THE DEVELOPMENT OF RESISTANCE BY CANDIDA SPECIES TO POLYENE ANTIBIOTICS IN VITRO

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PLATES XXVII AND XXVIII

Two polyene antibiotics, nystatin and amphotericin B, are the most successful substances currently available for the treatment of candida infections. They are also used in combination with antibacterial antibiotics to prevent the overgrowth of candida in patients treated primarily for bacterial infections. Experience with bacteria has suggested that the exposure of Candida species to sublethal concentrations of polyenes might lead to the development of resistant strains. This has led to criticisms of the use of combined antibiotics (Garrod and O'Grady, 1968).

Our study of the literature has failed to discover any convincing account of isolations of naturally occurring candida resistant to polyenes. On the other hand, several groups of workers have succeeded in inducing a moderate degree of resistance in vitro (Ito et al., 1955; Stout and Pagano, 1956; Littman, Pisano and Lancaster, 1958; Lones and Peacock, 1959; Sorensen, McNall and Sternberg, 1959; Bradley and Farber, 1960; Francois and De Vos, 1962; Hebeka and Solotorovsky, 1962, 1965; Patel and Johnston, 1968). The cultures made resistant by Hebeka and Solotorovsky also showed a partial loss of virulence.

The present paper reports the results of sensitivity tests on 2015 clinical isolates of candida, none of which was found to be resistant to nystatin or amphotericin B, and the induction of resistance to one or more polyenes, and the associated changes, in isolates of C. albicans, C. tropicalis, C. pseudotropicalis, C. parapsilosis, C. krusei, C. stellatooides, and C. guilliermondii (see also Athar, 1969).

MATERIALS AND METHODS

The cultures used were isolated from clinical specimens received in the hospital laboratories and identified by standard methods (Lodder and Kreger-van Rij, 1952; Denny and Partridge, 1968). Unless otherwise specified, the following three culture media were used: peptone-dextrose agar (PDA); peptone-dextrose broth (PDB); and penassay base agar (PBA). The cultures were grown on PDA slopes for 24 hr at 37°C, stored at room temperature and subcultured every 3 mth. The following polyene antibiotics were used: nystatin, amphotericin B, candicidin, amphotericin A, hamycin, pimaricin, filipin and rimocidin. They were stored in powdered form at −21°C. Solutions were stored at −21°C and used over a period of 10 days during which no loss of potency was noted.

Determination of the minimum inhibitory concentration (MIC). An agar dilution technique was used. The antibiotics were dissolved in dimethylsulphoxide; dilutions made in sterile distilled water were mixed with melted penassay base agar containing 0.5 per cent. dextrose, and poured into sterile petri plates, which were dried at 40°C for 5–10 min. Yeast

Received 1 Dec. 1970; accepted 26 Jan. 1971.

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suspensions were prepared in sterile distilled water from 24-hr slope cultures grown at 37°C, and standardised to contain c. 10^7 cells per ml. Antibiotic plates were divided into eight equal areas on which the suspensions were streaked. Results were recorded after 24 hours’ incubation at 37°C. The lowest concentration of the antibiotic completely inhibiting growth was taken as the MIC.

Development of resistance. Suspensions of eight different candida isolates (10^7 cells per ml) were uniformly streaked on plates containing a series of antibiotic concentrations, the lowest being slightly less than the predetermined MIC. After 24 hours’ incubation at 37°C growth on plates containing more than the MIC of antibiotic was emulsified in sterile distilled water. These fresh suspensions were standardised and used to inoculate plates containing still higher antibiotic concentrations. The candida isolates were tested for purity at regular intervals.

The numbers of isolates used in resistance studies with nystatin were: C. albicans (32); C. tropicalis (32); C. pseudotropicalis (12); C. krusei (10); C. parapsilosis (11); C. stellatoidea (3); C. guilliermondii (3); in resistance studies with amphotericin: B: C. albicans (32); C. tropicalis (24); C. pseudotropicalis (7); C. krusei (10); C. parapsilosis (11); C. stellatoidea (3); C. guilliermondii (3); in resistance studies with candididin: C. albicans (6); C. tropicalis (6); in resistance studies with pimaricin: C. albicans (6), and in resistance studies with filipin: C. albicans (6).

Morphology. The sensitive parent and derived resistant isolates of each of the Candida species were grown on PDA plates. After incubation at 37°C for 24–48 hr, the shape and appearance of their colonies were compared, and the colony sizes measured. The ability to produce germ tubes was studied by inoculating C. albicans isolates into ox serum and incubating for 2–3 hr at 37°C. The development of pseudomycelium and chlamydospores was determined by streaking cultures into cornmeal agar plates, making deep uncovered streaks at an angle, and incubating at 26°C from 1 to 4 days.

Suspension stability. It was noted that resistant cultures settled to the bottom of tubes of liquid media more rapidly than normal. This was investigated by preparing suspensions in physiological saline of 24-hr cultures of resistant and sensitive isolates adjusted to contain approximately 2 × 10^7 cells per ml. Equal volumes of the standardised suspensions were left undisturbed at room temperature and their suspension stability was observed after 2 hr.

Growth rates. PDB cultures of sensitive and resistant isolates, standardised to 2–4 × 10^6 organisms per ml, were inoculated into PDB tubes and incubated at 37°C. Tubes were removed from the incubator after 2, 4, 6, 8 and 24 hr. The cells were washed, resuspended in 10 ml sterile distilled water, and filtered through membranes, which were weighed after drying overnight at 80°C.

Biochemical properties. Isolates were inoculated into 3 per cent. solutions of glucose, maltose, sucrose, lactose, raffinose, and galactose, and incubated at 37°C for several days. Sensitive and resistant isolates were also grown on bismuth-glucose-glycine (Bi GGY) medium to test their ability to reduce the bismuth complex (Nickerson, 1953).

Ergosterol content. The total ergosterol content of the sensitive parent and derived resistant isolates was estimated according to the method of Breivik and Ovades (1957).

Antigenicity. Antibody was obtained by immunising a young Dutch rabbit with a formalised suspension of C. albicans. Antigens were prepared by Mickle extraction and centrifugation. The double-diffusion technique of Ouchterlony was used for antigen–antibody reactions.

Pathogenicity studies. Using the technique of chorio-allantoic membrane (CAM) inoculation (Partridge, Athar and Winner, 1971), we examined resistant isolates of the following five species of candida for pathogenicity in the chick embryo: C. albicans, C. tropicalis, C. pseudotropicalis, C. parapsilosis and C. krusei. A 0·1 ml aqueous inoculum containing 10^5 cells was used for C. albicans and C. tropicalis. This inoculum was insufficient to produce lesions with C. krusei, C. pseudotropicalis and C. parapsilosis, for which it was increased ten-fold.

The fungus was inoculated into 10-day embryos; 0·1 ml of sterile distilled water was inoculated into control eggs.
The viability of the inoculated embryos was checked throughout the incubation period by candling. Surviving embryos were finally harvested 9 days after inoculation.

Cross-resistance. Nystatin- and amphotericin B-resistant isolates were tested for cross-resistance to other polyenes by the agar dilution technique.

Fluctuation and Newcombe tests. The fluctuation (Luria and Delbrück, 1943) and Newcombe (Newcombe, 1949) tests were performed on two C. albicans isolates that were originally sensitive to polyenes; one had developed high resistance to nystatin and the other to amphotericin B. Suspensions containing approximately $10^4$ cells were prepared in sterile distilled water from 18-hr (37°C) slope cultures. The final concentrations of the antibiotics in the plates were 15 units nystatin, and 0.4 µg amphotericin B per ml. The plates were incubated at 37°C and colonies were counted after 48 hr (Demerec, 1948). The technique of the Newcombe test was a slight modification of that described by Bornschein, Dittrich and Höhne (1951).

Stability of resistance. Seven isolates of polyene-resistant candida were grown on PDA plates at 37°C and subcultured on to fresh antibiotic-free plates every 24 hr. The MIC of the subcultured isolates to the relevant antibiotic was determined daily by the agar dilution technique for the first ten transfers and subsequently after 15, 30, 50 and 100 transfers. Sensitive and resistant isolates of different species were grown separately in peptone-dextrose broth for 18 hr, mixed in various combinations, incubated at 37°C for 24 hr, and then streaked on PBA containing various antibiotic dilutions.

To investigate the transfer of resistance from one isolate to another of the same species, one of the isolates was labelled with radioactive phosphorus (P³²) by the method of Kozinn et al. (1959).

RESULTS

Minimum inhibitory concentrations. The MIC of nystatin and amphotericin B found for different species of candida are shown in tables I and II respectively.

Development of resistance. The numbers of candida isolates that developed resistance in vitro to one or more of the polyenes are shown in table III. The patterns of the progressive increase in resistance are shown in figs. 1, 2 and 3 respectively.
### Table II

*MIC of amphotericin B for 1307 Candida isolates at 24 hr*

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of isolates tested</th>
<th>Number of isolates inhibited by amphotericin B at a concentration (mg per ml) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>900</td>
<td>136</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>248</td>
<td>0</td>
</tr>
<tr>
<td><em>C. pseudotropicalis</em></td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>51</td>
<td>5</td>
</tr>
<tr>
<td><em>C. stellatoidea</em></td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1307</strong></td>
<td><strong>150</strong></td>
</tr>
</tbody>
</table>

### Table III

*Number of Candida isolates developing resistance to various polyenes*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Organism</th>
<th>Number of isolates tested</th>
<th>Number showing resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nystatin</td>
<td><em>C. albicans</em></td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>C. tropicalis</em></td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>C. pseudotropicalis</em></td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>C. krusei</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>C. parapsilosis</em></td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><em>C. stellatoidea</em></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>C. guilliermondii</em></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td><em>C. albicans</em></td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>C. tropicalis</em></td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><em>C. pseudotropicalis</em></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>C. krusei</em></td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>C. parapsilosis</em></td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>C. stellatoidea</em></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>C. guilliermondii</em></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Candididin</td>
<td><em>C. albicans</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Pimaricin</td>
<td><em>C. albicans</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Filipin</td>
<td><em>C. albicans</em></td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
Fig. 4.—Size of colonies produced by *C. albicans* after 48 hr at 37°C. Top: amphotericin B-sensitive parent. Bottom: amphotericin B-resistant derived.

Fig. 5.—Settle-tubes of *C. albicans* (a) nystatin-resistant (b) nystatin-sensitive. Suspensions containing $2 \times 10^7$ cells per ml in normal physiological saline after 2 hr at room temperature.

Fig. 7.—Growth of candida isolates on bismuth-glucose-glycine (Bi GGY) medium (48 hr at 37°C). 1: *C. albicans* nystatin-sensitive. 2: resistant. 3: *C. tropicalis* amphotericin B-sensitive. 4: resistant. 5: *C. pseudotropicalis* nystatin-sensitive. 6: resistant. 7: *C. krusei* amphotericin B-sensitive. 8: resistant. 9: *C. parapsilosis* amphotericin B-sensitive. 10: resistant.
Fig. 9.—Appearance of chorio-allantoic membrane (CAM) after inoculation with nystatin-sensitive *C. albicans*. 9-day infections. ×0·5.

Fig. 10.—Appearance of CAM after inoculation with nystatin-resistant *C. albicans* derived from isolate infecting CAM shown in fig. 11. 9-day infection. ×0·5.

Fig. 11.—Section of CAM 9 days after infection with amphotericin B-sensitive *C. albicans*. Hyperplasia of ectoderm and thickening of mesoderm with cellular migration. Haematoxylin and eosin. ×120.

Fig. 12.—Section of CAM 9 days after infection with amphotericin B-resistant *C. albicans*. Much less reaction in ectoderm and mesoderm than is shown in fig. 11. Periodic acid-Schiff. ×120.
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Fig. 1.—Increase in resistance to nystatin on transfer in antibiotic-containing medium.

Fig. 2.—Increase in resistance to amphotericin B on transfer in antibiotic-containing medium.

Fig. 3.—Increase in resistance to candicidin, pimaricin, and filipin on transfer in antibiotic-containing medium.
Morphology. Colonies of polyene-resistant isolates were much smaller than those of polyene-sensitive isolates (fig. 4). No other differences were found in their shape or appearance. The production of germ tubes in resistant *C. albicans* isolates invariably took at least twice as long as in their sensitive parents. Resistant isolates produced pseudomycelia and chlamydomycetes less readily than their sensitive parents.

Suspension stability. Resistant isolates of *C. albicans* and *C. tropicalis* settled to the bottom of tubes more rapidly than sensitive isolates (fig. 5).

Growth rates. Figure 6 indicates that growth is slower in nystatin-resistant than in sensitive isolates. Amphotericin B-resistant isolates gave an almost identical graph, and similar differences were found in all the candida species that developed resistance.

![Graph showing growth rates](image)

Biochemical properties. Fermentation reactions of the resistant isolates were slower and did not become manifest until 48 or 72 hr later than those of the sensitive isolates. Otherwise the reactions showed no change.

The growth on Bi GGY medium of various isolates is shown in fig. 7. Resistant isolates gave a poor growth and failed to reduce the bismuth complex. In order to confirm that this decreased reduction of the inorganic sulphite was not merely a manifestation of slow growth, the inoculated Bi GGY plates were left in the incubator for 1 wk. At the end of this period, the resistant isolates still showed very little reduction.

Ergosterol content. The ultraviolet absorption spectra of extracts of typical amphotericin B-sensitive isolates of *C. albicans* and of their resistant derivatives are shown in fig. 8. Similar curves were given by *C. tropicalis*. The ergosterol contents of the resistant derived isolates were invariably considerably less than those of their sensitive parents.

Antigenicity studies. No antigenic differences were detected between the sensitive and resistant isolates of *C. albicans*.
Pathogenicity studies. Resistant isolates caused a less severe infection of chorio-allantoic membranes than sensitive isolates and the lesions produced were less pronounced (figs. 9–12). Embryos succumbed more rapidly and showed a lower survival rate when infected by polyene-sensitive than when infected by resistant isolates. Similar results were found with all Candida species (fig. 13).

To exclude the possibility that the reduced pathogenicity of resistant isolates was due to repeated subculture, two polyene-sensitive C. albicans isolates were subcultured 75 times. The resultant and the original cultures were simultaneously inoculated on to CAMs; they were found to be equally virulent.

![Graph 1](image1)

**Fig. 13.—Survival rates of chick embryos inoculated with polyene-sensitive and -resistant C. albicans.**

0.1 ml of a standardised suspension (4 × 10⁷ cells per ml) of C. albicans was injected intravenously into each of 10 Swiss mice weighing c. 25 g. Four types of C. albicans were used: nystatin-sensitive and -resistant; amphotericin B-sensitive and -resistant. 0.1 ml of sterile distilled water was inoculated into control mice. The animals were observed daily for a period of 30 days and deaths were recorded. Survival rates are given in table IV. These results indicate that resistant isolates were less pathogenic to mice than the parent sensitive isolates, and confirmed the findings on the CAMs.

Nature of resistance. Almost all the isolates made resistant either to nystatin or to amphotericin B showed an increased MIC for the other polyenes tested, as illustrated in table V. The results of the fluctuation and Newcombe tests* suggested that resistance was acquired by mutation rather than by

* Data illustrating the results of these tests may be obtained from H. I. W.
adaptation. Resistant isolates became sensitive again after growth on an antibiotic-free medium (table VI), with the reappearance of normal colony size, ergosterol content and ability to grow on Bi GGY medium, although the reduction of the bismuth complex remained poor. No significant change was observed in the diminished ability of the resistant isolates to produce pseudo-mycelia and germ tubes or in their loss of suspension stability.

**Table IV**

*Survival of mice inoculated with polyene-sensitive and -resistant C. albicans isolates*

<table>
<thead>
<tr>
<th>Number of days after inoculation</th>
<th>Number of mice surviving after inoculation of</th>
<th>nystatin-sensitive isolate</th>
<th>derived nystatin-resistant isolate (28-fold resistance)</th>
<th>amphotericin B-sensitive isolate</th>
<th>derived amphotericin B-resistant isolate (500-fold resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>30</td>
<td></td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

There was no change in the MIC for sensitive species after mixed cultivation with resistant species, nor in polyene-sensitive isolates of *C. albicans* on mixed culture with resistant isolates. Transformation experiments showed no change in the MIC of nystatin-sensitive *C. albicans* after growth in the presence of various concentrations of crude Mickle extracts of their resistant derivatives*.

* Illustrative data may be obtained from H. I. W.
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DISCUSSION

No natural resistance to nystatin or amphotericin B was found in over 2000 clinical isolates of Candida spp. All the evidence now available suggests that resistance to these antibiotics does not occur in strains found naturally in man.

In the present investigation much higher degrees of resistance to nystatin and amphotericin B than those previously reported (Donovick et al., 1955; Littman et al., 1958; Hebeka and Solotorovsky, 1965) were induced in vitro in a number of species. Of the seven species examined, only two, C. stellatoidea and C. guilliermondii, failed to show resistance to both antibiotics, and few isolates of these species were tested. The present work also reports the development of resistance to candicidin, pimaricin, and filipin. No such previous reports have been traced.

Isolates of C. albicans readily developed resistance to all five polynes, but at different rates for the different antibiotics. The development of resistance to one antibiotic was often, but not invariably, accompanied by resistance to others. This was previously observed by Stout and Pagano.

The reduced colony size observed in polyene-resistant isolates of all Candida species tested is presumably due to slowing of their growth rates. All species showed a decreased ability to produce pseudomycelia, and C. albicans showed lessened production of chlamydospores and germ tubes. The increased tendency of resistant isolates to settle to the bottom of tubes of saline indicates alterations in their surface properties.

Isolates of resistant candida species reduced the bismuth complex in Bi GGY medium more slowly than their parent sensitive isolates. Since reduction is
due to utilisation of the reducing substances formed by the cells during glucose metabolism (Nickerson, 1953), decreased reduction probably indicates a decrease in the rate of glucose metabolism.

**Ergosterol.** It is known that the inhibition of fungal growth by polyenes can be prevented by the addition of sterols to the medium. Gottlieb *et al.* (1958, 1959) reported that the presence in the medium of cholesterol, ergosterol, stigmasterol and a number of other sterols can antagonise the inhibition of fungal growth by polyenes. This was confirmed by Perritt, Phillips and Robinson (1960), Ramachandran (1961), Ghosh and Ghosh (1963), and Kinsky (1964). Zygmunt and Tavormina (1966) tested a number of sterol compounds for antagonism to polyenes. They found that ergosterol was much the most effective and was the only one to show marked antagonism to nystatin and to four other polyenes, including amphotericin B, filipin, and pimaricin.

*Mycoplasma laidlawii* lacks sterols and is normally resistant to polyenes. However, Feingold (1965) found that amphotericin B inhibited its growth and brought about lysis after it had been grown in the presence of cholesterol and had therefore presumably incorporated some into the cell. The organism could again be made resistant by continuing its growth in a cholesterol-free medium. Loss of amphotericin-B sensitivity was found to be accompanied by the disappearance of some of the cholesterol from the cell. Weber and Kinsky (1965) also observed a similar reversible effect of filipin on *M. laidlawii*. Several other organisms known to contain sterols are sensitive to polyene antibiotics, such as snails (Seneca and Bergendahl, 1955) and higher algae (Hunter and McVeigh, 1961; Lampen and Arnow, 1961).

Andreoli, Dennis and Weigl (1969) have shown that in artificial bimolecular membranes the interaction of amphotericin B with membrane-bound cholesterol results in the formation of aqueous channels or pores. These do not develop in the absence of either amphotericin B or cholesterol. Some similar phenomenon at the yeast cell surface may account for the sensitivity of yeasts to polyenes. This provides a possible reason why our resistant isolates with reduced ergosterol content show a reduced polyene sensitivity. These observations have wide implications in cytology, and require further study.

**Pathogenicity studies.** The development of resistance in micro-organisms is often found to be associated with a decrease, and sometimes a complete loss, of their virulence (Manten, 1963; Garrod and O'Grady, 1968). Decreased virulence of *C. albicans* resistant to polyenes was reported by Ito *et al.* (1955), Lones and Peacock (1959) and Hebeca and Solotorovsky (1962, 1965). This may be related to their reduced growth rate, which would impair their ability to proliferate in infected tissues and give the host a greater opportunity to combat and restrict infection.

In the first report on the development of resistance (Stout and Pagano), the degree of acquired resistance was so low that the authors considered that it probably would not present any clinical problems. However, even a slight increase in the amount of amphotericin B required to inhibit a pathogenic agent may prevent the therapeutic use of this somewhat toxic drug, and the possible development of cross-resistance to other polyenes may hinder therapy.
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None the less, it appears that any danger of the development of polyene-resistant isolates of candida is more than counterbalanced by their loss of virulence. The most virulent candidas known cannot be said to be very virulent by the standards of other pathogenic microbes. Any reduction of their virulence, such as that accompanying the development in vitro of polyene resistance, would seem to rule out the danger of such organisms producing disease in man.

For this reason we are not alarmed by the possible development of resistance in naturally occurring isolates in man. We stress, however, that this has not yet occurred.

The nature of resistance. Our experiments suggest that the origin of resistance is a process of mutation rather than selection, but because the fluctuation test is considered not to be valid under all conditions (mainly because of failure to rule out all other causes of variability or fluctuation) the evidence is not conclusive.

In our studies, no fall in the MIC of the amphotericin B-resistant C. albicans occurred until the 15th subculture in antibiotic-free medium. With further subculture the proportion of smaller, resistant candida colonies decreased. The simplest explanation for the reversion to sensitivity is that some sensitive organisms remain within a predominantly resistant population and that when antibiotic is removed they overgrow the more slowly growing resistant colonies.

SUMMARY

The MIC of nystatin was determined for 1389 successive isolates of candida from clinical specimens, and of amphotericin B for 1307 successive isolates. A further 626 isolates of C. albicans were tested for sensitivity to nystatin and a further 708 to amphotericin B. All these were shown to be sensitive. After gradual exposure on solid media to increased antibiotic concentrations, isolates of seven candida species became resistant to nystatin, amphotericin B, candididin, pimaricin, and filipin. Compared with the polyene-sensitive parent isolates, the resistant cultures showed decreased growth rate, reduced production of germ tubes, slower production of chlamydospores, reduced suspension stability, reduced ergosterol content, and reduced pathogenicity. Isolates resistant to one polyene were found to show cross-resistance to other polyenes. The fluctuation and Newcombe tests suggested that the development of resistance was due to mutation. Resistant isolates showed reversion to sensitivity and some return of their normal properties when no longer exposed to polyenes. There was no evidence of the transfer of resistance from one isolate to another.

This work was supported by a grant from Messrs E. R. Squibb & Sons Ltd.

REFERENCES


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