duction) is separate from the receptors specific for prostaglandins or beta-adrenergic catecholamines.

3) Norepinephrine-RSA-Sepharose did not bind leukocytes, an observation which correlates with its relatively low potency in producing cell surface effects. We are presently testing the ability of histamine-RSA-Sepharose to stimulate leukocyte adenyl cyclase. Such experiments should help in determining whether the free portions of the histamine on Sepharose are sufficient for both biologic effect and binding.

We have demonstrated in this study that methods used to define the relationships of cell surfaces to large proteins or haptens are applicable to small pharmacologically active agents. The insolubilized histamine can be used to separate morphologically similar cells to test whether they are biologically different. The histamine receptors of circulating leukocytes are not confined to the small number of cells which contain intracellular histamine [the basophils (*7*)], thus setting the stage for a chemical basis of cell cooperation or interaction. Furthermore, we may now be able to distinguish between intra- and extracellular effects of amines by studying the effects of free hormone on cells which do not contain receptors to histamine. Finally, the possible significance of multiple hormone receptors on the same cells and the relationship of cell function to the presence of a receptor might also be defined by application of our techniques to problems in inflammation and immunology.

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References and Notes

1. J. P. Hecht, J. A. Dellacha, A. C. Paladini, E. Hurwitz, M. Sela, *Fed. Eur. Biochem. Soc. Lett.* **20**, 83 (1972); M. Rodbell, L. Birnbaumer, S. L. Pohl, F. Sundby, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 909 (1971); T. Oka, Y. J. Topper, *ibid.*, p. 2066; I. Schorr, R. Rathman, B. B. Saxena, R. L. Ney, *J. Biol. Chem.* **246**, 5806 (1971).
2. P. Cuatrecasas, *Proc. Nat. Acad. Sci. U.S.A.* **63**, 450 (1969).
3. S. Segal, A. Globerson, M. Feldman, J. Haimovich, D. Givol, *Nature* **223**, 1374 (1969); C. F. Shaffer, *ibid.*, p. 1375; A. Sulica, J. Haimovich, M. Sela, *J. Immunol.* **106**, 721 (1971); S. F. Schlossman, A. Yaron, S. Ben-Efraim, H. A. Sober, *Biochemistry* **4**, 1638 (1965); S. Leskowitz, V. E. Jones, S. J. Zak, *J. Exp. Med.* **123**, 229 (1966).
4. H. Wigzell and B. Anderson, *J. Exp. Med.* **129**, 23 (1969); P. Truffa-Bachi and L. Wofsy, *Proc. Nat. Acad. Sci. U.S.A.* **66**, 685 (1970); P. Cuatrecasas and C. B. Anfinsen, *Annu. Rev. Biochem.* **40**, 259 (1971).
5. H. R. Bourne, R. I. Lehrer, M. J. Cline, K. L. Melmon, *J. Clin. Invest.* **50**, 920 (1971); H. R. Bourne and K. L. Melmon, *J. Pharmacol. Exp. Ther.* **178**, 1 (1971); H. R. Bourne, K. L. Melmon, L. M. Lichtenstein, *Science* **173**, 743 (1971); H. R. Bourne, L. M. Lichtenstein, K. L. Melmon, *J. Immunol.*, in press.
6. J. Porath, R. Axen, S. Ernback, *Nature* **215**, 1491 (1967).
7. J. J. Pruzansky and R. Patterson, *Int. Arch. Allergy Appl. Immunol.* **37**, 98 (1970).
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Amphotericin B Potentiation of Rifampicin as an Antifungal Agent against the Yeast Phase of Histoplasma capsulatum

Abstract. *Rifampicin, at high concentrations, inhibited growth and RNA synthesis in the yeast phase of* Histoplasma capsulatum. *These effects were potentiated by low concentrations of amphotericin B. The combination of the two agents was fungicidal, whereas each alone, at much higher concentrations, was only fungistatic.*

The dimorphic fungus *Histoplasma capsulatum* is an important human pathogen which is worldwide in distribution and endemic in the mid and southern central United States. The polyene amphotericin B is the only effective therapeutic agent for the disseminated form of this infection; but it is only fungistatic, requires long-term therapy, and carries with it a high degree of toxicity (*1*). This report shows that rifampicin alone at high concentrations inhibited RNA synthesis in *H. capsulatum* and decreased the viability of the yeast-like phase. The effect of rifampicin was enhanced by amphotericin B, and the interaction of the two agents against *H. capsulatum* could be characterized as synergistic.

In tissue, *H. capsulatum* is found in

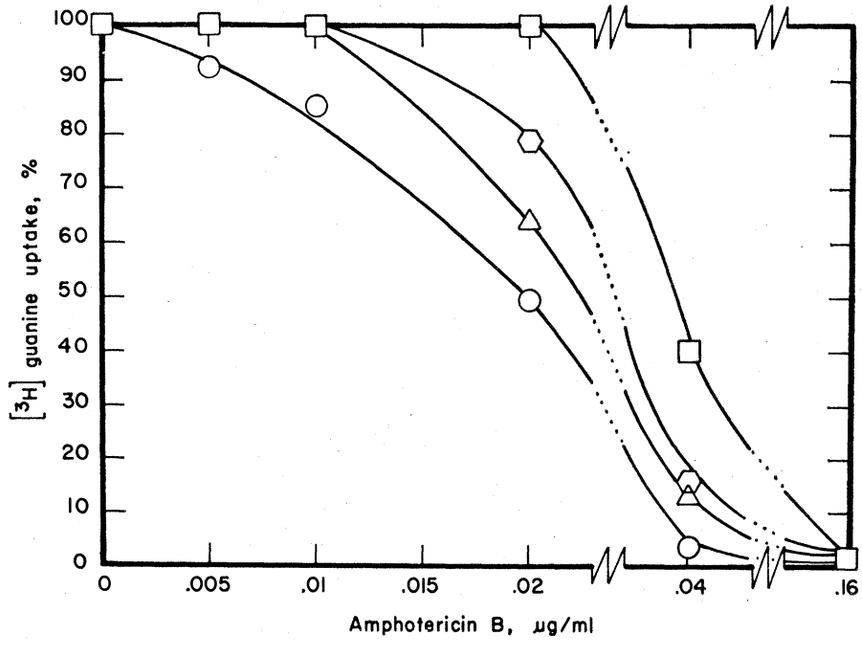

Fig. 1. Dose response to amphotericin B (Squibb) of [^{3}H]guanine incorporation in the presence or absence of rifampicin. Ten-milliliter volumes of a stock suspension of the yeast phase of *H. capsulatum* (Downs) (2×10^5 to 4×10^5 cell/ml) were dispensed into 50-ml erlenmeyer flasks with the appropriate agents and 0.5 μc of [^{3}H]guanine (specific activity, 13 c/mmole) per milliliter. Twenty-four hours later a portion of the culture was removed and precipitated with an equal volume of 10 percent trichloroacetic acid and filtered, and incorporation was determined. Duplicate samples were also removed and plated in triplicate to determine viability by colony counts. Symbols: □, no rifampicin; ○, 5 μg of rifampicin per milliliter; △, 10 μg of rifampicin per milliliter; ○, 20 μg of rifampicin per milliliter.

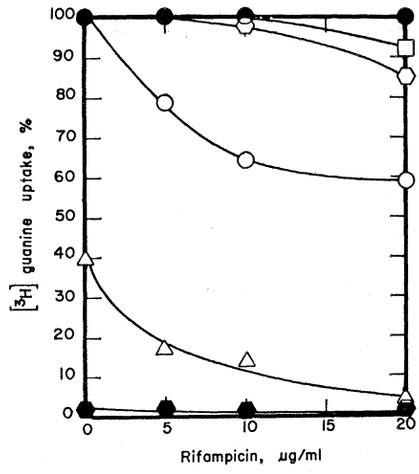

Fig. 2. Dose response to rifampicin of [^{3}H]guanine incorporation in the presence or absence of amphotericin B. Symbols: ●, no amphotericin B; □, 0.005 μg of amphotericin B per milliliter; ○, 0.01 μg of amphotericin B per milliliter; ○, 0.02 μg of amphotericin B per milliliter; △, 0.04 μg of amphotericin B per milliliter; ●, 0.16 μg of amphotericin B per milliliter.

its yeast-like phase. For this reason, our studies on three clinical isolates (*2*) were done on organisms converted to the yeast-like phase and subsequently maintained in this unicellular condition at 37°C on 2 percent glucose, 1 percent yeast extract, 1.5 percent agar (*3*). For these studies a 7-day-old yeast phase culture was transferred to Salvin's broth in sufficient quantity to yield 2×10^5 to 4×10^5 cells per milliliter of stock suspension which was then incubated at 37°C with constant agitation for 24 hours (*4*). After a prolonged lag phase, incorporation of [^{3}H]guanine was linear for 24 to 48 hours, and growth studies revealed the cells to be budding and in logarithmic growth. The effects of drugs on incorporation of [^{3}H]guanine and on viability of cells in colony counts were measured on replicate logarithmically growing cultures. The minimum inhibitory concentration (MIC) for each drug was defined as the lowest concentration that measurably decreased RNA synthesis and viability. Viability studies were performed on samples taken at 0 time and after 24 hours' incubation. All subcultures were incubated at 25°C for at least 2 weeks before they could be read. Because of the filamentous growth, colony counts could only be estimated. Only tenfold differences could be appreciated by comparing growth of the treated cultures with controls at various dilutions. Synergy was defined as a more than additive effect of the drugs in combination on [^{3}H]guanine incorporation into nucleic acid, and a decrease of at least 2 logs in colony counts when compared to the effects of either drug alone.

The MIC determined for amphotericin B alone against all three isolates was 0.04 μg/ml (Fig. 1), and confirmed the values previously reported (*5*). The MIC of rifampicin for the three isolates was 80 μg/ml. At 100 μg of rifampicin per milliliter there was a 76 percent drop in [^{3}H]guanine incorporation into the trichloroacetic acid–insoluble fraction of the cell (data not shown).

Low concentrations of amphotericin B potentiated the antifungal effects of rifampicin against the yeast-like phase of *H. capsulatum* (Figs. 1 and 2) on all three isolates. Our previous studies have shown that rifampicin was also an effective agent against the yeast *Saccharomyces cerevisiae* when it was used with amphotericin B (*6*). This effect was shown to be caused by an alteration of the permeability barrier of the cytoplasmic membrane of these yeasts by amphotericin B and an increased penetration of the second agent into the cell.

The concentrations of amphotericin B and rifampicin at which synergy against *H. capsulatum* was observed concentrations well below the respective MIC of each drug. The effect on [^{3}H]-guanine incorporation of both drugs together was greater than the additive effect of the drugs given separately. A similar effect was observed on viable colony counts.

The combination of both drugs, at concentrations well below the MIC, decreased colony counts by at least 2 logs, whereas each drug alone at these concentrations had no visible effect on viability. The combination containing 0.04 μg of amphotericin B per milliliter and 20 μg of rifampicin per milliliter was fungicidal, whereas a fungicidal effect could not be achieved with each drug alone even at much higher concentrations.

These in vitro studies may have important clinical implications. The combination of amphotericin B and rifampicin was fungicidal at concentrations readily attainable in vivo and this may be important in decreasing the prolonged period of treatment required for cure of histoplasmosis. This observation will have to be further evaluated with in vivo studies.

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References and Notes

1. P. Reddy, D. F. Gorelick, C. A. Brasher, H. Larsh, *Amer. J. Med.* **48**, 629 (1970).
2. Subcultures of primary human isolates of *Histoplasma capsulatum*, G217 A and G217 B, were obtained in the yeast phase of growth from Dr. Ram P. Tewari, Rutgers University, New Brunswick, N.J.; the Down's isolate is from the stock culture collection of this laboratory.
3. G. S. Kobayashi and P. Guilacci, *Sabouraudia* **5**, 180 (1967).
4. S. B. Salvin, *J. Bacteriol.* **59**, 312 (1950).
5. G. Hildick-Smith, H. Blank, I. Sarkany, *Fungus Diseases and Their Treatment* (Little, Brown, Boston, 1964).
6. G. Medoff, G. S. Kobayashi, C. N. Kwan, D. Schlessinger, P. Venkov, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 196 (1972).
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Chemical Spraying as Reported by Refugees from South Vietnam

Abstract. *Ninety-eight refugees who had been exposed to chemical sprays in South Vietnam were interviewed in Hanoi. Most reported effects on eyes and skin and gastrointestinal upsets. Ninety-two percent suffered fatigue, prolonged or indefinite in 17 percent of cases. Reports of abortions and monstrous births in sprayed humans and animals and of substantial numbers of deaths among fish, fowl, and pigs were also given.*

In the years since the initiation of the extensive use of chemical agents such as defoliants and harassing agents by the United States in Indochina, considerable attention has been devoted to the potential ecological consequence of this massive chemical intrusion (*1*, *2*). However, little attention has been paid, in the evaluation of the ecological and human effects of the defoliants, to their direct consequences for the sprayed population, despite the fact that for several years claims have been appearing that humans and animals exposed