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ANTIFUNGAL AGENTS USEFUL IN THERAPY OF SYSTEMIC FUNGAL INFECTIONS

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INTRODUCTION

After 24 years of use, amphotericin B (AmB) is still the drug most frequently used to treat systemic fungal infections. It continues to have potent fungistatic and fungicidal properties against many different organisms, only a few of which have developed resistance. A large volume of work has been published on the mechanism of action, toxicity, and pharmacology of AmB. It is possible, therefore, to review the clinical information on AmB and try to correlate these data with results obtained using artificial and biological membranes, cells in culture, and animal models. For these reasons, this review concentrates on AmB and attempts to explain how this drug works.

Miconazole and ketoconazole are representatives of a group of newer agents, developed at a time when the incidence of systemic fungal infections seen in clinical practice is rising. 5-Fluorocytosine (5-FC) is used only infrequently as a single agent to treat serious disease, but the combination of AmB and 5-FC is thought to have a synergistic effect against several different kinds of fungal infections (1). The imidazoles and 5-FC are covered in less detail in this review and we present what we think are the advantages, limitations, and toxicities of these newer agents.

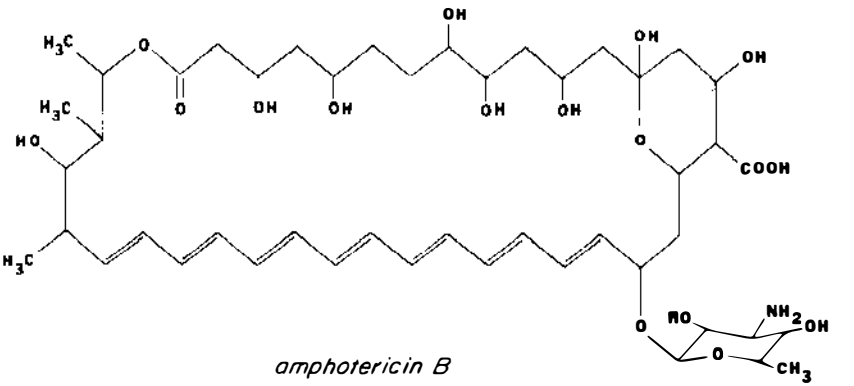
AMPHOTERICIN B

Structure and Physical Properties

AmB belongs to the group of polyene macrolide antibiotics characterized by a macrolide ring of carbon atoms closed by the formation of an internal ester or lactone. Its chemical structure was determined in 1970 and is shown in Figure 1. It is a heptane macrolide antibiotic with a mycosamine linked by a glycosidic bond to the hydroxyl at C-19 on the macrolactone ring. Analysis of the UV spectrum of AmB and also X-ray crystallography analysis (2) indicate that all of the double bonds are in trans conformation. The seven conjugated double bonds form a hydrophobic part of the molecule while the hydroxyl groups and the mycosamine sugar moiety form a hydrophilic part. AmB should therefore be regarded as an amphiphile and, as with other amphiphiles, AmB dissolved in water can achieve segregation of its hydrophobic portion from water by self-aggregation. Its electronic absorption spectrum in water is different from that observed in polar organic solvents, being shifted to the blue range, and exhibiting a new intense band at about 350 nm. Furthermore, a very intense signal centered at the same wave length appears in its circular dichroism (C.D.) spectrum. These features have been shown to be consistent with the existence of two levels of organization: oligomerization and aggregation of the oligomers (3, 4, 5).

Complexes with Sterols and Phospholipids

One of the results of AmB action on cells is a decrease in intracellular K^+ ions. Since the plasma membrane is the integral unit of the cell responsible for the maintenance of selective permeability, it was hypothesized very early that this was the principal site of action of the polyenes. This concept is now well established and, as a consequence, studies on the mechanism of action of AmB have focused on its interaction with membrane compo-



amphotericin B

nents. This notion has been extended and it is now believed that the anticellular action of AmB is due to an interaction with sterol in the cell membrane; this is presented in several excellent reviews (6, 7, 8, 9, 10). Results obtained since the articles were written, however, permit further evaluation of this concept.

The so-called "sterol hypothesis" is supported by three main lines of evidence:

1. The presence of sterol in natural or artificial membranes makes them sensitive to AmB. Bacteria do not contain sterol and are insensitive.
2. Free sterols antagonize the effects of AmB on *Saccharomyces cerevisiae* and *Candida albicans.*
3. Several different physical-chemical methods demonstrate an AmB-sterol interaction in water or in water-methanol mixtures.

Most of the recent studies have attempted to analyze the mechanisms of action of AmB at a molecular level using multilamellar vesicles (MLV), small sonicated unilamellar vesicles (SUV), and black lipid membranes (BLM). Numerous studies have shown that AmB induces permeability to K^+, Na^+ and H^+ ions or to small nonelectrolytes in these phospholipid bilayers which, therefore, may be considered good models for the study of the AmB-induced permability to K^+ ions observed in the cells.

The interactions between AmB and these model membranes have also been measured by several different kinds of spectroscopic methods including electronic absorption, fluorescence, resonance Raman, circular dichroism (C.D.), electron paramagnetic resonance (E.P.R.), and nuclear magnetic resonance (N.M.R.). The optical spectroscopic techniques are especially suitable for these studies because the absorption wavelength field of the polyene part of AmB is different from that of the phospholipid or sterol molecules and the AmB extinction coefficient is very high, permitting detection of low concentrations of AmB. However, problems related to the scattering of light by large particles prevent extension of these techniques to the study of the interaction of AmB with cells.

Some background information on the different spectroscopic and magnetic methods is necessary to understand the experimental results we discuss. In the electron absorption spectra, the four bands between 360–450 nm seen with free AmB in water are shifted in the presence of phospholipid or sterol molecules, while the intensity of the band at about 350, characteristic of self-aggregation, decreases (11). Unfortunately, these changes are independent of the type of phospholipid or sterol, so the only information that can be obtained from the absorption spectra is the extent of the interaction. This is a limitation of this technique. AmB resonance Raman does not

appear to add anything to the information provided by electronic absorption since only the vibrations of the polyene are observed and the spectra are not very sensitive to the environment (12).

C.D. is the absorption difference between left and right circularly polarized radiations. In contrast to the electronic absorption spectra, C.D. spectra are very sensitive to AmB conformational changes that occur when the drug interacts with membrane components (13). As a result, there are wave length, sign, and intensity shifts that depend on the nature of the phospholipids or sterols in the membrane. Furthermore, there is a very intense C.D. doublet that can monitor self-aggregation of AmB. Consequently, C.D. measurements can be considered "finger prints" of the interactions of AmB and should be preferred to absorption measurements.

Fluorescence has also been used to measure AmB interactions, but the significance of the results obtained using this technique is now open to question because a recent study has found that AmB may not be fluorescent (14).

The optical spectroscopic techniques described above give direct information on the AmB molecules, the large majority of which interact with the membranes. In contrast, magnetic techniques reveal the effects of AmB on the mobility of the surrounding sterol or phospholipid molecules (spin-labeled by the nitroxide groups when E.P.R. is used). Much higher concentrations of AmB than those used in biological studies are required to allow discrimination between responses of perturbed and unperturbed molecules. Despite this problem, the technique is useful. The E.P.R. signal of the nitroxide group is very sensitive to motion and, by using the probe in different locations along the fatty acid chain or attached to sterols at different positions, it is possible to monitor the motion of the molecule at different levels inside the membrane.

In the single ^{1}H N.M.R. study performed up to now, the interaction of AmB with SUV was monitored by the disappearance of the choline peak. At the high AmB concentration used, this was shown to be indicative of the destruction of the vesicles (15).

The existence of AmB-sterol complexes in water has been documented by electronic absorption (11), fluorescence (16, 17, 18), and circular dichroism (19, 20). The method of addition of the sterol to aqueous suspensions of AmB as well as the volume of solvent may alter the nature of the suspension formed. Complexes are also evident in binary mixtures such as water-alcohol, which mimic to some extent the membrane environment. Brown & Sidebottom (21) have made a start in identifying the structure of the complexes by noting 28 separate proton resonances in the N.M.R. spectrum of AmB dissolved in dimethylsulfoxide.

The existence of a sterol-AmB complex in water is suggestive, but not

proof of its existence in membranes. To deal with this point, studies have been performed with sterols embedded in phospholipid model membranes. In these experiments, the sterol content of the bilayer and the AmB concentration were varied and the stoichiometry (and ipso facto the existence of the complex) was estimated from the dependence of some property of the lipid layer on the AmB/sterol ratio. For instance molar ratios of 1/3.9, 1/3.3, or 1/0.7 have been proposed from absorption measurements or from the AmB induced K^+ efflux from lecithin MLV (22), while monitoring the order parameter changes of nitroxide spin-labeled membrane components (E.P.R.) gave a stoichiometry of approximately 1/1 with lecithin in SUV (23) and with lecithin planar multilayers (24). It is important to note that these results may not be relevant to interactions at the level of the cell membrane. The location of the AmB-sterol complex inside the phospholipid bilayer of the SUV has been questioned because the spin-labeled sterol used could be slowly removed from the lipid phase into the AmB aggregates (25), and AmB-cholesterol complexes may be expelled from the lipid phase into the aqueous medium some time after the interaction has taken place or when the SUV are heated through the transition temperature (20).

Direct evidence for an AmB-sterol complex in phospholipid-sterol membranes was proposed from fluorescence energy transfer with dehydroergosterol (26) but was later retracted (27). Recently another kind of direct evidence was derived from Scatchard analysis of the binding of AmB to lecithin-sterol SUV monitored by electronic absorption, and a single complex of 1 : 1 stoichiometry was proposed (28). However in the same conditions, circular dichroism, which is more sensitive to conformational changes, indicated the existence of not one, but several competing species (13); E.P.R. studies supported the circular dichroism results and demonstrated that below and above an AmB-ergosterol ratio of 0.7, two different types of interaction were observed (24). Therefore, no indisputable proof of the existence of an AmB-sterol complex in membranes has yet been presented, although most workers in the field believe it occurs. Furthermore, several other observations made during the last few years indicate that other factors may be involved in the membrane action of AmB and seem to offer an alternative to the sterol hypothesis.

First, it was demonstrated that the presence of sterols in membranes is not necessary for the binding of AmB to those membranes (of course "binding" is not necessarily equated to induction of permeability). Experiments were performed with sterol-free SUV or MLV and AmB was monitored by electronic absorption (11, 29) or by circular dichroism (13). The initial rate of AmB-phospholipid association was enhanced with shorter fatty acyl chains in saturated phospholipids, and with increasing numbers

of double bonds in the fatty acyl chain. In other words the initial rate of association was higher when the phospholipid molecules were in the liquid crystalline state than when they were in the gel state. The reverse was observed for the association constant: it was higher when the phospholipid molecules were in the gel state. This is evidenced by the fact that when AmB, which exchanges readily between vesicles (30, 31), was added to a mixture of vesicles in the gel state and in the liquid crystalline state, the circular dichroism spectrum typical of AmB bound to vesicles in the gel state was observed. This AmB-phospholipid interaction is not surprising: the amphiphile AmB is expected to interact with any other amphiphile, including phospholipids, proteins, or acyl fatty acids. In accordance with this, free fatty acids (32) and concentrated solutions of proteins decrease the anticellular action of AmB (33).

Second, the presence of sterol in the membranes is not necessary for the AmB-induced permeability. It has been shown that AmB or AmE, the methyl ester of AmB, are able to induce glucose (34) and K^+ (35) permeability in sterol-free SUV consisting of phospholipid in the gel state (dipalmitoyl phosphatidylcholine at room temperature) or egg yolk phosphatidylcholine at 2°C. Furthermore, the presence of cholesterol in the vesicles does not increase the sensitivity further under the same conditions, nor does it modify the conformation adopted by AmB in the bilayer, as monitored by circular dichroism (13). AmB has little or no effect on kinetics of spin-label reduction (23) or on permeability to monovalent cations (29) of sterol-free MLV made up of phospholipids in the liquid crystalline state. In contrast, AmB can induce permeability to protons in sterol-free SUV composed of phospholipid in the liquid crystalline state, but the sensitivity to AmB is less than in the presence of sterol. In this case AmB action is thought to be due to a detergent effect (13).

In summary, it seems that AmB is able to induce permeability in sterol-free model membranes, but, at least with vesicles in the liquid crystalline state, to a lesser extent than in the presence of membrane sterols. We emphasize that the mechanism of action is different with sterol-free vesicles in the liquid crystalline and in the gel state. In the latter case the presence of sterol in the vesicles does not contribute to the modification of the characteristics of the interaction. Because the role of membrane sterols to AmB action on cells is well established, we cannot reject its importance; however, the work on sterol-free model membranes indicates that other factors may also be involved in the interaction.

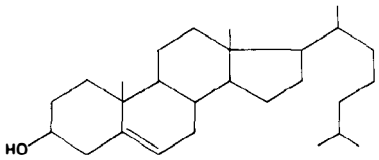
If we accept that the presence of sterol in a membrane is a major factor in conferring sensitivity to AmB, it should be possible to find a correlation between the induction of permeability and the level of sterol in the membrane. In fact, Kasumov et al (36) have demonstrated AmB channels responsible for the permeability induction and they have recently shown that

an increase of cholesterol concentration in BLM increases the mean lifetime of these channels in the active state. Other studies have also revealed an influence of the polyene/sterol ratio on membrane sensitivity, but the relationship with the sterol level is not straightforward. For example, it has been shown that AmB induces maximal K^+ release from MLV containing 20% cholesterol (35), but release decreases with higher cholesterol levels (29, 37). Circular dichroism studies (13) have revealed that AmB may adopt different conformational states according to the cholesterol content, but these changes also depend on the time elapsed after mixing with vesicles, the number of AmB molecules per phospholipid molecule, and the physical state of the phospholipids. On the other hand, E.P.R. studies conducted with nitroxide spin labels incorporated at various positions along the phospholipid fatty acyl chain have shown that at low polyene-sterol ratios AmB accumulates near the head group region of the membrane and that after some critical polyene ergosterol concentration is reached, AmB enters the bilayer, disordering the membrane (24). Therefore, it seems that the proportionality between the amount of sterol in the membrane and AmB-induced permeability exists only in a restricted domain of concentrations corresponding to well-defined AmB/phospholipid and sterol/phospholipid ratios. The domains of existence of the permeability-inducing species are limited by the appearance of competing species that may not induce permeability. The interactions are complicated further by the existence of rapid exchanges of AmB between artificial membranes (30, 31) present in the experimental system. This exchange, which may also occur between natural membranes, complicates the interpretation of data, especially those involving kinetics of the reaction.

In agreement with these considerations, studies on fungi have also failed to reveal an exact correlation between sterol level and fungal sensitivity. The results of all of these studies suggest that natural membrane sensitivity to polyene antibiotics is determined by overall membrane organization, including the level of sterols, rather than by the level of a single membrane component (38).

Sterol Specificity

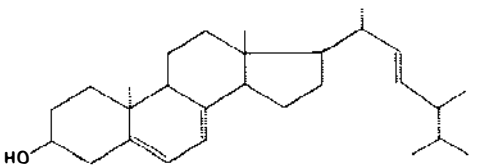
Since both host and fungal cells contain sterol, it has been suggested that the relative specificity of AmB for fungi is based on a difference in the nature of the sterols involved; ergosterol is the principal fungal sterol, whereas cholesterol is the sterol found in animal cell membranes. In agreement with this notion, it was demonstrated that the anticellular effects of AmB were more inhibited by extracellular ergosterol than cholesterol (39) and lower AmB concentrations were sufficient to induce K^+ leakage from ergosterol-containing liposomes than from cholesterol-containing liposomes (29).



cholesterol

Recently, two new procedures for measuring AmB electronic absorption have made it possible to determine the selectivity of AmB-sterol binding. Both methods are based on observations of sterol-induced changes in AmB absorption spectra at 408–409 nm, the wave length characteristic of monomeric AmB in water. Gruda et al (40) have measured sterol effects on AmB differential spectra. By adding alcohol to an AmB and sterol dispersion in water, they found conditions in which AmB-ergosterol, but not AmB-cholesterol, complexes could exist. In a study by Readio & Bittman, Scatchard analysis of the electronic absorption spectra of AmB bound to egg phosphatidylcholine-sterol SUV (28) revealed that AmB was bound to ergosterol containing SUV about 10-fold more tightly than to cholesterol containing SUV.

The basis for the preferential binding of AmB to ergosterol, as compared to cholesterol, is unknown. Ergosterol differs from cholesterol by having a methyl group at C-24 in the side-chain and two additional double bonds, one at C-7 in ring B of the nucleus and the other at C-22 in the side chain (Figures 2 and 3). On the basis of AmB effects on the conductivity of artificial membranes, it was proposed that the significant structural element in ergosterol which determines its greater ability to interact with AmB was the double bond at C-7 in ring B (41). However, the question still remains whether the differential effect of ergosterol as compared to cholesterol originates solely from a preferential binding of AmB to ergosterol, or whether it relies on an indirect effect resulting from a differential perturbation of the membrane properties by ergosterol and by cholesterol, which then results in differences in susceptibility to AmB. In this regard, it has been proposed that a disruptive interaction between polyene and membrane requires that the membrane be in a disordered state (34); either cholesterol



ergosterol

or ergosterol could contribute more or less to this, thereby influencing interaction with AmB in a differential manner.

Models for the Induction of Permeability by AmB

Direct observations have been made of the interaction of polyenes with plasma membranes using freeze etch electron microscopy of erythrocytes and *Acholeplasma laidlawii* (42). Structural alterations such as pits, doughnut-shaped craters, and protrusions in the membranes have been shown. It has been assumed, but not proven, that these structural changes are responsible for the enhanced permeability of the membrane.

Freeze-etch electron microscopy of *Epidermophyton floccosum* and *Saccharomyces cerevisiae* (43, 44) have shown that AmB can also induce profound ultrastructural changes in these fungi, consisting of aggregation of membrane-associated particles, and the formation of depressions or craters on the inner faces of the membranes. From this study it was proposed that the complexing of AmB to sterol could withdraw the sterol from its interaction with membrane phospholipids, thus altering the membrane fluidity and causing severe damage leading to leakage of cellular constituents. The same hypothesis was made from the results obtained on the AmB-induced permeability in MLV in the gel state (34).

However, the most frequently accepted explanation of the results of AmB interaction in membranes is different: it is based on the selectivity of the induced permeability and proposes that aqueous channels made by the antibiotic and the sterol (45, 46) are formed and possibly shuttle up and down in the bilayer or from double half pores. A recent polarized resonance Raman spectroscopy study of oriented macrodomains of phospholipids containing AmB has shown that only a part of the polyene structure or moeity is actually oriented parallel to the phospholipid fatty acyl chain (47). In addition, the studies of Aracava et al (25) on the effect of AmB on the E.P.R. spectra of paramagnetic lipid probe are in disagreement with the presence of highly structured pores. They are much more consistent with the notion that the antibiotic-sterol complex may involve discontinuities at phase boundaries.

The Relevance of Studies with Model Membranes

It is apparent from the studies we have reviewed on how AmB interacts with artificial membranes, that even with these relatively simple models the mechanism of action is complicated; consequently, we cannot expect a unique straightforward interaction with the membranes of cells. The studies with model membranes, however, have allowed us to specify the characteristics of AmB-induced permeability. Because the observations on the model membranes are not irrelevant to the in vivo action of AmB, we believe it

is worthwhile to continue to use them to study more thoroughly the kinetics of interaction, the characteristics of the exchange of the antibiotic between cells, and the origin of sterol specificity, and to compare the action of the various polyene antibiotics. It will be most rewarding if the results with artificial membranes can be correlated with those using other experimental models, including cells. To do this it will be necessary to use comparable experimental conditions. For example:

1. The studies on artificial membranes should use polyene antibiotics at a concentration of about 10^{-6}M and not the concentrations that have been used (10^{-4}M or 10^{-3}), which are one hundred or one thousand fold higher than those required to affect natural membranes.

2. It is necessary to standardize the experiments and to use the same membrane model, preferably large unilamellar vesicles, which more closely resemble natural membranes than SUV or MLV; the very small radius of SUV imparts unique properties to these membranes and the multilamellar structure of the MLV complicates the interpretation of results because a large number of phospholipid molecules are hidden from the environment.

Finally, it is evident from physiological studies (see below) that induction of permeability is far from being the only effect of AmB on cells and that trying to explain all its toxic and stimulatory effects requires the use of other kinds of model systems.

As a start, vesicles reconstituted from cell membranes could be used; ultimately whole cells should be studied. Perhaps such studies could be accomplished by utilization of other physical-chemical methods not described here, such as photobleaching recovery (48) or Raman spectroscopy.

Physiological Effects of AmB

AmB is more toxic for fungi than for animal cells (49). This fact is probably the basis for its clinical usefulness. The relative specificity for fungi may be due to the drug's greater avidity for ergosterol, the sterol found in fungal cell membranes, than for cholesterol, the principal sterol in animal cell membranes (see above).

It is apparent from several different kinds of experiments that AmB action on fungi and animal cells occurs in several distinguishable stages (50, 51). At low AmB concentrations, a stimulatory effect occurs which is manifested in particular by an increase in colony-forming units of fungi (52) and proliferating animal cells in culture (Brajtburg, et al, unpublished) and an enhancement of incorporation of labeled precursors into DNA and RNA (53, 54). The basis for these effects is unknown. At equivalent or higher AmB concentrations, cell membrane permeability to monovalent cations is affected; this is manifested by a leakage of K^+ from the cell and a decrease

in intracellular K^+ or a flow of extracellular Na^+ or Rb^+ into the cell (50, 51, 55). Cells may shrink or swell because of the disturbance in ionic balance. At this stage, cell growth is inhibited and cell death may also result. The ionic composition of the medium, the availability of energy sources, the temperature of incubation, and the rate of cell recuperation from the damage are all important determinants of what happens to the cells at this stage (56, 57, 58). At high AmB concentrations cells are lysed or killed, probably by direct disruptive action of AmB on membranes (50, 56).

The increase in incorporation of labeled precursors of DNA and RNA into cells is only one example of the "stimulatory" action of AmB. AmB is also known to stimulate the function of specific cell types. Polyclonal B cell activation (PBA) of lymphocytes was first described by Hammarström & Smith (59, 60). Little and collaborators (61, 62, 63) have confirmed and extended these studies using amphotericin B methyl ester as the B cell activator and trinitrophenylated sheep erythrocytes (TNP-SRBC) in direct plaque-forming assays. The stimulatory effect of AmB on macrophages has been demonstrated in several laboratories. AmB can activate macrophages to attach to plastic, to increase levels of prostaglandin E_2 (64), and to kill bacteria (65), parasites (66), and tumor cells in culture (67). These stimulatory effects occur at specific AmB concentrations; higher concentrations are toxic to cells and decrease chemotaxis of polymorphonuclear leukocytes and phagocytosis and killing by macrophages (68).

The variety of in vitro results we have discussed gives some insight into the complexity of the in vivo action of AmB. The toxic effects on fungi manifested by decreased cell viability clearly occur at lower AmB concentrations than the toxic effects on mammalian cells. It is possible, however, that comparable AmB concentrations induce inhibition of parasite growth and stimulation of some specific cell type or types in the host. If this stimulation results in an increased immune response, the final therapeutic effect of AmB may be attributed both to AmB stimulatory action on host cells and toxicity against parasitic cells.

Animal Experiments

Mice infected with *Candida albicans, Cryptococcus neoformans, Aspergillus* species, or several other kinds of fungi respond to AmB therapy by increased mean survival time or by decreased tissue load documented by the number of organisms infecting selected organs (69, 70). In some cases, the response is difficult to understand because AmB blood levels are low and there is no evidence of penetration of drug to specific areas of infection such as the central nervous system. As mentioned earlier, this puzzling discrepancy between the clinical and in vitro results also occurs in human infection and led some members of our group at Washington University to postulate

Table 1 Effect of AmB on the secondary response in BALB/c mice[a] (61)

1° Immunization	2° Immunization[b]	Days after 1°	Days after 2°	PFC/1 X 10^6 spleen cells[c] IgM	IgG
AmB + TNP-HSA	TNP-HSA	20	7	25.1 ± 6.1	6.9 ± 12.0
TNP-HSA	TNP-HSA	20	7	18.0 ± 2.9	0
AmB	TNP-HSA	20	7	11.0 ± 4.3	0
AmB + TNP-HSA	TNP-HSA	27	7	30.2 ± 8.1	12.7 ± 8.8
TNP-HSA	TNP-HSA	27	7	21.6 ± 6.7	0
AmB	TNP-HSA	27	7	20.3 ± 6.0	0
AmB + TNP-HSA	TNP-HSA	34	7	37.4 ± 9.2	18.1 ± 11.6
TNP-HSA	TNP-HSA	34	7	20.6 ± 4.9	0
AmB	TNP-HSA	34	7	12.1 ± 4.3	0

[a] All mice were 7- to 8-week-old BALB/c males immunized by separate ip injections of each agent. Groups of mice were given either 300 μg AmB in 0.2 ml 5% dextrose solution and 100 μg TNP-HSA in 0.2 ml saline, 100 μg TNP-HSA in 0.2 ml saline and 0.2 ml dextrose solution, or 300 μg AmB in 0.2 ml 5% dextrose solution.

[b] At intervals of 13, 20, and 27 days after primary immunization, all mice received 100 μg TNP-HSA in 0.2 ml saline solution ip.

[c] Spleens from each group of three mice were pooled and made in single-cell suspensions. The values given represent the arithmetic mean (±1 SD) of six separate PFC assays for each spleen cell suspention using TNP-SRBC as target cells. Parallel control PFC assays performed with unconjugated SRBC were done routinely and never yielded greater than three direct PFC or one indirect (IgG) PFC per 1 X 10^6 spleen cells. No correction was made in the data shown for the control assays.

that AmB may have an immunostimulant effect on the host that results in an increased response to the pathogen. This notion was investigated in animal models in several different ways.

In the first series of experiments, AmB and its methyl ester (AME) were tested to see if they could augment the immune responses of inbred strains of mice to several different antigens. Humoral immunostimulation by AmB (or AME) was consistently observed in mice immunized with the hapten-protein conjugate trinitrophenylated human serum albumin (TNP-HSA) (61). The immunoadjuvant effects of AmB in adult Balb/c mice are shown in Table 1. Neither AmB alone nor antigen alone induced the formation of any detectable IgG antibody-producing cells in the secondary response to TNP-HSA. However, IgG antibody-producing cells were consistently observed in the spleens of mice that received a single dose of AmB along with the primary antigenic stimulation. All mice received the same "secondary" stimulus of 100 μg TNP-HSA in saline solution. Table 1 also shows a consistently augmented IgM response in the groups of animals that received antigen plus AmB. These results and other similar experiments clearly show that AmB has potent humoral immunostimulant effects in mice. They also indicate that AmB enhances the switching of lymphoid cells from IgM to

IgG antibody production, a change known to be dependent on stimulation by helper T cells.

In addition to enhancing murine humoral immunity, it has also been shown that AmB augments cell-mediated immune responses to dinitrofluorobenzene (DNFB) and oxazalone (62). When AmB was administered to AKR mice at the time of skin sensitization with dilute DNFB, a significant enhancement of ear swelling occurred following a challenge application of DNFB. Other mice sensitized in the same way with DNFB plus AmB showed augmented lymph node cell proliferation when these cells were restimulated in culture with dinitrobenzenesulfonate (DNBS) (63). This result supports other data which show that AmB acts by enhancing the immune reactivity of lymphoid cells rather than by amplifying the inflammatory reaction as might have resulted from a generalized increase in cellular permeability.

In the analysis of the probable mechanism of the immunoadjuvant effects of polyenes, it is important to note that AmB partially ablated tolerance to DNFB induced by a single injection of DNBS in AKR mice. Immune tolerance in this system has been shown to be mediated by suppressor T cells; tolerance ablation by AmB suggests that AmB may be selectively toxic for suppressor T cells (71). It may be that multiple cell types are affected by AmB, but a toxic effect on suppressor T cells could account for the immune enhancement produced by AmB as well as the interference with tolerance induction. The effect on suppressor T cells also predicts that AmB could be immunosuppressive through interference with other effector functions of the suppressor cells. Toxic effects on helper cells or B cells when large doses of AmB are given to animals would also result in an immunosuppressive effect. Both immunostimulant and immunosuppressive effects of AmB have been observed using different experimental conditions (72).

An important question is whether these demonstrated effects of AmB on the immune system are correlated with altered responses to infection. Several different kinds of experiments indicate that this is the case. When AmB was injected into mice 24 h before infection with *Listeria monocytogenes,* mouse survival was significantly increased (73). In contrast, AmB injected 24 h after infection increased the mortality over that of controls. The number of colony-forming units of *L. monocytogenes* isolated from the livers and spleens of infected mice also depends on the time of treatment with AmB. The number of viable bacteria was greater in the livers and spleens of mice treated with AmB 24 h after infection than in those of control animals, and mice treated with AmB 24 h before infection had fewer bacteria in their livers and spleens. It is important to remember that AmB has no direct antimicrobial activity against *L. monocytogenes.*

In a similar type of experiment, a single dose of AmB increased resistance in mice to a challenge with *Schistosoma mansoni cercariae,* and schistosomular recovery from the lungs of the treated mice 5 days after challenge was reduced by 33–40% (66).

Perhaps the most dramatic demonstration of the immune effects of AmB is its antitumor action (74). About 20–80% of AKR mice bearing an advanced syngeneic leukemia were cured by treatment with a combination of AmB and the antitumor agent 1,3-bis (2-chloro-ethyl)-1-nitrosourea (BCNU). Neither drug alone induced cures, and the survivors of the drug combination therapy were solidly resistant to rechallenge with 10^6 leukemia cells. Spleen colony assays for leukemia cells have shown that mice treated with the combination of AmB and BCNU harbored viable leukemia cells for up to eight days following treatment and there was then an abrupt fall in the leukemia load. The kinetics of the decrease in the number of the leukemic cells to therapy were consistent with an immune response. In addition, the cured mice were resistant to rechallenge with the tumor cells, and tumor resistance could be adoptively transferred to virgin AKR mice using spleen cells from cured mice. This result further supports the immune basis of the antitumor effect.

The animal experiments indicate that the basis of the therapeutic effects of AmB in fungal and some parasitic infections is probably very complex. On the one hand, the drug has a direct toxic effect on the parasite, which is what we would expect from an antibiotic. Like other antibiotics, it is also toxic to host cells when given in large doses. On the other hand, AmB also enhances host responses to the parasite. Both effects probably contribute to the therapeutic efficacy of AmB and it is not clear which is more important. The unequal distribution of AmB in the body and variability in cell sensitivity add to the complexity of the situation. Thus, the same concentration of AmB might be toxic for kidney cells and stimulatory for spleen cells. Another factor influencing the cellular action of AmB is its binding to lipoproteins (Brajtburg et al, unpublished). AmB binding to cholesterol in lipoproteins may decrease the stimulatory or toxic effects of AmB on host cells, which also contain cholesterol, without affecting antibiotic action on fungal cells, which contain ergosterol. The transport in the body of AmB complexed to lipoproteins may also lead to localization of the drug in certain organs and may also influence its uptake into cells. AmB injection into mice and humans leads to dramatic changes in specific lipoproteins (75); the meaning of these changes in relation to the therapeutic and toxic effects of AmB is unknown. It is difficult using in vivo models to dissect out any one of these effects for study and dissociate it from the others. For this reason the in vitro experiments described above have been used in an attempt to understand mechanisms of AmB action.

Clinical Use

AmB is the only antibiotic sufficiently nontoxic and tolerated parenterally to be used in the treatment of systemic disease in humans. Because of the multiplicity of its possible effects, its use is largely based on pragmatic criteria. AmB is insoluble in nonorganic solvents and is marketed as Fungizone, a bile salt complex that forms a colloidal dispersion when hydrated. It is supplied in glass vials under nitrogen as a dry powder consisting of 50 mg AmB, 41 mg sodium deoxycholate, and 25.2 mg of sodium phosphate. After addition of 10 ml of sterile distilled water, vigorous shaking results in a clear yellow suspension. Before injection, the initial suspension must be diluted in sterile 5% glucose solution to a final concentration that should not exceed 0.1 mg/ml. The pH should be between 4.2 and 6.5 to minimize spontaneous decomposition and aggregation. Sodium-containing diluents must be avoided because they will precipitate the drug. These diluted suspensions can be used intravenously or for local injection into infected body space.

As discussed above, AmB has no activity against bacteria. It is effective against many species of fungi such as yeasts (e.g. *Candida, Cryptococcus neoformans*), dimorphic fungi (e.g. *Histoplasma capsulatum, Blastomyces dermatitidis,* and *Coccidioides immitis*), dermatophytes (e.g. *Trichophyton, Microsporium,* and *Epidermophytes*), and molds (e.g. *Aspergillus* and *Penicillium*). The antifungal activity of the polyenes appears to be highly variable against these different fungi. However, it is difficult to compare results of different studies because experimentally derived minimum inhibitory concentrations (MIC) vary among different laboratories. Among the factors that may affect the MIC are inoculum size, temperature and duration of incubation, and medium composition (76). Therefore, only if strictly standardized conditions are adhered to can susceptibility data from different centers be compared.

In addition to antimycotic activities, many polyenes are toxic to protozoa of medical importance such as trichomonads, *Entamoeba, Naeglaria, Leishmania,* and trypanosomes.

In general, there is good correlation between the in vitro effectiveness of AmB and the clinical results of therapy. However, some limitations in regard to the relevance of in vitro susceptibility testing should be considered. The poor solubility and stability of AmB complicate the assay procedures and lead to problems in interpretation.

Some infections do not respond to AmB therapy, possibly because the MIC of the causative organism is greater than the blood level. Although fungal resistance to the drug has been thought to be rare, a recent report indicates that it may occur under some circumstances (77). Sometimes there

is a lack of clinical response even when the organism is very susceptible to the drug in vitro. In addition, some infections respond to AmB even when in vitro susceptibility tests indicate that the MIC is higher than that thought to be achievable in blood. This may be secondary to the immune effects described above in the section on animal experiments. The poor correlations between the in vitro susceptibility of the organisms to AmB and the clinical response of patients may also be due to several other factors, including the special nature of the disease. For example, the large clots on prosthetic valves seen with fungal endocarditis caused by species of *Candida* or *Aspergillus* may make the disease unresponsive to therapy even though the organisms are susceptible in vitro. Penetration to the site of infection can also influence the clinical response. The poor penetration of AmB into closed body cavities such as the central nervous system lowers the cure rate in these kinds of infections (78). The state of the host is another important factor. For example, the patient with a malignancy who has no white blood cells and has other deficiences of host resistance will have difficulty handling any kind of infection, even though potent drugs are used in treatment. In addition, in vitro susceptibility tests do not reflect the toxic effects of drugs on the host.

The critical factors in determining dosage of AmB for treatment of fungal infections are not well defined. Most infections are successfully treated with doses of AmB, which achieve a blood level of 1 to 2 μg/ml as measured by bioassay (0.5 to 1 mg per kilogram of body weight per day). The dosage schedules have been established empirically and are based on what is known about toxicity, convenience of use, and what seems to have worked in practice. There are disagreements regarding the details of administration of the drug to patients, and recommendations are often made purely on the basis of anecdotal clinical experience. We believe that the initial dose of AmB should be 1.0 mg given over a period of two to six hours; on each subsequent day the dose can be doubled until the desired maintenance dose is reached. In critically ill patients, an accelerated dosage schedule should be used, and the 1.0 mg test dose can be followed immediately by 0.25 mg per kilogram of body weight; 0.5 mg per kilogram can be given the following day. In our experience, it is seldom necessary to use more than 0.5 mg per kilogram per day, but if higher doses are required, up to 1 mg/kg/day can be administered.

An occasional patient will not tolerate even the 1 mg test dose, and chills, fever, and hypotension may occur. In this event, initial doses may have to be lowered to 0.1 mg and then increased slowly as tolerance allows. Even the low doses may cause chills and fever, and the addition of 25 to 50 mg of hydrocortisone sodium succinate to the infusion bottle may be required to control the reactions. It may also be necessary to premedicate the patient

with aspirin, diphenhydramine (Benadryl), or meperidine hydrochloride (Demerol) to control chills and fever. Prochlorperazine (Compazine) is frequently used to control nausea.

A low-dose regimen has been recommended for certain types of infections with *C. albicans* in which the patient receives a total dose of 100 to 200 mg of AmB over 7 to 10 days (79). Although experience with the regimen has been limited, it appears to be particularly successful in patients with mucocutaneous, esophageal, or lower urinary tract fungal infections.

The dosage of AmB should not be reduced in patients with preexisting renal dysfunction. Because only a small amount of the drug is excreted acutely in the urine, the reduced renal function has little or no effect on the serum levels of AmB. Immediately after intravenous injection of the drug, up to 10% of the bioactivity is found in the plasma, strongly bound to plasma proteins (including lipoproteins). Blood levels up to 10 μg/ml have been reported at this time, but then fall quickly to 1–2 μg/ml; these levels are maintained over the 24–48 h after administration (80). The affinity of AmB for cholesterol suggests that most of the administered AmB is bound to sterol-containing membranes in many different tissues. The distribution and excretion of the drug are not well characterized, but it appears that it is concentrated in the liver (81). The drug is excreted in active form in the urine, but is cleared very slowly. AmB can be found in the urine of patients for weeks after the last dose and the total amount in urine is only a small percentage of the total dose of the drug administered (82).

Intrathecal as well as intravenous administration of AmB is indicated in the treatment of coccidioides meningitis and in some resistant cases of cryptococcal meningitis. From 0.1 to 1.0 mg of the drug can be injected into the lumbar theca or cisterna magna or by means of a prosthesis into the lateral ventricles (83). The latter route is required if direct injections into cerebrospinal fluid are to be continued beyond two to three weeks. Before initiating this mode of therapy, the physician should be thoroughly familiar with the technical details of administration and the formidable problems that may arise during treatment. Intraarticular injections of AmB may be useful in the treatment of coccidioides arthritis and in refractory cases of articular sporotrichosis (84). Irritation commonly follows local injections of AmB, but systemic reactions are unusual.

Renal dysfunction is the most important toxic effect of AmB (85). Soon after the beginning of therapy, the glomerular filtration rate (GFR) falls about 40 per cent in nearly every patient. After repeated doses, the GFR frequently stabilize at 20 to 60% of normal and remains at this level throughout the course of therapy. If renal function continues to deteriorate beyond this level, the drug should be discontinued for two to five days. Renal function almost always improves with brief cessation of therapy, and

treatment with the previously administered dose can then be reinstituted. Nephrotoxicity has been reduced in laboratory animals by concomitant administration of mannitol intravenously or sodium bicarbonate orally, but these measures have not worked in patients (86).

In most patients, creatinine and inulin clearances and the blood urea nitrogen level return to nearly normal some months after cessation of AmB therapy, but the degree of permanent GFR reduction, when it occurs, has not been documented in a large series. A few patients, who cannot be singled out before therapy, sustain as much as a 50% reduction in GFR for many months after therapy but remain asymptomatic.

Monitoring of AmB toxicity requires measurement of hematocrit, serum potassium, blood urea nitrogen, creatinine, and carbon dioxide values, and also a urinalysis. These tests should be obtained as a base line before treatment and during treatment, twice weekly for the first four weeks of treatment and weekly until administration of the drug is discontinued.

Renal abnormalities other than azotemia are frequent but are not used as a guide to dosage. Among these abnormalities are cylindruria, mild renal tubular acidosis, and other disturbances of renal tubular function. Hypokalemia occurs in about one fourth of patients and requires potassium chloride supplementation. As a result of decreased erythrocyte production, the hematocrit frequently falls to a stable value of 22 to 35% of the starting level. Thrombocytopenia, hepatic dysfunction, and allergic reactions are rare toxic manifestations of treatment with AmB.

AmB is still the most effective antifungal agent. Despite its toxicity and problems with administration, it is the drug of choice for most systemic fungal infections, particularly when these occur in the immunocompromised host. Unfortunately, there are no good objective data on the appropriate length of therapy with AmB required for most fungal infections. Most patients are treated with a total dose of 1.5 to 2.0 gm of AmB over a 6 to 12 week period. It is reasonable, however, to expect that the length of therapy should vary with the organism, type of disease, and clinical response.

IMIDAZOLES

Mechanism of Action

There are several proposed mechanisms for anticellular effects of the imidazoles. Some studies have emphasized the rapidly induced membrane damage and loss of viability of fungi (87). Other studies with model membrane systems confirm the striking rapid destructive action of the imidazoles. Especially sensitive are model membranes containing free unsaturated fatty acids. This may explain some of the selectivity of imidazole action, because

fungi and gram-positive bacteria, which are sensitive to imidazoles, are rich in free fatty acids, whereas mammalian cells and gram-negative bacilli, resistant to imidazoles, have low levels of free fatty acids. As far as can be determined, direct damage to the membrane occurs with miconazole, but not ketoconazole; this correlates with data that show that ketoconazole is only a fungistatic agent. However, it is not clear if this membrane effect is relevant in vivo, because it occurs at concentrations above those achievable in patients.

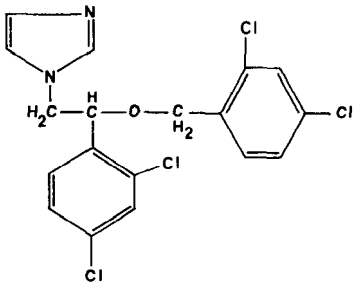
Several investigators have demonstrated that both miconazole and ketoconazole inhibit ergosterol synthesis in fungi by blocking C-14 demethylation (87). This results in a decrease in ergosterol and an accumulation of C-14 methyl sterol intermediates, such as lanosterol. Since lanosterol cannot support the growth of yeast in the absence of ergosterol, this may be the primary antifungal mechanism of the imidazoles. The selectivity of the imidazoles may result from a requirement for endogenous sterol synthesis in many fungi, whereas mammalian cells can incorporate exogenous sterol. Van Den Bossche et al (personal communication) have recently discovered that both miconazole and ketoconazole selectively affect the cytochrome P450 of yeast microsomes at concentrations equal to or slightly higher than 10^{-8}M. These results have the best correlation with the in vivo effects of the imidazoles. The effect on cytochrome P450 is related to or may be responsible for the observed accumulation of C-14 methylated sterols (e.g. lanosterol) in the fungal cells (88).

Evidence has also been presented that ketoconazole addition to mitochondria from *C. albicans* results in a decrease of cytochrome aa3 levels (89, 90). This work indicates a specific inhibition by ketoconazole of cytochrome c oxidase. These results are also consistent with the specificity of the imidazoles for fungi because the cytochrome c oxidase of fungi is more sensitive to the inhibitory effects of ketoconazole than cytochrome c oxidase isolated from animal cells. They would also explain the effect on ergosterol synthesis since oxidation is required for the C-14 demethylation. At this point, we do not know which of these is the most likely primary mechanism of action of the imidazoles on fungi.

Miconazole

Miconazole is a β substituted 1-phenethylimidazole derivative with a broad spectrum of antifungal and antibacterial activity (Figure 4). The antimicrobial spectrum of miconazole includes most fungi and many pathogenic gram-positive bacteria, although its action against gram-positive bacteria is too weak to be of clinical relevance (91).

Miconazole is poorly absorbed by the oral route of administration, so the drug must be given intravenously. The peak blood levels occur about two



miconazole

hours after administration and then fall off rapidly so that drug must be given every eight hours to maintain therapeutic blood levels (92). Another disadvantage is its poor penetration into the central nervous system, which has necessitated intrathecal therapy for fungal meningitis.

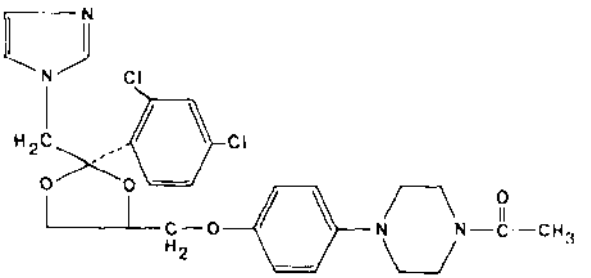
Miconazole is not excreted by the kidneys, but is broken down in the liver to an inactive metabolite. Therefore, its dosage should not be altered in patients with compromised renal function. Its suggested dosage ranges from 400 to 1200 mg given every eight hours. The duration of therapy has not been established, but 3 to 12 weeks or longer has been suggested, depending on the type of infection being treated and the response of the patient.

The reported toxicity has involved immediate problems arising during infusion, such as anaphylaxis, tachycardia, arrhythmias, fever, chills, nausea, and phlebitis. Pruritus, anemia, and hyponatremia are relatively common, and leukopenia, thrombocytopenia, and elevated liver enzymes have also been reported. Increased cholesterol and triglycerides secondary to the cremophor carrier of miconazole have also been reported (93).

Studies of patients with coccidioidomycosis and paracoccidioidomycosis suggest that miconazole is effective in these infections; however, long periods of treatment are required and there is a high rate of relapse when the drug is stopped. In addition, intrathecal drug is required in patients with meningitis. The appropriate role of miconazole in the therapy of candidiasis, blastomycosis, histoplasmosis, sporotrichosis, and cryptococcal infection remains to be defined. For these reasons, it should not be considered as a first-line antifungal agent and should be reserved for patients who cannot tolerate or have not responded to AmB.

Ketoconazole

Ketoconazole is the most recently developed antifungal imidazole derivative (Figure 5). It is water soluble and well absorbed from the gastrointestinal tract.



ketoconazole

Ketoconazole's spectrum of action is similar to that of miconazole, but the newer drug appears to be about five times more active in vitro against *Coccidioides immitis* (94). The increased activity against *C. immitis* in vitro has been confirmed by in vivo studies, which have shown excellent results in treating coccidioidomycosis in mice.

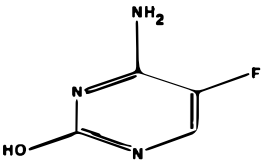
Ketoconazole is supplied as 200-mg tablets, and the suggested dose is 200 to 400 mg by mouth daily. The duration of therapy required for cure of systemic fungal infections is unknown. Ketoconazole is metabolized by the liver; inactive metabolites appear in bile, feces, and urine. The dosage should not be adjusted in patients with compromised renal function. Toxicity studies are incomplete, but pruritus, elevated liver-function tests and nonspecific gastrointestinal findings have been observed. More recently an inhibition of steroid and testosterone synthesis by the adrenals has been reported (95). It is not known whether prolonged treatment will result in adrenal insufficiency.

The drug is especially attractive because it allows oral therapy for systemic fungal infection. Not all infections have responded in a satisfactory manner. The results in treating coccidioidomycosis, histoplasmosis, and mucocutaneous candidiasis have been good; however, the responses of patients with sporotrichosis and blastomycosis have been poor (Dismukes et al, unpublished). A disturbing feature has been the high rate of recurrence of disease when the drug is stopped. In addition, there is some indication that the drug may not be effective in treating fungal infections in patients with impaired host response. Perhaps the drug would be more effective if higher doses were used or if it were administered over a longer period of time. However, until these issues are settled by more experience, we would not recommend its use to treat systemic fungal infections in immunocompromised patients.

5-FLUOROCYTOSINE

5-FC is a synthetic oral antimycotic agent that is effective in the treatment of some systemic fungal infections, in particular those due to yeasts (Figure 6). Its antifungal properties may result from its conversion to the antimetabolite 5-fluorouracil (96). Absence or low activity of cytosine deaminase, which catalyzes fluorocytosine formation in yeast, has been observed in animal cells and is considered the basis for the drug's low toxicity in the patient. As for the mode of action, evidence suggests that in addition to inhibiting RNA synthesis, 5-FC acts to interrupt DNA synthesis (97). In this reaction, the drug is converted to 5-fluoro-2'deoxyuredylic acid, which inhibits thymidylate synthetase. Resistance to 5-FC may be produced by deficiency of an enzyme at any step of this pathway or by a surplus of normal compound in the environment (or synthesized de novo), which would compete with the fluorinated antimetabolite. In our opinion, there are few indications for therapy with 5-FC alone because of natural resistance in yeasts and also the development of resistant organisms during therapy (98, 99, 100, 101). 5-FC can be used for *Candida* infections of the urinary, respiratory, and gastrointestinal tracts. It can also be used for chromomycosis. In most instances, however, it should be used in combination with AmB to treat candida and cryptococcal infections and probably chromomycosis as well (102).

The currently recommended dosage of 5-FC is 150 mg per kilogram of body weight per day, given in divided doses at intervals of 6 h. Absorption from the gastrointestinal tract is rapid and virtually complete. When kidney function is normal, the half-life in serum is 3 to 5 h. Penetration into the cerebrospinal fluid and other body fluids and tissue is excellent. The exact distribution of 5-FC in body fluids and organs is not known, but approximately 80 to 90% of the oral dose is excreted unchanged in the urine. Therefore, reduced doses have to be employed when renal function is impaired. One recommendation is to administer the drug at intervals determined by the creatinine clearance: a dose is administered every 6 h if clearance is 20 to 40 ml per minute, and every 24 h if clearance is 10 to 20



5-fluorocytosine

per minute (103). The drug can be removed from blood by dialysis, and in patients with end-stage renal disease 20 mg per kilogram of body weight should be given after each dialysis (104).

5-FC has been well tolerated by patients, even when given in large doses over extremely long periods. Gastrointestinal toxicity has caused nausea, vomiting, diarrhea, and rarely, intestinal perforation. Disturbed hepatic function has been indicated mainly by elevated titers of transaminases and alkaline phosphatase, and bone-marrow depression by neutropenia and thrombocytopenia. Usually, these toxic manifestations are mild and reversible when the dosage is reduced or the drug is stopped (105).

Many of the toxic manifestations, particularly the hematologic effects, appear to be related to serum concentrations of 5-FC in excess of 100 μg per millilider. This relation has been particularly evident when 5-FC has been given in combination with AmB, and it may involve potentiation of its toxicity by AmB or elevated blood levels of 5-FC caused by the AmB-induced renal impairment. In any case, it is useful to monitor blood levels of 5-FC, particularly in patients with impaired renal function, and to keep peak blood levels between 50 and 75 μg per millilider, and certainly below 100 μg per millilider.

CONCLUSIONS

Over the past few years, several new antifungal agents have been developed and new information has accumulated about how the oldest drug, AmB, works. Until more information and experience have been obtained on the imidazoles, we believe that AmB is still the most effective antifungal agent, particularly in the immunocompromised host. 5-FC should only be used in combination with AmB to treat certain infections with *Candida* or *C. neoformans* (93).

It is apparent that the pharmacology of all of the antifungal agents is complex. In the case of AmB, the outcome of therapy in a patient is probably the result of toxic and stimulatory effects on the parasite and host respectively. Moreover, each effect is modified by AmB interactions with serum lipoproteins, other serum proteins, and extent of uptake into specific organs. Much less is known about the newer agents, but we presume that the same complex interactions occur with them as well. The molecular or cellular basis of the effects of all of these agents can only be studied and understood by using appropriate in vitro models. However, the in vitro models are useful and clinically meaningful only when they are constantly compared with in vivo results for positive correlations.

We believe that a better understanding of how the newer and older antifungal agents work will be a very important step in improving therapy

of systemic fungal infections. In simple terms, we need to know more about the mechanism of action of these agents at every level. We have to know how they act at the subcellular level, and what kind of effect they have on cell function. We have to be able to recognize and differentiate toxic and stimulatory effects on the host so that we can avoid the former and exploit the latter to improve therapy. This is an extremely important concept and one that has not received a lot of attention. Finally, better and more reproducible susceptibility tests can help to determine appropriate dosage and more information on pharmacokinetics will allow the utilization of antifungal agents according to the best possible schedules.

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Literature Cited

1. Medoff, G., Kobayashi, G. S., Kwan, C. N., Schlessinger, D., Venkov, P. 1972. Potentiation of rifampicin and 5-fluorocytosine as antifungal antibiotics by amphotericin B. *Proc. Natl. Acad. Sci. USA* 69:196–99
2. Mechlinski, W., Schaffner, C. P., Ganis, P., Avitabile, G. 1970. Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. *Tetrahedron Lett.* 44:3873–76
3. Ernst, C., Grange, J., Rinnert, H., DuPont, G., Lematre, J. 1981. Structure of amphotericin B aggregates as revealed by UV and CD spectroscopics. *Biopolymers* 20:1575–88
4. Strauss, G., Kral, F. 1982. Borate complexes of Amphotericin B: polymeric species and aggregates in aqueous solutions. *Biopolymers* 21:459–70
5. Mazerski, J., Bolard, J., Borowski, E. 1982. Self-association of some polyene macrolide antibiotics in aqueous media. *Biochim. Biophys. Acta.* In press
6. Norman, A. W., Spielvogel, A. M., Wong, R. G. 1976. Polyene antibiotic-sterol interaction. In *Advances in Lipid Research,* ed. R. Paoletti, D. Kritchevsky, 14:127–70. New York: Academic. 268 pp.
7. Hamilton-Miller, J. M. T. 1973. Chemistry and biology of the polyene macrolide antibiotics. *Bacteriol. Rev.* 37: 166–96
8. Hamilton-Miller, J. M. T. 1974. Fungal sterols and the mode of action of the polyene antibiotics. *Advan. Appl. Microbiol.* 17:109–34
9. Hammond, S. M. 1977. Biological activity of polyene antibiotics. In *Progress in Medicinal Chemistry,* ed. G. P. Ellis, G. B. West, 14:105–79. Amsterdam: Elsevier/North Holland Biomedical. 308 pp.
10. Kerridge, D. 1979. The polyene macrolide antibiotics. *Postgrad. Med. J.* 55:653–56
11. Chen, W. C., Bittman, R. 1977. Kinetics of association of Amphotericin B with vesicles. *Biochemistry* 16:4145–49
12. Bunow, M. R., Levin, I. W. 1977. Vibrational Raman spectra of lipid system containing amphotericin B. *Biochim. Biophys. Acta* 464:202–16
13. Bolard, J., Seigneuret, M., Boudet, G. 1980. Interaction between phospholipid bilayer membranes and the polyene antibiotic amphotericin B. Lipid state and cholesterol content dependence. *Biochim. Biophys. Acta* 599:280–93
14. Petersen, N. O., Henshaw, P. F. 1981. Separation of fluorescent impurities from amphotericin B. *Can. J. Chem.* 59:3376–78

15. Gent, M. P. N., Prestegard, J. H. 1976. Interaction of the polyene antibiotics with lipid bilayer vesicles containing cholesterol. *Biochim. Biophys. Acta* 426:17–30
16. Bittman, R., Fischkoff, S. A. 1972. Fluorescence studies of the binding of the polyene antibiotics filipin III, amphotericin B, nystatin and lagosin to cholesterol. *Proc. Natl. Acad. Sci. USA* 69:3795–99
17. Schroeder, F., Holland, J. F., Bieber, L. L. 1972. Fluorometric investigation of the interaction of polyene antibiotic with sterols. *Biochemistry* 11:3105–11
18. Patterson, J., Holland, J., Bieber, L. L. 1979. Studies on the competition of polyene antibiotics for sterols. *J. Antibiot.* 32:646–53
19. Ernst, C., Lematre, J., Rinnert, H., Dupont, G., Grange, J. 1979. Interaction d'un heptaene antifongique, l' "amphotericine B", savec le cholesterol *in vitro*, detectee par dicroisme circulaire et absorption. Influence de la temperature. *C. R. Acad. Sci. Ser. D* 289: 1145–48
20. Bolard, J., Cheron, M., 1982. Association of the polyene antibiotic amphotericin B with phospholipid vesicles: perturbation by temperature changes. *Can. J. Biochem.* In press
21. Brown, J. M., Sidebottom, P. J. 1981. The proton magnetic resonance spectrum of amphotericin B. *Tetrahedron* 37:1421–28
22. De Kruijff, B., Gerritsen, W. J., Oerlemans, A., Demel, R. A., Van Deenen, L. L. M. 1974. Polyene antibiotic-sterol interaction in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. I. Specificity of the membrane permeability changes induced by the polyene antibiotics. *Biochim. Biophys. Acta* 339:30–43
23. Ohki, K., Nozawa, Y., Ohnishi, S. I. 1979. Interaction of polyene antibiotics with sterols in phosphatidylcholine bilayer membranes as studied by spin probes. *Biochim. Biophys. Acta* 554: 39–50
24. Oehlschlager, A. C., Laks, P. 1980. Nitroxide spin-probe study of amphotericin B-ergosterol interaction in egg phosphatidylcholine multilayers. *Can. J. Biochem.* 58:978–85
25. Aracava, Y., Smith, I. C. P., Schreier, S. 1981. Effect of amphotericin B on membranes: a spin probe study. *Biochemistry* 20:5702–7
26. Archer, D. B. 1975. The use of a fluorescent sterol to investigate the mode of action of amphotericin B methyl ester, a polyene antibiotic. *Biochem. Biophys. Res. Comm.* 66:195–201
27. Archer, D. B. 1975. Correction. *Biochem. Biophys. Res. Comm.* 66:1088
28. Readio, J. D., Bittman, R. 1982. Equilibrium binding of amphotericin B and its methyl ester and borate complex to sterols. *Biochim. Biophys. Acta* 685: 219–24
29. Teerlink, T., De Kruijff, B., Demel, R. A. 1980. The action of pimaricin, etruscomycin and amphotericin B on liposomes with varying sterol content. *Biochim. Biophys. Acta* 599:484–92
30. Van Hoogevest, P., De Kruijff, B. 1978. Effect of amphotericin B on cholesterol containing liposomes of egg phosphatidylcholine and didocosenoyl phosphatidylcholine: a refinement of the model for the formation of pores by amphotericin B in membranes. *Biochim. Biophys. Acta* 511:397–407
31. Bolard, J., Vertut-Croquin, A., Cybulska, B. E., Gary-Bobo, C. M. 1981. Transfer of the polyene antibiotic amphotericin B between single-walled vesicles of dipalmitoylphosphatidylcholine and egg-yolk phosphatidylcholine. *Biochim. Biophys. Acta* 647:241–48
32. Iannitelli, R. C., Ikawa, M. 1980. Effect of fatty acids on action of polyene antibiotics. *Antimicrob. Agents Chemother.* 17:861–64
33. Brajtburg, J., Kobayashi, D., Medoff, G., Kobayashi, G. S. 1982. Antifungal action of amphotericin B in combination with other polyene and imidazole antibiotics. *J. Infect. Dis.* 146:138–45
34. Hsu Chen, C. C., Feingold, D. S. 1973. Polyene antibiotic action on lecithin liposomes: effect of cholesterol and fatty acyl chains. *Biochem. Biophys. Res. Comm.* 51:972–78
35. Archer, D. B. 1976. Effect of the lipid composition of Mycoplasma Mycoides subspecies *Capri* and phosphatidylcholine vesicles upon the action of polyene antibiotics. *Biochim. Biophys. Acta* 436: 68–76
36. Kasumov, Kh. M., Borisova, M. P., Ermishkin, L. N., Potseluyev, V. M., Silverstein, A. Yo., Vainshtein, V. A. 1979. How do ionic channel properties depend on the structure of polyene antibiotic molecules? *Biochim. Biophys. Acta* 551:229–37
37. Singer, M. A. 1975. Interaction of amphotericin B and nystatin with phospholipid [redacted] membranes: effect of cholester[redacted] *J. Physiol. Pharmacol.* 53:1072–79

38. Jirku, V., Čejkova A., Páca, J. 1981. Effect of growth factor deficiency on nystatin sensitivity in *Saccharomyces cerevisiae*. *Experentia* 37:39–40
39. Kotler-Brajtburg, J., Price, H. D., Medoff, G., Schlessinger, D., Kobayashi, G. S. 1974. Molecular basis for the selective toxicity of amphotericin B for yeast and filipin for animal cells. *Antimicrob. Agents Chemother.* 5:377–82
40. Gruda, I., Nadeau, P., Brajtburg, J., Medoff, G. 1980. Application of differential spectra in the ultraviolet-visible region to study the formation of amphotericin B-sterol complexes. *Biochim. Biophys. Acta* 602:260–68
41. Feigin, A. M., Belousova, I. I., Yakhimovich, R., Vasilevskaya, V. N., Tereshin, I. M. 1979. Role of the number and position of double bonds in the four ring sterol nucleus in reaction with polyene antibiotics. *Biofizika* 24:330–31
42. Verkleij, A. J., De Kruijff, B., Gerritsen, W. F., Demel, R. A., Van Deenen, L. L. M., Ververgaert, P. H. J. 1973. Freeze-etch electron microscopy of erthyrocytes, *Acholeplasma laidlawii* cells and liposomal membranes after the action of filipin and amphotericin B. *Biochim. Biophys. Acta* 291:577–81
43. Nozawa, Y., Kitajima, Y., Sekiya, T., Ito, Y. 1974. Ultrastructural alterations induced by amphotericin B in the plasma membrane of *Epidermophyton floccosum* as revealed by freeze-etch electron microscopy. *Biochim. Biophys. Acta* 367:32–38
44. Kitajima, Y., Sekiya, T., Nozawa, Y. 1976. Freeze-fracture ultrastructural alterations induced by filipin, pimaricin, nystatin and amphotericin B in the plasma membranes of *Epidermophyton, Saccharomyces* and red blood cells. A proposal of models for polyene ergosterol complex-induced membrane lesions. *Biochim. Biophys. Acta* 455: 452–65
45. Finkelstein, A., Holz, R. 1973. Aqueous pores created in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. In *Membranes,* ed. G. Eisenman, 2:377–408. New York: Dekker. 1191 pp.
46. DeKruijff, B., Demel, R. A. 1974. Polyene antibiotic-sterol interaction in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic-cholesterol complexes. *Biochim. Biophys. Acta* 339:57–70
47. Harrand, M., Peticolas, W. L., Dupeyrat, R. 1982. Polarized Raman spectroscopy of oriented macrodomain of phospholipids containing amphotericin. *Biochem. Biophys. Res. Comm.* 104: 1120–26
48. DeLaat, S. W., Van Der Saag, P. T., Elson, E. L., Schlessinger, J. 1980. Lateral diffusion of membrane lipids and proteins during the cell cycle of neuroblastoma cells. *Proc. Natl. Acad. Sci. USA* 77:1526–28
49. Kwan, C. N., Medoff, G., Kobayashi, G. S., Schlessinger, D., Raskas, H. J. 1972. The potentiation of the antifungal effects of antibiotics by amphotericin B. *Antimicrob. Agents Chemother.* 2:61–65
50. Brajtburg, J., Medoff, G., Kobayashi, G. S., Elberg, S., Finegold, C. 1980. Permeabilizing and hemolytic action of large and small polyene antibiotics on human erythrocytes. *Antimicrob. Agents Chemother.* 18:586–92
51. Brajtburg, J., Medoff, G., Kobayashi, G. S., Elberg, S. 1980. Influence of extracellular K^+ or Mg^{2+} on the stages of the antifungal effects of amphotericin B and filipin. *Antimicrob. Agents Chemother.* 18:593–97
52. Brajtburg, J., Elberg, S., Medoff, G., Kobayashi, G. S. 1981. Increase in colony-forming units of *Candida albicans* after treatment with polyene antibiotics. *Antimicrob. Agents Chemother.* 19:199–200
53. Kotler-Brajtburg, J., Medoff, G., Schlessinger, D., Kobayashi, G. S. 1977. Amphotericin B and filipin effects on L and HeLa cells: dose response. *Antimicrob. Agents Chemother.* 11:803–8
54. Kitagawa, T., Andoh, T. 1978. Stimulation by amphotericin B of uridine transport, RNA synthesis and DNA synthesis in density-inhibited fibroblasts. *Exp. Cell Res.* 115:37–46
55. Rozengurt, E., Mendoza, S. 1980. Monovalent ion fluxes and the control of cell proliferation in cultured fibroblasts. *Ann. NY Acad. Sci.* 339:175–90
56. Chen, W. C., Chou, D. L., Feingold, D. S. 1978. Dissociation between ion permeability and the lethal action of polyene antibiotics on *Candida albicans*. *Antimicrob. Agents Chemother.* 13:914–17
57. Malewicz, B., Jenkin, H. M., Borowski, E. 1980. Dissociation between the induction of potassium efflux and cytostatic activity of polyene macrolides in mammalian cells. *Antimicrob. Agents Chemother.* 17:699–706
58. Malewicz, B., Jenkin, H. M., Borowski, E. 1981. Repair of membrane alterations induced in baby hamster kidney

cells by polyene macrolide antibiotics. *Antimicrob. Agents Chemother.* 19: 238–47
59. Hammarström, L., Smith, E. 1976. Mitogenic properties of polyene antibiotics for murine B cells. *Scand. J. Immunol.* 5:37
60. Hammarström, L., Smith, C. I. E. 1977. *In vitro* activating properties of polyene antibiotics for murine lymphocytes. *Acta Pathol. Microbiol. Scand. Sect. C.* 85:277–83
61. Little, J. R., Blanke, T. J., Valeriote, F., Medoff, G. 1978. Immunoadjuvant and antitumor properties of amphotericin B. in *Immune Modulation and Control of Neoplasia by Adjuvant Therapy,* ed. M. A. Chirigos, pp. 381–87. New York: Raven. 501 pp.
62. Shirley, S. F., Little, J. R. 1979. Immunopotentiating effects of amphotericin B. I. Enhanced contact sensitivity in mice. *J. Immunol.* 123:2878–82
63. Shirley, S. F., Little, J. R. 1979. Immunopotentiating effects of amphotericin B. II. Enhanced *in vitro* proliferative responses of murine lymphocytes. *J. Immunol.* 123:2883–89
64. Stewart, S. J., Spagnuolo, P. J., Ellner, J. J. 1981. Generation of suppressor T lymphocytes and monocytes by amphotericin B. *J. Immunol.* 127:135–39
65. Lin, H. S., Medoff, G., Kobayashi, G. S. 1977. Effects of amphotericin B on macrophages and their precursor cells. *Antimicrob. Agents Chemother.* 11:154–60
66. Olds, G. R., Stewart, S. J., Ellner, J. J. 1981. Amphotericin B-induced resistance to Schistosoma mansoni. *J. Immunol.* 126:1667–70
67. Chapman, H. A. Jr., Hibbs, J. B. Jr. 1978. Modulation of macrophage tumoricidal capability by polyene antibiotics: support for membrane lipid as a regulatory determinant of macrophage function. *Proc. Natl. Acad. Sci. USA* 75:4349–53
68. Hauser, W. E. Jr., Remington, J. S. 1982. Effect of antibiotics on the immune response. *Am. J. Med.* 72:711–16
69. Kitahara, M., Kobayashi, G. S., Medoff, G. 1976. Enhanced efficacy of amphotericin B and rifampicin combined in treatment of murine histoplasmosis and blastomycosis. *J. Infect. Dis.* 133:663–68
70. Arroyo, J., Medoff, G., Kobayashi, G. S. 1977. Therapy of murine aspergillosis with amphotericin B in combination with rifampin or 5-fluorocytosine. *Antimicrob. Agents Chemother.* 11:21–25
71. Stobo, J. D., Paul, S., Van Scoy, R. E., Hermans, P. E. 1976. Suppressor thymus-derived lympocytes in fungal infection. *J. Clin. Invest.* 57:319–28
72. Ferrante, A., Rowan-Kelley, B., Thong, Y. H. 1979. Suppression of immunological responses in mice by treatment with amphotericin B. *Clin. Exp. Immunol.* 38:70–76
73. Thomas, M. Z., Medoff, G., Kobayashi, G. S. 1973. Changes in murine resistance to *Listeria monocytogenes* infection induced by amphotericin B. *J. Infect. Dis.* 127:373–77
74. Medoff, G., Valeriote, F., Lynch, R. G., Schlessinger, D., Kobayashi, G. S. 1974. Synergistic effect of amphotericin B and 1,3-Bis (2-chlorethyl)-1-nitrosourea (BCNU) against a synergistic transplantable AKR leukemia. *Cancer Res.* 34:974–78
75. Shore, V. G., Shore, B., Lewis, S. B. 1978. Isolation and characterization of two threonine-poor apolipoproteins of human plasma high density lipoproteins. *Biochemistry* 17:2174–79
76. Kitahara, M., Seth, V. K., Medoff, G., Kobayashi, G. S. 1976. Antimicrobial susceptibility testing of six clinical isolates of Aspergillus. *Antimicrob. Agents Chemother.* 9:908–14
77. Pappagiannis, D., Collins, M. S., Hector, R., Remington, J. 1979. Development of resistance to amphotericin B in *Candida lusitaniae* infecting a human. *Antimicrob. Agents Chemother.* 16:123–126
78. Binschadler, D. D., Bennett, J. E. 1969. A pharmacologic guide to the clinical use of amphotericin B. *J. Infect. Dis.* 120:427–36
79. Medoff, G., Dismukes, W. E., Meade, R. H. III, Moses, J. M. 1972. A new therapeutic approach to *Candida* infections: a preliminary report. *Arch. Intern. Med.* 130:241–45
80. Louria, D. B. 1958. Some aspects of the absorption, distribution and excretion of amphotericin B in man. *Antibiot. Med. Clin. Ther.* 5:295–301
81. Jagdis, F. A., Monji, N., Lawrence, R. M., Hoeprich, P. D., Schaffner, C. P. 1976. Distribution of radiolabelled amphotericin B methyl ester and amphotericin B in non-human primates. *Clin. Res.* 24:453A
82. Medoff, G., Kobayashi, G. S. 1972. Pulmonary mucor mycosis. *N. Engl. J. Med.* 286:87
83. Diamond, R. D., Bennett, J. E., 1973. A subcutaneous reservoir for intrathecal

therapy of fungal meningitis. *N. Engl. J. Med.* 288:186–88
84. Serstock, D. S., Zinneman, H. H. 1975. Pulmonary and articular sporotrichosis: report of two cases. *J. Am. Med. Assoc.* 233:1291–93
85. Butler, W. T., Bennett, J. E., Alling, D. W., Wertlake, P. T. 1964. Nephrotoxicity of amphotericin B: early and late effects in 81 patients. *Ann. Intern. Med.* 61:175–87
86. Hellebusch, A. A., Salama, F., Eadie, E. 1972. The use of mannitol to reduce the nephrotoxicity of amphotericin B. *Surg. Gynecol. Obstet.* 134:241–43
87. Sud, I. J., Feingold, D. S. 1981. Heterogeneity of action mechanisms among antimycotic imidazoles. *Antimicrob. Agents Chemother.* 20:71–74
88. Van Den Bossche, H., Willemsens, G., Cools, W., Cornelissen, F., Lauwe s, W. F., Van Cutsem, J. M. 1980. *In vitro* and *in vivo* effects of the antimycotic drug ketoconazole on sterol synthesis. *Antimicrob. Agents Chemother.* 17: 922–28
89. Uno, J., Shigematsu, M. L., Arai, T. 1982. Primary site of action of ketoconazole on *Candida albicans*. *Antimicrob. Agents Chemother.* 21:912–18
90. Shigematsu, M. L., Uno, J., Arai, T. 1982. Effect of ketoconazole on isolated mitochondria from *Candida albicans*. *Antimicrob. Agents Chemother.* 21: 919–24
91. Botter, A. A. 1971. Topical treatment of nail and skin infections with miconazole, a new broad spectrum antimycotic. *Mykosen* 14:187–91
92. Deresinski, S. C., Lilly, R. B., Leving, H. B., Galgianis, J. N., Stevens, D. A. 1977. Treatment of fungal meningitis with miconazole. *Arch. Intern. Med.* 137:1180–85
93. Medoff, G., Kobayashi, G. S. 1980. Strategies in the treatment of systemic fungal infections. *N. Engl. J. Med.* 302:145–55
94. Dixon, D., Shadomy, S., Shadomy, H. J., Espinal-Ingroff, A., Kerkering, T. M. 1978. Comparison of the *in vitro* antifungal activities of miconazole and a new imidazole R41,400. *J. Infect. Dis.* 138:245–48
95. Port, A., Williams, P. L., Loose, D. S., Feldman, D., Bochra, C., Reitz, R. E., Stevens, D. A. 1982. Ketoconazole inhibits adrenal steroid synthesis. *Clin. Res.* 30:32A
96. Diasio, R. B., Lakings, D. E., Bennett, J. E. 1978. Evidence for conversion of 5-fluorocytosine to 5-fluorouracil in humans: possible factor in 5-fluorocytosine clinical toxicity. *Antimicrob. Agents Chemother.* 14:903–8
97. Polak, A., Scholer, H. J. 1975. Mode of action of 5-fluorocytosine and mechanisms of resistance. *Chemotherapy* 21:113–30
98. Drouhet, E., Mercier-Soucy, L., Montplaisir, S. 1975. Sensibilite et resistance des levures pathogenes aux 5-fluoropyrimidiens. I. Relation entre les phenotypes de resistance a la 5-fluorocytosine, le sterotype de *Candida albicans* et l'ecologie de differentes especes de *Candida* d'o igine humaine. *Ann. Microbiol. B* Paris 126:25–39
99. Scholer, H. J. 1976. Grundlagen und Ergebnisse der antimykoti chen chemotherapie mit 5-fluorocytosin. *Chemotherapy* 22:Suppl. 1:103–46
100. Polak, A., Scholer, H. J. 1976. Combination of amphotericin B and 5-fluorocytosine. In *Chemotherapy*, ed. J. D. Williams, A. M. Geddes, 6:137–42. New York: Plenum. 395 pp.
101. Block, E. R., Jennings, A. E., Bennett, J. E. 1973. 5-Fluorocytosine resistance in *Cryptococcus neoformans*. *Antimicrob. Agents Chemother.* 3:649–56
102. Bennett, J. E., Dismukes, W. E., Duma, R. J. 1979. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N. Engl. J. Med.* 301: 126–31
103. Cutler, R. E., Blai , A. D., Kelly, M. R. 1978. Flucytosine kinetics in subjects with normal and impaired renal function. *Clin. Pharmacol. Ther.* 24:333–42
104. Christopher, T. G., Blair, A. D., Forrey, A. W., Cutler, R. E. 1976. Hemodialyzer clearances of gentamicin, kanamycin, tobramycin, amikacin, ethambutol, procainamide and flucytosine, with a technique for planning therapy. *J. Pharmacokinet. Biopharm.* 4:427–41
105. Kauffman, C. A. Frame, P. T. 1977. Bone marrow toxicity associated with 5-fluorocytosine therapy. *Antimicrob. Agents Chemother.* 11:244–47