INTRODUCTORY ADDRESS

Antifungal therapy, 1978

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Summary
The past 40 years have brought great advances in the knowledge of morphology, immunology and epidemiology of fungal infections in man. Each general advance in the science of medicine has had its counterpart in man's improved facility to deal with these infections therapeutically. Although satisfactory control of fungal infections of man is still lacking, productive paths of investigation seem to have been found and the continued application of rigorous discipline to research efforts should provide even more efficient treatment in the future.

In spite of the emphasis being placed by this conference on the therapy of fungal diseases it seems to me that the understanding of where we have been and to what point we have arrived in the treatment of this group of diseases requires us to consider the broader accomplishments of our quest for understanding of our subject. That is to say that a simple recitation of what drugs, incantations, and manipulations were used and are being used and the names and drinking habits of the men who first published on these activities simply will not do. If perspective is desired then a search for understanding is essential.

I have been unable to find any citation or letters to the editor to explain the rationale of what must have been the earliest treatment by our ancestors of itchy toes; that is amputation. Nevertheless, I believe that it is clear to us all what motivated the action. On the other hand Hippocratic logic in using local bleeding as treatment for ulcers on the toes occasionally caused by fungi is a bit obscure unless one accepts the master's rationale that bleeding an area is a means to dry it (Adams, 1971).

Although Giovan Cosimo Bonomo was the first to relate a human disease to the activities of a small companion, in his case scabies caused by Acarus scabiei, in 1787, the field of mycology has the honour of having given the field of infectious diseases its scientific beginnings with the discovery by Langenbeck of the thrush fungus and by Schoenlein of the cause of favus, both in 1839. This was the age of descriptive medicine and so it is natural that understanding of effective treatments, such as they existed, was limited to the simple observation that these treatments did, indeed, work. Again, based on description only, it is clear why diseases of the skin should have been so prominent among the literature of the 19th century. The list of treatments for the wide variety of ringworm infections, for example, is long and consists of unguents, lotions, and powders all of which either discoloured the skin so badly that the continuously flourishing infection was invisible or caused the skin to peel or slough off so that, once again, the physician and, even more importantly, the patient, could no longer see the lesion. This latter approach was actually effective with 2 slight drawbacks: the infection frequently returned and the results of overuse or overconcentration of the therapy gave rise to skin lesions that made the fungal infection a pleasure by comparison. Not to be underestimated in assessing these various treatments was the circumstance of remuneration to the therapist which generally was, over the period of many recurrences, considerable. Considering the drama of apparent cure, albeit short-lived, and the comfort of a consistent income, it is a wonder that the great break-through of griseofulvin was greeted with such enthusiasm.

The beginnings were rather unimpressive . . . and surprisingly early, 1939. Oxford, Raistrick and Simonart (1939) reported discovery of the substance from the mould Penicillium griseofulvin and something of its chemical nature. This period of pre-World War II was a time in the history of medicine when the beginnings of modern biochemistry of the 1920s were being applied to medicine of the 1930s. That was the time when this biochemical approach was particularly appealing for the microbiologist since the 1930s had witnessed the beginnings of the chemotherapy of bacterial diseases with the sulphonamides. So it was natural that the effect of biologically generated substances should be looked...
at as possible influences on the life cycle of microorganisms. The drama of penicillin was about to explode upon society but 1939 was a little too early for anyone to try out this apparently interesting but not important substance, griseofulvin, against fungi, of all things!

And so the appearance of antibiotics had to happen with great drama, seemingly as a by-product of the tragedy of World War II. Antibiotics came along with empirical regularity, penicillin, streptomycin, the first of the tetracyclines, para-amino salicylic acid, and in 1949 nystatin, discovered by Brown and Hazen. At the same time the first words were printed about the effect of stilbamidine and then 2-hydroxy stilbamide on blastomycosis; and in 1956 we were given amphotericin. But more about all of them later; it is back to the first discovered griseofulvin for more detail.

In 1958 Gentles reported on this drug as having an effect on experimental ringworm in guinea-pigs and in the same year Williams, Marten and Sarkany (1958) reported on treatment of ringworm in man with this substance. In 1959, the flood gates were opened by Harvey Blank and the drug was off and running (Blank and Roth, 1959). But what gave this substance that had lain fallow for 20 years the push into some studies that led to discovery of its exciting characteristics? Perhaps it was the words of the authors of 1939 who wrote of this substance in the context of biochemistry of the mother organism and thereby aroused the curiosity of a susceptible Dr Gentles or perhaps it was something much more banal: curiosity to see what would happen if the substance was cooked with the one group of pathogenic fungi that seemed resistant to all the newer antifungal agents that had been described up to that point. After all, nearly everything except the dermatophytes was being treated at least in the laboratory, so why not try it out! I am not sure what came first, the success clinically in guinea-pigs or the observation that the hyphae of the dermatophytes were curled by this substance; but, in any case, the enquiring mind was primed to make the clinical observation and now we have a drug that works.

But we have now grown more sophisticated and are no longer satisfied merely to say it works and curls hyphae into the bargain. We have advanced in the 1970s to finding a cellular biological explanation for all these phenomena, such as killing fungi and so we have arrived at the conclusions that griseofulvin inhibits DNA synthesis, destroys cytoplasm and kills the fungus. The words are impressive but I have a hunch that we really do not understand it all and the 1980s will have to unravel how all this works, what reactor sites are blocked and how it really does what it does. Perhaps we will start to learn here at this conference.

Ageing of humans has been given rather bad publicity of late but I have to admit that it is not as bad as I once thought it might be. Advantages take some looking after but they do exist, and one of them is the feeling that one has lived through great days of discovery and can report to another generation on such great moments. My first stutterings into mycological mysteries were at the knee of my teacher and father in medicine, Jan Schwarz, and our first efforts were directed towards North American blastomycosis. Our mutual affection and enthusiasm served us both well: I learned a tremendous amount about medicine, especially fungus diseases, from him, and he obtained translations of his words from English to English from me. As we pursued our interests in blastomycosis we were impressed that the available treatment using iodides and various immunization programmes using blastomycin were terribly ineffective and did little to improve the horrible prognosis of disseminated disease. We toyed with a substance called ethyl vanillate and even went so far as to treat one man for a short time with this foul potion. What stopped us was the unfortunate circumstances of Dr Schwarz’s tasting the witch’s brew one day before giving it to the hapless patient. Jan was and still is a ‘chicken-heart’ and could not bear tormenting the poor fellow with that brackish brew with so little hope of success. Either in spite of or because of the treatment the patient did not improve. We played in the laboratory with various agents and were thrilled to find a miracle in the form of stilbamidine which was used by Dr Emanuel Schoenbach in New York for blastomycosis. His reports in 1948 and 1951 were the first reporting success in the treatment of this terrible disease (Schoenbach and Greenspan, 1948; Schoenbach et al., 1951). Why Dr Schoenbach was working in such an area is not clear but this was a time of intense interest in chemical interference with various disease processes; one has only to think of the plethora of agents that were being used for metabolic defects or the exciting discoveries that were being made in enzymology at the same time. Cell biology as we know it today was only the dream of a few physicists and biologists but metabolic pathways and defects therein discovered preoccupied many investigators. Thus the aromatic diamidines found their way into the hands of an astute clinical investigator, and blastomycosis became treatable. Needless to say the toxicity was great and led to the use of the 2-hydroxy derivative but that was a technicaity; the stroke of genius was Schoenbach’s and the validation was found to come from another classical clinical investigator, Isadore Snapper, only a few months later (Snapper and McVay, 1952).

But now we come to the big story of the last 30 years: the discovery of nystatin by 2 intrepid ladies.
who were working for the New York State Department of Health. Drs Brown and Hazen isolated a species of *Streptomyces* on the Nourse dairy farm in Fouquier County, Virginia, and from the locale came the organism's name, *S. noursei*. They found 2 antibiotic substances produced by the fungus, actidiolone and nystatin (Brown and Hazen, 1949; Hazen and Brown, 1950). The former was useful in its own right but the primary discovery was that a polyene substance had unique properties, among them its ability in low concentration to inhibit the growth of several fungi *in vitro* and, in high concentrations, to kill them. The good news was that *in vitro* the spectrum included Candida spp., *Cryptococcus neoformans*, Histoplasma capsulatum, *Blastomyces dermatitidis*, Paracoccidioides brasiliensis, *Sporothrix schenckii* (these latter in their yeast forms) and a wide variety of the dermatophytes. The bad news was that the substance was all but completely insoluble in water and, therefore, its use was limited to direct application to the offending fungus.

Never mind, this was a major discovery both from the point of view of the direct results over the years of use of nystatin and from the fact that it introduced to the investigator a new family of substances that has proved to be highly effective in the treatment of deep fungus infections in man. Keeping in mind the time of the discovery we find ourselves in the halcyon days of the post-World War II search for new antibiotics.

In addition to the contribution made to the treatment of *Candida* infections, nystatin has become a tool to unravel the mode of action of polyene antibiotics on the susceptible micro-organisms. Lampen and his colleagues (Lampen, Morgan and Slocum, 1956, 1957) pointed out that nystatin attaches itself to the cell wall of the yeast cell and renders it more permeable, first increasing its O₂ consumption and then causing it to fall to nothing, with subsequent cell death. This latter appeared to be due to loss of potassium through the permeable cell membrane followed by inhibition of utilization of glucose both *via* aerobic and anaerobic pathways (Marini, Arnow and Lampen, 1961).

As a clinician, my own interests were more in the area of deep fungus infections; thus I found nystatin disappointing but in the area of surface troubles with *Candida* in debilitated patients, for example, the effects of this substance were amazing. It was always my feeling that it lacked only a colourful personality such as that of gentian violet, the treatment of another era for *Candida* overgrowth. I probably was not the first to realize that a few drops of gentian violet added to the suspension of nystatin applied to tongue, mouth, ear, finger-tips or vaginal mucosa added tremendously to the impact of the treatment on the patient.

Now the stage was set for the discovery by Gold *et al.* (1956) of amphotericin. As is well known, this substance (really 2 substances) was found to be the product of *Strep. nodosus* which had been isolated in the Orinoco river valley. The 2 forms of amphotericin, named A and B, were separated by Steinberg, Jambor and Suydam (1956) and the work of Gold and his colleagues (1956) was extended and supported. This was THE polyene antibiotic and it had not only *in vitro* effect on a wide variety of deep fungi but it also worked against infections in animals and man. As a budding student of mycology in Dr Schwarz's laboratory I spent many a day and night injecting animals with amphotericin and other lesser substances as we, along with many other investigators, exploited the promise implied by the earlier reports. By the late 1950s it was clearly established that this drug worked and, in spite of its relative insolubility, it could be given intravenously, and by this route did, indeed, fulfil its destiny as an effective clinical tool. Switching hats again I participated both with Dr Schwarz and with the Veterens Administration Cooperative Studies in helping to demonstrate that histoplasmosis, blastomycosis, sporotrichosis, systemic candidiasis and cryptococcosis were all effectively treated with amphotericin B. During this same period, several workers in the western United States demonstrated the significant impact of amphotericin B on coccidioidomycosis.

The mechanism of action was found to be consistent with that of nystatin but a stroke of genius was expressed by Kobayashi *et al.* (1972) when they exploited the permeability effect of amphotericin to the cell wall and added rifampicin to the mix and got a considerable enhancement of the anti-DNA effect, apparently owing to the increased penetration of the cell by rifampicin.

But what of the toxicity of this drug? From a personal point of view I can add that both in my clinical experience with patients and as a participant in the Cooperative Studies I found that the bark of toxicity was considerably greater than the bite. Kidneys and bone marrow were found to be badly damaged but, by and large, not permanently so. Professor Symmers stated it brilliantly by coining the term 'pharmacophobia', pointing out that the greatest danger was in not using the drug where it was indicated (Symmers, 1973). These experiences were noted during the times of great achievement in cell biology, and investigations of this drug were therefore carried out. To this day, we are continuing to use this substance as a cell membrane-active agent and who knows what secrets may come through the cell walls thus rendered more permeable by amphotericin B. We also learned early on to use the clinical trial approach to finding out if a cure was really a cure and of course the Medical
Research Council of the U.K., the U.S. Public Health Service and the U.S. Veterans Administration all share the honour of developing and exploiting this method.

We come now to the story of 5-fluorocytosine, an agent which began its career in the shadow of its more toxic cousin, 5-fluorouracil, one of the earliest of the antineoplastic agents. 5-Fluorocytosine was discovered by Duschinski, Plevin and Heidelberger (1957) but its antifungal activity was first reported by a friend of many years, Emanuel Gruneberg, and his colleagues (Gruneberg, Titsworth and Bennet, 1964). I remember a meeting of the Cooperative Study group of the Veterans Administration dealing with fungus diseases in the early 1960s when Dr Gruneberg mentioned his discovery; and, in the 6 months between that first announcement and our next meeting, several investigators reported anecdotal experience with the drug. I complained to my colleagues that it was too bad that no animal work was being done to support these early in vitro claims and in the manner of many a dyspeptic non-believer, doubted the validity of the claims. As matters have turned out I was wrong in my conclusions even though I am convinced that my methodological complaints were justified. We have come to find 5-fluorocytosine a useful adjunctive drug to be used with amphotericin B in the treatment particularly of cryptococcal meningitis. It is useful in its own right as a treatment for candidal infections but in the case of both Candida and Cryptococcus resistance develops quickly, or is already present, and this limits the use of the agent as a single treatment.

Coming along as it did as a by-product of cancer chemotherapy, we must credit the zealous search for anti-neoplastic agents as being responsible for the discovery of 5-fluorocytosine. That was an interesting time, when millions of U.S. dollars were being spent in the race to find the cure for malignant disease. In the course of studies with this agent we learned that the effect on the yeast cell was actually achieved by 5-fluorouracil and the conversion of 5-fluorocytosine to 5-fluorouracil occurred within the yeast cell. This conversion does not occur within the human cells because man lacks the necessary enzyme, a very convenient difference between yeast and human cells indeed! This, then, accounts for the relative lack of toxicity in man of this drug and rationalizes its use against yeast infections.

This brings us to a family of drugs that has appeared since 1969. Clotrimazole and miconazole were both reported upon in those times (Plempel et al., 1969, 1970; Godefroi et al., 1969). Both these drugs were found to be active in that they increased membrane permeability, a familiar story with antifungal agents. With clotrimazole, in spite of its promise of activity against Aspergillus, Candida and some of the dermatophytes, it was soon found that blood levels were impossible to maintain even with very high doses of the drug. The mechanism for this strange phenomenon appears to be the activation, after initial exposure, of liver enzymes which destroy the drug. With miconazole, the effect has proved to be more reliable, and the drug appears to be a reliable oral treatment for candidiasis, some dermatophyte infections and, of particular note, for coccidioidomycosis (Stevens, Levine and Deresinski, 1976). It is in this latter role that the most dramatic implications arise since disseminated coccidioidomycosis remains a difficult infection to treat and amphotericin B, although effective, is far from the ideal agent. Only time will clarify the role of this new, promising agent.

As a recent development, Dr Levine (1976) in California has reported experience with a dioxolane imidazole, R-3400, which has shown promising results in experimental coccidioidomycosis. Perhaps we are about to find another effective agent in this exciting chemical family.

In order to finish this brief encounter with the past and present of antimycotic chemotherapy let me deal a moment with 2 agents that were received by the world of mycologists with anticipation but which faded from the scene after a brief moment of fame. These are not the only such agents to have come and gone in the past 40 years but they are the ones I dealt with and of which I can recount some personal reactions.

In 1961, Gruneberg, Berger and Titsworth reported on the antibiotic they obtained from *Strep. novum*, x-5079-c, a name with adventurous implications but marketing limitations. Subsequently it became known as saramycetin, named after Dr Gruneberg's daughter. More importantly it had significant activity against *H. capsulatum*, and *B. dermatitidis* and was proposed for extensive clinical trials after the usual preliminary statements of success. Dr Gruneberg approached the Veterans Administration (V.A.) and, although they expressed interest, the company that manufactured the drug discontinued it owing to the high cost of production and the relatively small market potential and agreed to a franchise arrangement if someone would pay for the production. A grant was given by the National Cancer Institute to cover the cost of the drug, $75 000 for the first kilo. By this time, the Food and Drug Administration had set up its strict procedures, and extensive laboratory testing was required before the clinical trials. When this was completed, the V.A. was ready to start on the big test but they had used up that first kilo and ordered a second. As the trial was about to get started the Food and Drug Administration exploded their bombshell by stating that with a new batch new basic data would be required.
Such a ‘crafty’ approach was too much even for the V.A. and saramycetin faded away and has not been heard of since. We must always wonder what might have been but, frankly, the early results were no better than with existing treatment so perhaps we didn’t lose too much.

Of even more personal connection was the story of hamycin, a polyene antibiotic that was discovered in India by Thirumalachar, Menon and Bhatt (1961) and developed in the U.S. by Williams, Bennett and Emmons (1965). The drug was found to be active against \textit{B. dermatitidis} and in clinical practice was found to be a potential alternative to amphotericin B and hydroxystilbamidine in the treatment of North American blastomycosis. The preliminary work was done and the drug was accepted by the Food and Drug Administration for clinical trials. Again the V.A. was involved and it was my responsibility to develop the protocol and help to organize the co-operating hospitals. I spent nearly a year getting things into good order and had arranged for the final protocol to be printed by the V.A. with the blessings of the statisticians. As a courtesy I sent the last rough copy to the drug company representatives with whom I had been working closely. After a long silence, I was told that the company was discontinuing importation of the drug because the potential market did not seem to them to justify the cost. Who knows what we may have lost and who can say what we might have learned about cell–drug interaction or dynamics of organism biochemistry.

We are about to embark on a conference to hear the latest news. What has gone before was a reflection of the needs, the times but especially the curiosity and industry of the men who did the work. I have not in any way cited all the major contributions made in this field but I have tried to pick out some of the early discoveries. There is an American bias to this presentation but for this I have no excuses, only an explanation. This is but a preamble to the conference. The one matter which we can apply from past experience to future activity is the rigorous insistence on careful dissemination of data and, even more, a careful interpretation of them.

As we open this conference my clinical bias prompts me to express questions which I hope may be touched upon. Are there \textit{in vitro} indices which can be used as guides to the length of antifungal therapy? Are all known fungal infections necessarily to be treated with anti-fungal agents? Is the host response a matter of only divine intervention, or may we expect more effective and longer lasting tools such as transfer factor, B.C.G., levamisole and thymic hormone to be available to the clinician? Is immunization a means significantly to reduce morbidity and mortality from fungal infections?

\textbf{References}


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