CYTOPATHIC EFFECTS OF PATHOGENIC AND NON-PATHOGENIC SPECIES OF *CANDIDA* ON CULTURED MOUSE EPITHELIAL CELLS: RELATION TO THE GROWTH RATE AND MORPHOLOGY OF THE FUNGI

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IN most diphasic fungi there is a morphological distinction between the parasitic and saprophytic modes of growth; the organisms are pathogenic in the tissue yeast (Y) phase, and saprophytic in the mycelial (M) phase. Workers on candidosis have extrapolated from this observation and speculated that pathogenicity in *Candida* spp. is likely to be related to the phase of growth. As both phases are seen in lesions, each has been stigmatised by different workers as the pathogenic form.

The yeast phase is seen on microscopy of direct films made from healthy body surfaces infected with *Candida*. Kozinn and Taschdjian (1962), studying gastro-intestinal candidosis, equated the appearance of mycelium in smears of faeces with the signs and symptoms of illness, and Rogers (1957) made similar observations. Mankowski (1963) concluded from experimental studies that the blastosporic phase was the more pathogenic, and Dr Sujata G. Dastidar (1967, personal communication) noted that often only yeasts are seen in direct films made from the lesions of superficial thrush. Both Haley (1965-66) and Rogers (1966) drew attention to the danger of too readily accepting that the appearance of mycelium in clinical specimens denoted a pathological condition, and pointed out that the nature of the specimen itself might promote filamentation.

In previous experiments, Stanley and Hurley (1967) showed that cell culture systems were suitable models for studies of the pathogenic mechanisms of *Candida*. It is the purpose of this paper to compare the cytopathic effects of pathogenic and non-pathogenic species of *Candida* on cultured mouse epithelial cells, and to show the relationship between the degree of lethality of the fungi, their growth rates and the morphological phase of growth (see also Stanley, 1968).

MATERIALS AND METHODS

Organisms

The cultures studied belonged to two groups of *Candida* species: the pathogenic group, which comprised 7 species that have been isolated from human sources, and the non-pathogenic group of 6 species isolated originally from plants, from animals other than man,

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or from inanimate sources. In the pathogenic group were: *Candida albicans* (1 culture), *C. stellatoidea* (1), *C. krusei* (1), *C. tropicalis* (1), *C. pseudotropicalis* (1), *C. parapsilosis* (*C. parakrusei*) (2) and *C. guilliermondii* (2); and in the non-pathogenic group were one culture each of *C. diddensii*, *C. blankii*, *C. ingens*, *C. cacaoui*, *C. kefyri* and *C. shehatae*. Although 5 of the cultures in the second group were not assigned specific status by Lodder and Kreger-Van Rij (1967), they are kept as stock cultures by Miss Helen R. Buckley, by whom they were kindly supplied. A culture of *Saccharomyces cerevisiae* isolated from a vaginal swab was used as a control.

Standard suspensions of fungi (30,000 blastospores per ml) were prepared, as described by Stanley and Hurley, in tissue culture growth medium (TCGM), which consisted of "Wellcome" tissue culture medium 199 with the addition of 2 per cent. "Wellcome" calf serum no. 1; they were used to infect tissue cultures and for experiments on growth rates. In tests for pathogenicity, emulsions in saline (0.85 per cent. NaCl) were prepared from the overnight growth on glucose peptone agar, and were standardised by counting in a haemocytometer.

Tissue culture

The methods used for growing mouse renal epithelial cells, for infecting the tissue cultures and for determining the rates of killing of the cultured cells have also been described (Stanley and Hurley). The growth medium in a 5-day-old tissue culture was replaced by the standard suspension of fungus (30,000 blastospores per ml), and the preparation was incubated at 37°C or at 30°C. Cultures were examined microscopically at frequent intervals by transmitted light or by phase contrast, and some were stained intravitaly. At predetermined intervals, cultures were fixed, stained and examined histologically.

Growth rates of candida cultures

As the production of mycelium and pseudomycelium by *Candida* spp. in TCGM made optical density measurements and enumeration by pour plate techniques unsuitable, rates of growth were determined by measurements of dry weight. The yeasts (10 ml of a suspension in TCGM containing 30,000 blastospores per ml) were placed in ½ oz. siliconed and tightly stoppered bottles, and incubated as stationary cultures at 37°C. This simulated as closely as possible the conditions of growth in infected cultures, except that living cells were absent. At 0, 2, 4, 8, 16, 24, 48 and 72 hr, a drop of 4 per cent. formaldehyde in saline was added to each of two duplicated cultures, and the cultures were stored at 4°C when necessary. The growth was washed, resuspended in distilled water, and transferred to aluminium pans previously dried to constant weight. The samples were dried to constant weight *in vacuo* over CaCl₂. All weights were determined on a Mark 1 Microforce balance (C.I. Electronics Ltd).

Antigenic structure of the fungi

The antigenic structure of the species of *Candida* was studied by Miss Helen R. Buckley by methods described by Murray and Buckley (1966).

RESULTS

The distinction noted by Lodder (1934) between "mycelium" and "pseudomycelium" is used in this paper.

Killing of cultured cells, and relation to the morphology of the fungus

The pathogenic group. Observations on the rates of killing of cultured cells by members of the 7 species in the pathogenic group have been published

(Stanley and Hurley), and will be summarised briefly. The following stages in the process of degeneration of the cells were recognised.

- Stage 1. *Minimal degeneration.* Mitotic figures present.
- Stage 2. *Early degeneration.* Mitotic figures present.
- 2a. Less than 5 per cent. degeneration; occasional rounded, swollen cells; occasional pyknosis.
 - 2b. 5–25 per cent. degeneration; focal aggregates of cells at various stages of degeneration and pyknotic, shrunken, clumped cells; no obvious change in remainder of monolayer.
- Stage 3. *Subtotal degeneration.* Mitotic figures absent.
- 3a. Focal destruction of 25–50 per cent. of the monolayer; aggregates of degenerate and pyknotic, shrunken cells; remainder of monolayer intact and viable, with normal appearance.
 - 3b. Focal destruction of more than 50 per cent. of the monolayer; patches consisting of viable cells with normal appearance remain.
 - 3c. Generalised degeneration; about 50 per cent. of the cells are at various stages of degeneration with slightly or strongly pyknotic nuclei; karyorrhexis frequent. Degenerate cells interspersed with viable cells and no focal degeneration. Cytoplasm of many cells granular or patchily staining, and cell membrane often conspicuous.
- Stage 4. *Total degeneration.* Large clumps of strongly pyknotic, shrunken cells with eosinophilic cytoplasm. Much of the monolayer has retracted from the glass. Abundant cellular debris.

The rate of destruction of the tissue cells varied with the species of *Candida*, and the species were separated into groups 1, 2 and 3 according to the time of appearance of the cytopathic effects (Stanley and Hurley). Those species regarded from other evidence as the more pathogenic destroyed the cultures most rapidly. There was also a relation between the time of onset and of completion of degeneration and the morphological form of the fungi (table I).

C. albicans, *C. stellatoidea* and *C. tropicalis* all produced "germ tubes" and formed relatively more mycelium and less pseudomycelium than did *C. krusei*, *C. pseudotropicalis* and *C. parapsilosis*. *C. guilliermondii* produced only scanty pseudomycelium and *S. cerevisiae* produced blastospores only. The fungi producing most mycelium (group 1: *C. albicans*, *C. tropicalis*, *C. stellatoidea*) induced degenerative changes rapidly, and totally destroyed the cultures in 24 hr. Those producing a lesser amount of mycelium (group 2: *C. krusei*, *C. pseudotropicalis*, *C. parapsilosis*) totally destroyed the tissue cells in 48–72 hr, and *C. guilliermondii*, which produced occasional mycelium in tissue culture but not in the control medium, destroyed the cell cultures slowly and less extensively, though still more completely than the control yeast, *S. cerevisiae*, which produced blastospores only. All 7 species produce abundant blastospores.

The non-pathogenic group. Tissue cultures were infected with members of the 6 non-pathogenic species and were incubated in parallel at 37°C and at 30°C. Two temperatures of incubation were used because some of these fungi are near their maximum growth temperatures at 37°C. Epithelial cell cultures can be maintained at 30°C, and mitotic figures are seen. The effect of temperature on the relative preponderance of the morphological forms of the fungi could also be studied.

TABLE I
Effects of pathogenic* and non-pathogenic species of *Candida* on cultures of mouse renal epithelial cells

Group	Species of fungus ‡	Average time (hr) of onset of degeneration of stage †						Production of			Time of onset of acidity in growth medium
		1	2		3	4	blastospores	pseudomycelium	mycelium		
			a	b						a	
1	<i>C. albicans</i>	4	7	...	...	16	24	++++	++++	++++	16
	<i>C. tropicalis</i>	4	7	...	...	16	24	++++	++++	++++	16
	<i>C. stellatoidea</i>	4	7	...	...	16	24	++++	++++	++++	16
2	<i>C. krusei</i>	4	7-16	...	...	24	48	++++	++++	++	16
	<i>C. pseudotropicalis</i>	7	16	...	...	24-48	48-72	++++	++++	++	24
	<i>C. parapsilosis</i>	7	16	24	...	...	96	++++	++++	++	24
3	<i>C. cacaoli</i>	16	24	48	...	...	...	++++	++	—	16
	<i>C. diddensii</i>	16	24	48	72	96	...	++++	++	—	24
	<i>C. shehatae</i>	16	24	48	72	96	...	++++	++	—	24
	<i>C. cacaoli</i>	16	24	48-72	96	...	...	++++	++	—	24
	<i>C. blankii</i>	16	24	72	96	...	...	++++	++	—	48
	<i>C. guilliermondii</i>	24	48	72§§	...	...	...	++++	++	—	24
	<i>C. diddensii</i>	24	48	72§§	96	...	...	++++	++	—	24
	<i>C. kefyr</i>	24	48	72	96	...	...	++++	++	—	24
	<i>C. blankii</i>	24	48	72	96	...	...	++++	++	—	24
											48
4	<i>S. cerevisiae</i>	48	72	96	...	...	...	+	+	—	24
	<i>C. shehatae</i>	>96	...	...	...	...	...	+	+	—	72
	<i>C. ingens</i>	>96	...	...	...	...	...	+	+	—	24
	<i>C. ingens</i>	>96	...	...	...	...	...	+	+	—	24

* Results for pathogenic species are taken from Stanley and Hurley (1967).

† For explanation see text.

‡ Inoculum 3×10^4 blastospores per ml.

§ Mitotic figures present.

|| Mycelium not always produced.

Observations on these cultures are summarised in table I, in which the fungi are listed in descending order according to the rate at which they destroyed the cell cultures. Members of the non-pathogenic group did not have such a rapid action as did the 6 pathogenic species in groups 1 and 2. None of them destroyed the tissue cultures completely within the period of the experiment, and their cytopathic effects were of a different type from those observed in cultures infected with the pathogenic fungi. With fungi in groups 1 and 2, the cytopathic effects developed diffusely throughout the whole monolayer, which remained largely intact until pyknosis and degeneration were well advanced, but the mitotic figures disappeared early. A different effect was observed in cultures infected with most of the non-pathogenic candidas and with *C. guilliermondii*. The necrotic effects were localised, so that small areas of the monolayer became pyknotic, then clumped and disintegrated. These small areas of focal degeneration were surrounded by sheets of healthy, viable cells of normal appearance in which mitotic figures were still observed until destruction of the monolayer was well advanced. Later, the areas of focal degeneration increased in number and size, but even when 75 per cent. or more of the monolayer was totally destroyed patches of healthy, viable cells remained. Fungi that produced these changes were placed in group 3.

From table I it can be seen that members of group 3, which includes most of the non-pathogenic species, had a similar action on epithelial cell cultures, and that *C. guilliermondii* falls within this group. *C. parapsilosis*, although placed in group 2, produced cytopathic effects intermediate in type between those of groups 2 and 3. *C. cacaoi* at 30°C and at 37°C, *C. shehatae* and *C. diddensii* at 30°C and *C. blankii* at 37°C caused slightly more rapid destruction of the cell cultures than did *C. guilliermondii*. *C. diddensii* and *C. kefyri*, at 37°C and *C. blankii* at 30°C behaved like *C. guilliermondii*. *C. guilliermondii* and *C. diddensii* at 37°C, however, differed from the other members of group 3 in that the disappearance of mitotic figures occurred when only a small proportion of the monolayer had been destroyed. *C. shehatae* at 37°C and *C. ingens* at both temperatures of incubation had no effect on the cell cultures during the period of observation; along with *S. cerevisiae*, which produced relatively slight degeneration on prolonged incubation, they were placed in group 4.

All species in the non-pathogenic group, except *C. diddensii* at 37°C, produced some pseudomycelial filaments, but growth was predominantly blastosporic. Occasional fine freely branched mycelium was seen only with *C. blankii* and *C. kefyri*. After 24 hours' incubation the monolayers were still healthy apart from some early degeneration with *C. cacaoi* at 30°C and at 37°C, *C. diddensii* and *C. shehatae* at 30°C and *C. blankii* at 37°C. As with the pathogenic species there was ingestion of blastospores by the occasional, scattered macrophages present in the cultures, although this was rare with *C. ingens*, possibly because of its large size $(15-28) \times (5-10) \mu\text{m}$. Intracellular multiplication of fungi within the macrophages occurred with all species but particularly with *C. cacaoi*, *C. blankii*, *C. kefyri* and *C. diddensii*. Filaments grew out of the infected macrophages, and this was more apparent as the

incubation time was prolonged. Although *C. shehatae* produced filaments, the ingested blastospores were frequently lysed within cells at 37°C, and fairly numerous macrophages containing yeast-shaped vacuoles, or very weakly staining yeasts, were seen. With all species, intracellular yeasts were also seen in the epithelial cells; they were surrounded by a non-staining halo, and their position within the monolayer was not constantly related to the presence of

FIGURE.—Growth curves of *Candida* cultures in medium 199 containing 20 per cent. calf serum at 37°C, plotted from the average dry weight of 4 samples.
In all cases the initial inoculum concentration was 3×10^4 blastospores per ml.

mycelium or pseudomycelium. Invasion of the epithelial cells by filaments occurred, but was infrequent. *C. cacaoi* and *C. blankii* showed essentially similar morphology at 30°C and at 37°C, but the rate of killing was faster at 37°C, which was nearer to their maximum temperature for growth. *C. shehatae*, on the other hand, killed cells faster at 30°C, as did *C. diddensii*, which produced filaments at this temperature. *C. ingens* and *C. shehatae* at 37°C (group 4) had little effect on the tissue cells, and we believe that this was due to poor growth at this temperature and to the ingestion and lysis of blastospores by macrophages present in the culture, with consequent reduction of the effective inoculum.

Tests for pathogenicity

Since some of the fungi of the non-pathogenic group closely resembled *C. guilliermondii* and, to a lesser extent, *C. parapsilosis* in their qualitative behaviour towards cultured cells, two of them, *C. diddensii* and *C. kefyri*, were selected for further study. Each was injected intravenously into 6 rabbits in doses of 200 million organisms. Blood cultures at monthly intervals were negative and the animals showed no signs of illness. Yeasts were not cultured from random urine samples. The animals were killed after 6 mth, and the tissues were cultured and examined histologically *post mortem*. No fungi were grown, and there was no morbid anatomical evidence of fungal disease. Further tests are in progress.

Growth rates in the pathogenic Candida species and in S. cerevisiae

The growth rates of one culture each of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii* and *S. cerevisiae* were measured in duplicate on two occasions. Similar results were obtained in the two experiments. The mean constant dry weights of 4 replicate cultures in the two experiments are plotted against the incubation time in hours to give growth curves (figure).

The weight of the initial inoculum was closely similar for all the cultures. *C. albicans* and *C. tropicalis* (group 1) showed the greatest and most rapid weight gain. With *C. albicans*, the period of most rapid increase was between the 4th and the 8th hr, but the weight of growth continued to rise steadily throughout the remainder of the experiment. With *C. tropicalis*, the onset of rapid weight gain occurred about 4 hr later than with *C. albicans* and the period of maximum increase in weight occurred between the 8th and the 16th hr. The maximum weight was reached at 48 hr and then the fungal weight began to decrease. *C. krusei* and *C. parapsilosis* (group 2) showed an intermediate weight gain, but in *C. krusei* the rate of weight increase was faster than in *C. parapsilosis* after the first 24 hr. With both species the amount of growth produced at 24 hr was similar, but was less than with *C. albicans* or *C. tropicalis*. Between the 24th and the 48th hr *C. krusei* showed a weight increase of about 30 per cent. to its maximum weight, whereas *C. parapsilosis* showed a smaller increase (c. 18 per cent.) between the 24th and the 72nd hr. With *C. guilliermondii* (group 3) there was a long lag period of about 8 hr. The weight then increased to a maximum at 24 hr; it remained stationary for 24 hr, and then began to fall off. Despite the apparently poor growth of this species, microscopic examination of infected epithelial cell cultures showed heavy budding of the fungus.

The behaviour of *S. cerevisiae*, the control yeast, was similar to that of *C. guilliermondii*, although a greater maximum weight was achieved and there was a more rapid onset of weight increase. The maximum weight gain was of the order of four-fold for *C. albicans* and *C. tropicalis*, three-fold for *C. krusei* and two-fold for *C. parapsilosis*, *C. guilliermondii* and *S. cerevisiae*.

DISCUSSION

The fungi with the most rapid and extensive lethal effects on the cultures, that is, the 3 organisms of group 1, *C. albicans*, *C. tropicalis* and *C. stellatoidea*, produce "germ-tubes" and abundant mycelium; formation of pseudomycelium is not so heavy as in some other species. The fungi of group 2, *C. krusei*, *C. pseudotropicalis* and *C. parapsilosis*, produce an intermediate quantity of mycelium and abundant pseudomycelium, and are more slowly lethal. Group-3 fungi destroy cultured cells even more slowly, and include *C. cacaoi*, *C. diddensii*, *C. blankii*, *C. guilliermondii*, *C. kefyri* and *C. shehatae* (the last at 30°C only); of these, only *C. blankii* and *C. kefyri* produce any mycelium, whilst *C. guilliermondii* produces mycelium occasionally in the presence of mammalian cells. The remaining fungi (group 4), *C. shehatae* (at 37°C) and *C. ingens* (at 30°C and 37°C) have no discernible effect on the cultured cells and do not produce mycelium. *C. diddensii*, the only species in which it proved possible to suppress filament formation completely (at 37°C), is more destructive to the cultures in the filamentous phase (at 30°C).

It thus seems that there is an association between the production of mycelium and the cytopathic effect of the fungi on the cultured cells. To test this relation further, many attempts were made to suppress the M phase of growth in the cell cultures of fungi of the pathogenic species, and thus to study the effects of pure Y and pure M forms. The methods used included sulphur-free culture media, the addition of sugars, insulin, cysteine, glutathione or bile salts, and reduction in the amount and change in the type of serum added. Although it was possible to suppress the M form in control culture media, incubation with mammalian cells in modified media that were not toxic stimulated the M phase of growth. This suggests that cells themselves affect the phase of growth. Filamentation of *Candida* species, particularly *C. albicans*, in the presence of serum, is, of course, well known, and is the basis of the "germ tube" test for rapid identification of *C. albicans* (Taschdjian, Burchall and Kozinn, 1960). By means of organ cultures, Koch (1965) was able to induce filament formation in old stock cultures of *Candida* spp. including *C. albicans*, and Mackenzie (1965-66) showed that aqueous extracts of organs, particularly the brain and the kidney, stimulated filament formation in *C. albicans*. We have found that *C. guilliermondii* produces mycelium in cell cultures but not in the growth medium alone, suggesting that the effect was not serum dependent. Extracts of mouse kidney in medium 199, with and without added serum, did not induce the formation of mycelium in 72 hr, and this suggests that the presence of intact cells was the operative factor in our experiments. It thus seems that mammalian tissues or their aqueous extracts actively promote growth of some of these fungi in the filamentous (M) phase.

Experiments with pathogenic bacteria (Holland and Pickett, 1956; Shepard, 1957; Furness, 1958) have shown a correlation between the growth rate of the bacteria in cell cultures and their pathogenicity. The experiments described in this paper demonstrate that the speed and extent to which cultures of mouse

renal cells are destroyed are related to the increase in dry weight, which was taken as an index of the rate of growth of the fungi. The speed and extent of destruction of the cell cultures is also correlated with the amount of mycelium produced in the infected cultures (Stanley and Hurley, 1967).

Cantrell and Widra (1964) concluded that unrestricted growth of *C. albicans* *in vivo* is associated with the M phase, and our experiments, conducted *in vitro* with a model resembling conditions *in vivo*, confirm this view. The cytopathic effects of the candidas are a function of their growth rate and the mycelial phase predominates in the more rapidly growing species. Species such as *C. albicans* and *C. tropicalis* that show the earliest and most rapid weight increase also produce the most abundant mycelial growth and destroy the cell cultures considerably faster than a species such as *C. guilliermondii*, which grows slowly and rarely produces mycelial filaments.

Though this suggests that pathogenicity in *Candida* is closely related to the rate of mycelial proliferation, the cytopathic effect is not solely a function of the weight of infection. Although the more rapidly lethal species all attain a dry-weight increase of approximately 150 per cent. over the period required for total destruction of the cell cultures (see table II), there is no close correlation between the actual mass attained by the different fungi and the extent of their effect on the cell cultures, e.g., *C. parapsilosis* and *S. cerevisiae* at 24 hr and at 48 hr have a closely similar dry weight, but *C. parapsilosis* at this time has already caused considerable destruction of the cell cultures and *S. cerevisiae* has had no discernible effect. Similarly, *S. cerevisiae* produces an earlier and greater dry-weight gain than does *C. guilliermondii* although the latter species causes more rapid cytopathic effects. However, for individual fungal species, the time of onset and extent of the cytopathic effects are dependent upon the mass of fungal inoculum (Stanley and Hurley).

The period of maximum increase in dry weight is not correlated with the period of maximum rate of destruction of the cultured epithelial cells, although it corresponds fairly closely to the period of onset of cytopathic effects (stages 1 and 2) in all the *Candida* species but not in *S. cerevisiae* (table II). This period is not associated with any particular phase of growth.

It was thought that autolysing the cells of the fungi might release substances toxic to the cultured mammalian cells, but a comparison of the figure with table II shows that there was no correlation between the extent of degeneration in the tissue cultures and a falling off in the dry weight of the fungi. *C. albicans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* were still growing well when the cell cultures were completely destroyed. *C. guilliermondii* and *S. cerevisiae*, however, were apparently dying off as the cytopathic effects were becoming more marked. Five-day cell-free culture filtrates of these and other *Candida* species possessed no toxic activity for mouse renal cell cultures (Stanley and Hurley).

These findings suggest that the cytopathic effects depend upon specific aggressive mechanisms that are possessed to a high degree by species such as *C. albicans* and *C. tropicalis* and to a low degree by species such as *C. guilliermondii* and other group 3 species.

TABLE II
*Association, in six species of yeast, of increase in dry weight * and cytopathic effects on cultured mouse epithelial cells*

Species	Period of maximum increase in dry weight (hr)	Time of onset of stage-2 degeneration of culture (hr) †	Period of maximum killing of epithelial cells (hr) †	Maximum weight attained (mg)	Time maximum weight attained (hr)	Time of total destruction of cell cultures (hr †)	Per cent. dry weight increase required for destruction of cultures
<i>C. albicans</i> . .	4-8	4-8	16-24	> 9	> 72	24	150
<i>C. tropicalis</i> . .	8-16	4-8	16-24	9	48	24	180
<i>C. krusei</i> . .	0-8	4-16	24-48	6.9	48	48	180
<i>C. parapsilosis</i> . .	8-16	8-24	24-48	> 5.2	> 72	96	140 §
<i>C. guilliermondii</i> .	8-24	24-72	- ‡	3.8	24	> 168	50
<i>S. cerevisiae</i> . .	0-8	48-96	- ‡	4.5	24	> 168	80

* In cultures in medium 199 with 20 per cent. added calf serum; the initial inoculum was 3×10^4 blastospores per ml.

† From Stanley and Hurley (1967).

‡ Not attained within experimental period.

§ Measured by extrapolation from the figure.

|| In these cases the maximum weight was measured.

Weyman-Rzucidło (1965) suggested that virulence might be associated with the possession of specific antigens. Isenberg, Allerhand and Berkman (1963) noted immunological differences between mouse virulent and mouse non-virulent *C. albicans* and isolated toxic antigens from the virulent strains. There is no ready correlation between the antigenic structure of our cultures, as studied by Buckley, and their effects on cultured cells, but some of the more destructive of the non-pathogenic species are closely related to the pathogenic species; for example, *S. kefyri* Beijerinck (1889) is classified by Lodder and Kreger-Van Rij (1967) as *C. pseudotropicalis* var. *lactosa*. Our strain of *C. kefyri* is a subculture of the Beijerinck isolate.

The correlation of severe cytopathic effects with rapid growth in the mycelial phase may demonstrate the importance of the M phase in the genesis of infections with *Candida* spp., but cannot be taken as evidence that the M phase possesses aggressive mechanisms that are lacking in the Y phase. The present experiments and others in progress indicate that living cells are parasitised by candidas in two ways; the cells may engulf the yeast form, or they may be invaded by the filamentous form. Death of the cells is the result in either event, but the speed and extent of destruction of cultures is proportional to the amount of fungus produced. The ingested blastospores of most candidas develop intracellular filaments that eventually rupture the cell, but cells heavily parasitised by the blastospores of *C. diddensii* also die, despite the lack of filament formation. In non-pathogenic species such as *C. ingens* and *C. shehatae*, ingested blastospores are frequently lysed. Our experiments do not, therefore, demonstrate a qualitative difference between the cytopathic effects of M or Y, but they do demonstrate a relation between cytopathogenicity and the rate of growth. Rapid growth seems to be associated with the M phase, and with mycelium-producing rather than with pseudomycelium-producing species.

The presence of intact mammalian cells encourages filamentation, and this suggests that the presence of mycelium in direct smears (Rogers, 1957, 1966; Kozinn and Taschdjian, 1962) may denote that the fungi have been exposed to cellular exudates or transudates. It is possible that the sequence of events *in vivo* parallels that of the experimental system: the Y phase may initiate infection, thus accounting for the observation of pure Y-phase candidas in some films from candida lesions (Dr Sujata G. Dastidar, 1967, personal communication); and cellular damage may encourage filamentation, the presence of filaments being associated with a more exudative and less superficial type of lesion.

SUMMARY

The cytopathic effects of standard inocula (30,000 blastospores per ml) of *Candida albicans*, *C. tropicalis*, *C. stellatoidea*, *C. krusei*, *C. pseudotropicalis*, *C. parapsilosis* and *C. guilliermondii* (isolated from man) on murine renal epithelial cells in culture were compared with those of *C. cacaoui*, *C. diddensii*, *C. kefyri*, *C. blankii*, *C. ingens* and *C. shehatae* (isolated from non-human sources). The nature of the changes in the mammalian cells, and the extent

of their destruction, were shown to be correlated with the growth rate and with the proportion of mycelium in the growing fungi. The presence of mammalian cells induced filamentous change in some species.

These experiments demonstrate the greater importance of the M phase in the progression of candida lesions, because of the more widespread involvement of cells by organisms with rapid growth rates, but they do not demonstrate a qualitative difference in the cytopathic effects of the M and Y phases. They suggest that *in vivo* the Y phase may initiate infection, as it does experimentally, and that the M phase is associated with the more rapidly growing species and with extension of the lesion. The filamentous phase may be induced by exposure of the fungi to cellular exudates or transudates.

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