static interactions with cellular components involving van der Waals' forces or hydrogen bonding. The degree of polymerization may be an important factor in determining the hemolytic activity of these nonionic surface-active agents with the most highly polymerized compounds evidencing the least hemolytic activity. However, an insufficient range of compounds was available to establish this point.

TABLE 1

HEMOLYTIC ACTIVITY OF SELECTED SURFACE-ACTIVE AGENTS

Surface-active agent	Concentration (μg/ml) for 100-min hemolysis time	Surface tension (dynes/cm) at that concentration
Nonionic compounds		
Triton WR-1352	>10,000	(42)*
Triton A-20	>10,000	(33)
Triton M-3619	33	37
Triton N-100	30	40
Triton X-155	10	51
Triton WR-1363	10	40
Triton WR-1360	6	40
Triton WR-1364	4	57
Anionic compounds		
Dioctyl sodium sulfosuccinate (Aerosol OT)	20	52
Sodium lauryl sulfate (Duponol WA)	17	60
3,9-Diethyltridecanol-6 sodium sulfate (Tergitol 7)	5	57
Cationic compounds		
Cetyl dimethyl benzyl ammonium chloride	9	57
Cetyl pyridinium chloride (Ceepryn)	4	67
Cetyl trimethyl ammonium bromide (CTAB)	4	68

* Values in parentheses were measured at a concentration of 10,000 μg/ml.

It is of interest to record, in addition, that the two compounds observed to have such low hemolytic activity also displayed a low toxicity. Upon intraperitoneal injection into mice, both Tritons WR-1352 and A-20 had an LD_{50} (10-day observation period) of more than 2,500 mg per kg body wt. No higher concentrations were used, due to the viscosity of these compounds at this level. This is in contrast with the LD_{50}'s of the other compounds listed in Table 1, which ranged from 1 to 50 mg per kg for the cationic, 70 to 100 for the anionic, and 100 to 300 for the other nonionic compounds.

In summary, attention has been called to two nonionic surface-active agents capable of altering the physical properties of solutions but largely devoid of such deleterious biological effects as hemolytic activity and toxicity.

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The Inhibitory Effect of Three Antihistaminic Compounds on the Growth of Fungi Pathogenic for Man

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Recent observation in this laboratory revealed that several cases of *tinea pedis* (athlete's foot) responded dramatically to applications of a cream containing 2% pyribenzamine. This observation suggested that the antihistamines should be assayed for inhibitory activity against the pathogenic fungi. The following studies were conducted to determine whether the effect noted was solely against allergic manifestations incited by the etiologic agent (*4*) or whether such compounds possessed fungistatic or fungicidal properties as well.

Three crystalline antihistaminic compounds selected for investigation were pyribenzamine hydrochloride (Ciba),[1] antistine hydrochloride (Ciba),[2] and di-phenyl-pyraline (Nopco).[3] Final concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg/ml of the freely soluble drugs were prepared in Mycophil broth (Baltimore Biological Laboratory). These were dispensed to tubes in 5.0-ml quantities and autoclaved at 15 lb for 15 min. After sterilization the pH of the control broths as well as those containing the varying concentrations of di-phenyl-pyraline and antistine was 6.65; those containing pyribenzamine were pH 6.3.

The dermatophytic species tested included four strains of *Microsporum*, eight of *Trichophyton* and seven of *Epidermophyton floccosum*. Single strains of *Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum*, and *Blastomyces dermatitidis*, causative agents of systemic mycoses, were also employed in the study. With the exception of three organisms (*T. ferrugineum, C. albicans*, and *C. neoformans*) all strains had been isolated from human lesions within the previous six months.

The test media containing the antihistamines and the control media without antihistamine compounds were inoculated with ½-ml amounts of organism suspension prepared according to the method outlined in a previous publication from this laboratory (*1*). All cultures were incubated at 28° C and examined for comparative growth at three-day intervals for a period of two weeks.

[1] N,N-dimethyl-N'-benzyl-N'-(α-pyridyl)-ethylenediamine monohydrochloride.

[2] 2N-benzyl-N-phenyl-aminomethyl-imidazoline hydrochloride.

[3] 1-Methyl-piperidyl-4-benzhydryl ether.

TABLE 1

INHIBITORY EFFECT *in Vitro* OF THREE ANTIHISTAMINIC COMPOUNDS ON RECENTLY ISOLATED STRAINS OF PATHOGENIC FUNGI

Organism	No. strains tested	Compounds					
		Di-phenyl-pyraline		Antistine		Pyribenzamine	
		Negative*	Restricted†	Negative*	Restricted†	Negative*	Restricted†
Dermatophytes:							
Trichophyton mentagrophytes	2	0.50	0.10	1.00	0.25	>1.00	0.25
" *rubrum*	5	0.50	0.10	0.75	0.25	>1.00	0.25
" *ferrugineum*	1	0.25	0.10	0.50	0.10	0.50	0.10
Microsporum gypseum	3	0.50	0.10	1.00	0.50	>1.00	0.25
" *lanosum*	1	0.50	0.10	1.00	0.50	>1.00	0.25
Epidermophyton floccosum	7	0.50	0.10	0.50	0.25	0.75	0.25
Systemic fungi:							
Histoplasma capsulatum	1	0.25	0.10	0.75	0.25	0.50	0.25
Blastomyces dermatitidis	1	0.50	0.10	0.50	0.10	>1.00	0.50
Cryptococcus neoformans	1	0.50	0.10	0.25	0.25	1.00	1.00
Candida (Monilia) albicans	1	>1.00	...	1.00	0.75	>1.00	...

* Mg/ml of drug concentration completely inhibiting growth.

† Mg/ml of drug concentration that restricted growth to approximately 30%–40% of growth in control containing no antihistamine.

The results shown in Table 1 illustrate the comparative inhibitory effect exhibited by the three compounds following the two-week period of incubation. Growth was markedly restricted at 0.25-mg/ml concentrations of di-phenyl-pyraline and completely inhibited, macroscopically, at 0.5 mg/ml with all strains tested except *C. albicans*. The effective concentrations of antistine varied with the species tested and ranged from 0.5 to 1.0 mg/ml. Pyribenzamine was the least effective. Concentrations of 1.0 mg/ml failed to inhibit the growth of 13 of the 23 test organisms.

To determine the effect of hydrogen ion concentration on the inhibitory activity of the compounds, two sets of media were adjusted to pH 5.0 and 8.0 respectively. The subsequent addition of the test drugs did not alter the pH level of either medium. Each set was inoculated with three strains representative of the dermatophytic genera. The results obtained at pH 8.0 with the antistine and di-phenyl-pyraline compounds were similar to those shown in Table 1. Pyribenzamine, however, showed an improvement in its inhibitory capacity in that a concentration of 0.75 mg/ml was as effective at pH 8.0 as a > 1.0 mg/ml concentration at pH 6.3. Conversely, in media adjusted to pH 5.0, 1.0 mg/ml concentrations of each of the three compounds failed to inhibit growth of the organisms. The compounds are heat stable in that no detectable difference in inhibitory activity could be demonstrated between autoclaved and Seitz-filtered solutions of the drugs.

The efficacy of many reportedly inhibitory agents has been shown to be greatly reduced, and in many instances completely negated by the presence of serum in the test medium (*2, 3*). A further study was conducted employing the penicylinder method of assay (*2*) and Sabouraud's dextrose agar containing horse serum in a final concentration of 10%. Two sets of media, one adjusted to pH 5.0 and the other to pH 7.0, were prepared and autoclaved at 15 lb for 15 min. The media were cooled to 45° C for the addition of horse serum, and then were distributed in Petri dishes in 20-ml quantities and allowed to solidify. Aqueous solutions of the drugs were added to the penicylinders on plates streaked with a ten-day-old culture of *T. mentagrophytes*. After ten days' incubation at 28° C, zones of complete inhibition, measuring 4, 3, and 1.5 cm in diam were obtained at both pH levels with 2.0-, 1.0- and 0.5-mg/ml concentrations of di-phenyl-pyraline (see Fig. 1). The inhibitory activity of

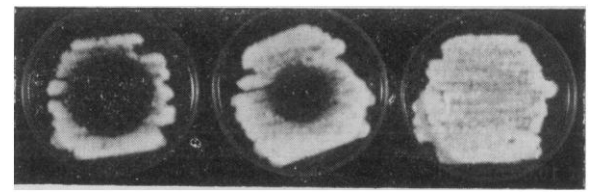

FIG. 1. Inhibitory activity of di-phenyl-pyraline against *Trichophyton mentagrophytes* cultured in Sabouraud's agar (pH 7.0) containing horse serum in a final concentration of 10%. The first penicylinder on the left contained 2.0 mg/ml of the drug, the second 1.0 mg/ml. The third is the organism control.

pyribenzamine against the growth of the test organism on serum agar adjusted to pH 7.0 resulted in zones of complete inhibition measuring 2.5 and 2.0 cm in diam with 2.0- and 1.0-mg/ml concentrations. A 1.5-cm zone was obtained with 0.5 mg/ml but inhibition was not complete. When tested on serum agar adjusted to pH 5.0, the 2.0-mg/ml concentration, showing an inhibitory zone of 1.5 cm, was the lowest level at which this drug was effective. Antistine, which appeared to be inhibitory in the absence of serum (Table 1), failed to inhibit growth at either pH level when serum was incorporated in the medium.

The growths of four systemic species (*C. neoformans* and yeast phase cultures of *H. capsulatum, B. dermati-*

tidis, and *Sporotrichum schenckii*) incubated on the horse serum agar (pH 7.0) at 37° C also were inhibited by 1.0-mg/ml concentrations of di-phenyl-pyraline and pyribenzamine. The growth of *C. albicans* was not affected by either drug.

Results obtained from these preliminary studies suggest that both di-phenyl-pyraline and pyribenzamine possess properties which are inhibitory to pathogenic fungus species and that their activities, therefore, are not limited to the alleviation of allergic manifestations incited by these organisms. The results also suggest that further clinical study is imperative to determine the effectiveness of these drugs *in vivo*.

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Triphenyltetrazolium Chloride in Tissue Culture[1]

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A number of reports have recently appeared in the literature describing a tetrazolium salt (2,3,5-triphenyltetrazolium chloride) as a possible indicator of viability of plant and animal tissues. Lakon (*3*), Cottrell (*1*), and Porter, Durell, and Romm (*4*) have used this compound to test the viability of seeds and wood cuttings, and Straus, Cheronis, and Straus (*6*) employed this salt to demonstrate reducing enzyme systems in living normal and neoplastic mammalian tissues.

In studies of the nucleic acids and of the effect of folic acid and its analogues on tissue cultures, it is occasionally important to determine cytologically the number of living cells present in a particular culture. Since the cytoplasm and nuclei of living cells do not stain with any of the vital dyes, and dead cells stain only diffusely, the use of tetrazolium as an indicator of cellular viability suggested itself. It is the purpose of this paper to report the failure of 2,3,5-triphenyltetrazolium chloride as an indicator of cell viability of tissue grown *in vitro*.

The method employed in testing this compound consisted of growing chick-heart fibroblasts under perforated cellophane (*2*) in a medium composed of equal parts of human fetal (umbilical cord) serum and Simms salt-diluted ultrafiltrate (*5*). After 72 hr, when sufficient migration and outgrowth of fibroblasts had occurred, the cultures were placed in a 1% solution of the tetrazolium salt in 0.9% NaCl. They were then examined grossly and microscopically, every 20 min for 6 hr, for evidence of reduction of the colorless tetrazolium to the red insoluble formazan. In none of the 16 cultures tested was there any observed reduction of the salt. Cultures left in this solution for 48 hr also gave negative results.

The failure of whole, uninjured, rapidly growing embryonal cells to reduce tetrazolium chloride indicates that this compound is not necessarily a measure of cellular viability. The action of tetrazolium chloride depends on combination with dehydrogenases which cause a reduction of the dye to its colored form. Apparently the failure of the compound to penetrate through the living cellular membranes is responsible for the negative results obtained.

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A Modification of the Hardy-Weinberg Law

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The Hardy-Weinberg law, as it is customarily specified or understood, applies to populations which are indefinitely large, which are panmictic, and in which gene frequencies are the same in both sexes. If, in addition to these conditions, gene frequencies are constant, genotype frequencies will also be constant through succeeding generations (*2–6*).

Whether gene frequencies are constant or not, the primary distribution (*primary* being used in the same sense as applied to sex ratio) of mean genotype frequencies for two alleles A and a, with respective frequencies p and q ($q = 1 - p$), is written $p^2AA:2pqAa:q^2aa$ when A and a are autosomal and ♀♀ ($p^2AA:2pqAa:q^2aa$) + ♂♂ ($pAY:qaY$) when they are sex-linked. But the frequency of one of the two alleles, say q, may be very small, and when it is sufficiently small it is evident that generations will occur in which homozygous recessives will not be produced; regardless of the smallness of q, the term q^2 then represents the probability of occurrence of genotype aa, but not its frequency, which of course is 0. Under this condition the genotypic distribution for a pair of autosomal genes A, a becomes $(p-q)AA:2qAa$, which provisionally may be called a limiting distribution. This can readily be seen, since under panmixis the proportions of pA and qa that recombine will be equal and will