discovered, it was not long before the toxin was produced *in vitro* and shown to be composed of two[3] and then three[4] interacting factors. This was before other toxins had been shown to be multicomponent molecules.

The discovery of the anthrax toxic complex was the aspect of this work that hit the headlines. However, there was another which had an equal impact on me: the demonstration of a striking difference in membrane permeability between anthrax organisms grown *in vivo* and *in vitro*[5]. Bacilli from infected guinea pigs were harvested in quantity (1.5–2.0 g dry weight from 100 animals) by differential centrifugation of peritoneal and thoracic exudates of animals that had been inoculated intraperitoneally and intrathoracically, respectively. The bacilli contained <0.1% of blood cell substance. After centrifugation in saline and suspension in water they became swollen and could no longer be deposited by similar centrifugation. This swelling did not occur for 16 samples of organisms obtained by growing the same strain of *Bacillus anthracis* in four different media and harvesting them at four different times. Furthermore, on adding 0.16% (w/v) ammonium carbonate to the aqueous suspension of organisms grown *in vivo*, they dissolved completely, but those grown *in vitro* did not[4]. The reason for this difference in permeability between the organisms grown *in vivo* and *in vitro* and the susceptibility of the membranes of the former to ammonium carbonate was not investigated. However, I was left in no doubt that these changes would have profound effects on metabolism and virulence determinant production.

Following this work on anthrax, I advocated the use of bacteria grown *in vivo* for studies on pathogenicity[3], but the idea did not catch on. At the time, pathogenicity was not a popular area of microbiology. Also, methods for investigating the properties of bacteria grown *in vivo* required large numbers to obtain meaningful results, and separating organisms from infected animals in quantity was difficult. In the 1970s and 1980s, the popularity of studies on bacterial pathogenicity increased owing to the application of genetics and molecular biology, and methods such as gel electrophoresis became available for analysing surface components of small numbers of organisms, which could be obtained relatively easily from infected animals. This led to an increase in studies of bacteria grown *in vivo*. However, it has been the recent development of new methods, such as *in vivo* expression technology, signature-tagged mutagenesis and differential fluorescence induction that has stimulated the current intense interest. This was clear at a recent Royal Society Discussion Meeting entitled 'The activities of bacterial pathogens *in vivo*'[6]. At last, the idea has caught on and I am delighted.

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Pathogenic fungi in the 21st century

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Medical mycology is one of the youngest sub-specialities within the area of medical microbiology and infectious diseases. I entered it, almost by accident, as a postgraduate, and found a fascinating and significantly unexplored research domain whose main economic reason for existence was two non-fatal diseases: ringworm and thrush. For the first part of the 20th century, its focus of interest lay in the recognition and identification of fungal species causing infections of skin, nails and mucosal tissues. Experts often spoke and wrote of their field as representing a 'Cinderella' left at home while the significant microbiologists enjoyed the Prince's Ball. When I started, it was possible to study molecular virulence attributes in fungi and look at the epidemiology of fungal isolates from patients as part of a single day's work.

The clinical importance of fungal diseases increased enormously in the last half of the 20th century, thanks mainly to the never-ending growth in medical and surgical technologies – including all forms of transplantation and cancer chemotherapy – involving

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 PII: S0966-842X(00)01752-2

immunosuppression as part of the procedure. Just as physicians were becoming accustomed to the idea that a patient deprived of circulating neutrophils was a major target for a fatal, opportunist invasion by any one of a number of fungi common in the human environment, the AIDS epidemic struck with exquisite timing and showed that specific elimination of $CD4^+$ lymphocyte function opened the door of host susceptibility even wider, to admit a new gamut of fungal infections.

The switch of emphasis in medical mycology from morbidity to mortality has been accompanied by a dramatic increase in fundamental research on pathogenic fungi, particularly on *Candida* species (the third or fourth most common microorganisms isolated from blood cultures in the USA, depending which survey you read), *Aspergillus fumigatus* (the scourge of bone marrow transplantation and a significant source of fatality in all types of haematological malignancy) and *Cryptococcus neoformans* (a major cause of AIDS-associated meningitis). As funding sources for the study of *Saccharomyces cerevisiae* have diminished with falling financial security for bakers, brewers, vintners and distillers, many big names from the so-called 'yeast' world have brought their talents to bear on the other yeast species that garner support from the medical side. The result has been a massive unravelling of fundamental biological processes in the main fungal pathogens. And it is here that investigators have discovered the real beauty of these organisms. It so happens that the study of virulence attributes in the two principal pathogenic yeasts has thrown light on diverse aspects of eukaryotic physiology that extend beyond their role in human infections.

Candida albicans is a maverick for biologists. It is equipped with genes homologous to those responsible for mating behaviour in other yeasts and moulds, yet it remains permanently saddled with a diploid genome, apparently incapable of mating. Survival without sex suggests that *C. albicans* must have developed other tricks to develop genetic diversity. *C. albicans* also seems to be unaware of the requirements of textbooks listing genetic codes, as it reads CUG as serine instead of leucine. This phenomenon could be the result of selective advantages conferred on the organism by codon ambiguity and has implications for evolutionary processes in general[1].

C. albicans is almost universally described as 'dimorphic', yet its cell morphology actually lies on a pleomorphic continuum from ovoid yeast cells to filamentous hyphae, rather than in the distinct saprophytic and parasitic morphological phases that are seen in the 'true' dimorphic pathogens (e.g. *Histoplasma capsulatum, Blastomyces dermatitidis* and *Coccidioides immitis*). Regulation of morphological differentiation in *C. albicans* has long been regarded as a possible paradigm for more general developmental processes in eukaryotes.

C. neoformans, by contrast, is almost never referred to as 'dimorphic', yet it undergoes a complete morphological change from encapsulated yeast forms to basidiomycete hyphae as a result of mating processes! Its putative virulence attributes to date include melanin production and the composition of its capsular polysaccharides. Moreover, virulence appears to be regulated by signal transduction pathways and by expression of genes at the *MAT* locus. These findings add novel functions to fundamental control systems in lower eukaryotes.

A theme linking *C. albicans* and *C. neoformans* that goes far beyond virulence attributes is that of rapid phenotypic switching – the ability of cell subpopulations to alter their gene expression, most probably by DNA rearrangements. This mechanism of regulation occurs at frequencies between those of ordinary mass responses to environmental change and those of random mutation[2]. 'Switching' could represent a mechanism of accelerated 'micro-evolution' in which 'survival of the fittest' means survival of the sub-strain type that can switch rapidly to facilitate adaptation to a new microniche. The study of switching mechanisms in *Candida* and *Cryptococcus* species is far advanced and could be poised to generate real breakthroughs in our general understanding of cell behaviour[2,3].

What we still think of as 'medical mycology' research has now become a significant player in the bigger worlds of general biology and microbiology, a wonderful and well-earned elevation of status for the former Cinderella. However, the perceptible shift of emphasis to fundamental research on fungal pathogens of humans has not been accompanied by many improvements on the applied side. Successful and dependable diagnostic methods for life-threatening mycoses have advanced little since the days earlier in my career when I worked on what then seemed to be very promising serological approaches, now regarded as relatively worthless. The armoury of clinically useful anti-fungal agents has expanded considerably, yet approved registrations of novel drugs over the past 25 years have been only for new molecular variations on already known chemical class themes.

As a society, we need to be forever vigilant in maintaining the balance between research that advances our knowledge and research that offers practical benefits in the short- and medium-term. Those who study human pathogens in fact have an exemplary history of sustaining interest on both sides. The laboratory scientists and the clinicians in medical mycology still know each other personally and sit together through seminars and symposia – I hope it stays that way.

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