in sufficient quantity to produce the effect. This aspect of the problem is being studied. An obvious hypothesis to explain the action of histamine herein described is that *T. mentagrophytes* requires histamine (or a closely similar compound) for growth and that the cellular chemical topography concerned is "blanketed" or blocked by the antihistaminics; the reversal is apparently competitive, for the degree depends on histamine concentration within certain limits. Since none of the antihistaminics tested

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3. Robbins, W. J., and Ma, R., *Am. J. Botany*, 1945, v32, 509.

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 themselves 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 LD_{50} 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-

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