YEAST FLORA OF THE MOUTH AND SKIN DURING AND AFTER IRRADIATION FOR ORAL AND LARYNGEAL CANCER

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SUMMARY. The quantitative and qualitative changes occurring in the fungal flora of 22 patients with oral and 9 with laryngeal carcinoma were studied during and after radiation therapy. Each patient received 6000 rad of externally applied radiation in divided doses for 5 weeks. The fungal flora was isolated from the patients' oral cavity and irradiated skin sites during irradiation and 2 weeks and 4–6 months afterwards. The number and types of fungi increased in both groups of patients after the start of irradiation and persisted at high levels for at least 4–6 months after treatment. *Candida albicans* and *C. tropicalis* were the principal yeasts isolated throughout the period studied but seven other species were also identified. All the yeast isolates were sensitive *in vitro* to miconazole, ketoconazole, amphotericin B and nystatin. Any of these antifungal agents should be appropriate for therapy.

INTRODUCTION

Radiotherapy administered for the treatment of oral neoplastic disease has profound effects on the oral flora. One of these effects is to cause an increase in the number of yeast colonies that can be isolated from the oral mucosa and saliva (Bodey, 1975). This increase is often associated with some form of superficial fungal infection, which may complicate the radiation therapy (Boggs, Williams and Howell, 1961; Shipman, 1979). These infections are thought to cause severe pain and to be associated with difficulties of swallowing and speech (Beumer, Curtis and Harrison, 1979). Such large numbers of oral yeasts may contribute to the disseminated fungal infections that are often found at post-mortem examination of patients who have had neoplastic disease (Cho and Choi, 1979).

Although fungal infection is known to be associated with radiotherapy, it is difficult for the clinician to anticipate when it will occur in relation to the progress of treatment or how long it will persist; the timing and duration of antifungal therapy is therefore difficult to decide.

Studies on the fungi associated with oral infections during radiotherapy have shown that *Candida* spp. are usually present, although *Aspergillus* spp.

and *Torulopsis glabrata* are sometimes also isolated (Bodey, 1975). The sensitivity of such yeasts to antifungal drugs is often not known and therapy has to be empirical. Moreover, it is not known whether the sensitivity of yeasts alters during radiation therapy.

Patients receiving externally applied radiotherapy for the treatment of oral and other lesions often suffer side effects at the skin site of irradiation. These usually take the form of dry or moist desquamative lesions accompanied by profound erythema (Moss, Brand and Battifora, 1973). These infections are thought to be predominantly bacterial and the presence of fungi has been reported only occasionally (Walter, Miller and Bomford, 1979).

The aim of this study was to identify the number and type of fungal colonies isolated from various sites in the mouth from patients receiving radiotherapy for the treatment of oral or laryngeal neoplasm. In addition, we decided to sample the skin sites of irradiation and in a similar manner to monitor any fungal changes. Fungi isolated before, during and after radiotherapy from each site were tested against three commonly used and one new antifungal agent: nystatin, amphotericin B, miconazole and ketoconazole.

PATIENTS AND METHODS

Patients and samples. Thirty-one patients were studied, 22 with oral neoplasm (the "oral group") and 9 with laryngeal neoplasm (the "laryngeal group"). The patients were selected from those attending for either inpatient or outpatient treatment at Weston Park Hospital, Sheffield. It was planned that the groups would be age and sex matched, but enough patients for precise matching could not be found. The oral group (18 males, 4 females) had an average age of 63 years (range 51–79). The neoplastic lesions being treated in this group were 21 squamous-cell carcinomas and one muco-epidermoid carcinoma. The squamous-cell carcinomas involved the tongue (5), floor of mouth (4), palate and antrum (9), submandibular gland (1) and tonsillar fossa (2). The muco-epidermoid carcinoma involved the floor of the mouth and submandibular gland. The laryngeal group (7 males, 2 females) had an average age of 58 years (range 43–70). The lesions involved the vocal cords (4), epiglottis (4) and pyriform fossa (1) and were all squamous-cell carcinomas.

Each patient received a total of 6000 rad at a beam distance of 100 cm and a field width of 7–10 cm. Twenty-nine patients (22 oral, 7 laryngeal) were treated with the linear accelerator set at a potential of 8 MV and two patients were treated with cobalt 60. Radiation was applied to alternate sides in 25 sessions during 5 weeks. Each patient was provided with the saliva substitute described by Shannon, John and Starcke (1978) *ad libitum* and aspirin as an analgesic when required; antibacterial or antifungal agents were not given. None of the patients had had previous surgery or any other treatment for their neoplastic lesions.

Each patient was examined before treatment and thereafter at the end of 1, 2, 3, 4 and 5 weeks of radiotherapy and 2 weeks after it ended. A final examination was made 4–6 months after completion of radiotherapy. At each examination plain cottonwool swabs were used to sample, by a standardised method, the mucosa of the palate, dorsum of tongue, floor of mouth in the region of the molar teeth, upper and lower buccal sulci, and the skin sites of irradiation.

Microbiological methods. Swabs were inoculated onto Sabouraud's agar (Lab M, London) within 2 h of sampling by a standardised technique; plates were incubated at 30°C for 72 h. The fungal colonies were counted on every plate and scored 0 for no colonies, 1 for 1–5 colonies, 2 for 6–15 and 3 in excess of fifteen. The total score for the patients in each group at every examination time was then calculated as a percentage of the maximal score possible. The percentage scores obtained for the oral and the laryngeal groups were then plotted against time in weeks since the start of the treatment. The different colony types from all the plates were subcultured onto Sabouraud's agar and identified by the methods of Dolan (1971) and the

sugar-assimilation tests of Martin and Schneidau (1970). Each colony identified was then subcultured onto the yeast-nitrogen-base (YNB) agar described by Shadomy (1969).

Sensitivity tests. Inocula for sensitivity testing were harvested from overnight growths at 30°C on YNB medium and adjusted to $c. 5 \times 10^6$ /ml in fresh YNB medium; 0.2 ml of this suspension was used for inoculation. The suspension of the individual organism was then spread evenly over the surface of YNB-agar plates containing either miconazole or ketoconazole 0.1–50 µg/ml, or amphotericin B 0.1–10 µg/ml or nystatin 1–30 units/ml. The amphotericin B was prepared from that used for intravenous injection diluted with sterile distilled water. Miconazole and ketoconazole were dissolved in dimethylsulphoxide to a concentration of 50 µg/ml and then further diluted with water. Nystatin, 5050 units/mg, was dissolved in dimethylformamide to a concentration of 1000 units/ml and then diluted with water as required. The inoculated sensitivity plates were incubated at 30°C for 72 h and the minimum inhibitory concentration (MIC) for each drug was recorded as the lowest concentration of the antifungal agent that completely inhibited visible growth. In every set of determinations, one plate per strain to which antifungal agent had not been added, was included to ensure that growth had occurred. Laboratory strains of *Saccharomyces cerevisiae*, *C. albicans*, *C. tropicalis* and *T. glabrata*, for which the MICs of the antifungal agents were known, were included in every set of determinations.

RESULTS

Quantitative changes in yeast flora

The majority of patients with either oral or laryngeal cancer yielded fungi from one or more oral sites before treatment (fig. 1), with less than five colonies being isolated from each site. In contrast, fungi were not isolated from the skin sites of irradiation before treatment in either group of patients (fig. 2). After the start of treatment the fungal isolations from the mouth in both

FIG. 1.—Isolation of fungi from mouth of patients with oral carcinoma (▲) or laryngeal carcinoma (●).

FIG. 2.—Isolation of fungi from skin sites of irradiation of patients with oral carcinoma (▲) or laryngeal carcinoma (●).

groups rose rapidly, the rise being faster in the oral group (fig. 1). By the end of the 4th week of treatment the majority of patients had grade-3 scores. After the end of radiotherapy (week 5) the fungal isolations did not fall but remained high in both groups; 4–6 months later little diminution in fungal isolations was seen.

Fungal isolation from the skin sites of irradiation showed a similar pattern for both groups (fig. 2). After the start of treatment there was an initial and rapid rise in the number of fungi, which by the 3rd week of treatment was approaching maximal values. Six months after the end of treatment a slight

TABLE I
Distribution of fungal species among sites of patients in the laryngeal group

Species	Number of isolates from						Total	Percentage of all isolates
	palate	tongue	floor of mouth	buccal sulcus		skin		
				upper	lower			
<i>Candida albicans</i>	51	37	46	41	37	44	256	59.3
<i>C. tropicalis</i>	13	16	5	19	14	7	74	17.1
<i>C. pseudotropicalis</i>	1	1	3	0	2	0	7	1.6
<i>C. guilliermondi</i>	3	7	3	1	4	0	18	4.2
<i>C. parapsilosis</i>	1	2	1	2	1	1	8	1.9
<i>C. mycoderma</i>	0	0	2	3	0	3	8	1.9
<i>Torulopsis glabrata</i>	0	1	2	0	6	12	21	4.9
<i>Saccharomyces</i> spp.	1	8	7	6	4	2	28	6.5
<i>Rhodotorula rubra</i>	0	0	0	0	1	0	1	0.2
Unidentified or not subcultured	2	0	3	0	3	3	11	2.5

decrease was noted in the isolations from the laryngeal group but the oral group still had almost maximal fungal scores.

Qualitative changes in yeast flora

At the first examination the majority of isolates (>96%) were *C. albicans*; occasionally *C. tropicalis* and *C. krusei* were identified. After 1 week of treatment the number of different isolates rose in both groups from all sites and remained varied for at least 4–6 months after treatment was complete. Tables I and II show the yeast flora for each site from each group. From the laryngeal group of patients, 432 isolates were identified; *C. albicans* and *C. tropicalis* accounted for 76.4% of them (table I). Four other species of *Candida* were identified together with *T. glabrata* and *Rhodotorula rubra*. Three isolates

TABLE II
Distribution of fungal species among sites of patients in the oral group

Species	Number of isolates from						Total	Percentage of all isolates
	palate	tongue	floor of mouth	buccal sulcus		skin		
				upper	lower			
<i>C. albicans</i>	103	123	109	134	143	59	671	65.8
<i>C. tropicalis</i>	41	26	36	18	12	31	164	16.1
<i>C. pseudotropicalis</i>	3	0	8	7	4	7	29	2.8
<i>C. guilliermondi</i>	7	3	1	2	0	1	14	1.4
<i>C. parapsilosis</i>	1	6	2	0	1	1	11	1.1
<i>C. krusei</i>	12	2	1	0	3	1	19	1.9
<i>C. mycoderma</i>	0	1	0	0	1	21	23	2.3
<i>T. glabrata</i>	1	0	2	3	4	14	24	2.4
<i>Saccharomyces</i> spp.	1	8	10	2	0	18	39	3.8
<i>R. rubra</i>	0	1	0	3	1	14	19	1.9
Unidentified	1	0	1	1	1	3	7	0.7

failed to grow after repeated subculture; they were yeast-like on gram-stained films. Eight other organisms that remained unidentified were *Candida* spp. but distinction between *C. albicans* and *C. stellatoidea* could not be achieved because of variability of the results of repeated fermentation and assimilation tests.

A similar distribution of isolates was seen in the oral group of patients, although more (1020) were identified (table II). The reason for this was a greater variety in their colonial appearance. *C. albicans* and *C. tropicalis* again accounted for the majority of isolates. *C. krusei* was also isolated from this group but not from patients in the laryngeal group. In addition, *C. mycoderma* was isolated predominantly from the skin sites.

From both groups of patients 87 samples (63 oral, 24 laryngeal) yielded growths of *Klebsiella* species on Sabouraud's agar. They were identified, by the methods of Cowan (1974), as mixtures of *Klebsiella aerogenes*, *K. pneumoniae* and, from four samples, *K. oxytoca*.

TABLE III
Minimum inhibitory concentration of nystatin for yeast isolates

			Number of isolates inhibited by nystatin at concentration (units/ml) of												
Species	Group derivation	Number tested	1	2	3	4	5	7.5	10	15	20	25	30		
<i>C. albicans</i>	O	671	...	1	48	2	52	116	433	11	8	...	...		
	L	256	1	...	22	4	83	5	136	...	5	...	...		
<i>C. tropicalis</i>	O	164	...	...	1	...	1	10	...	83	61	8	...		
	L	74	...	...	1	1	11	8	11	27	4	11	...		
<i>C. pseudotropicalis</i>	O	29	...	1	1	...	2	...	4	21	...	...	...		
	L	7	...	...	...	...	3	1	...	2	1	...	...		
<i>C. guilliermondi</i>	O	14	8	1	1	4	...	...	...	...	...	...	...		
	L	18	6	6	3	2	1	...	...	...	...	...	...		
<i>C. parapsilosis</i>	O	11	...	...	...	...	...	...	4	4	3	...	...		
	L	8	...	...	...	...	...	2	1	2	3	...	...		
<i>C. krusei</i>	O	19	...	...	...	...	...	...	...	7	...	4	8		
	L	0	...	...	...	...	...	...	...	...	...	...	...		
<i>C. mycoderma</i>	O	23	...	...	1	...	...	...	6	16	...	...	...		
	L	8	...	...	2	...	2	1	1	2	...	...	...		
<i>T. glabrata</i>	O	24	...	13	...	4	...	...	1	5	1	...	...		
	L	21	...	4	...	11	...	3	3	...	...	...	...		
<i>R. rubra</i>	O	19	...	...	...	3	2	6	8	...	...	...	...		
	L	1	...	...	...	...	...	1	...	...	...	...	...		

O = patients of the oral group; L = patients of the laryngeal group.

TABLE IV
Minimum inhibitory concentration of amphotericin B for yeast isolates

			Number of isolates inhibited by amphotericin B at a concentration ($\mu\text{g/ml}$) of							
Species	Group derivation	Number tested	0.1	0.5	1.0	2	4	5	10	
<i>C. albicans</i>	O	671	121	526	13	8	3	...	...	
	L	256	83	141	32	...	...	...	...	
<i>C. tropicalis</i>	O	164	3	23	101	23	8	6	...	
	L	74	19	31	11	10	2	1	...	
<i>C. pseudotropicalis</i>	O	29	...	10	18	1	...	...	...	
	L	7	1	3	3	...	...	...	...	
<i>C. guilliermondi</i>	O	14	8	3	3	...	...	...	...	
	L	18	11	6	1	...	...	...	...	
<i>C. parapsilosis</i>	O	11	4	...	2	...	1	4	...	
	L	8	...	...	3	2	3	...	...	
<i>C. krusei</i>	O	19	...	4	4	4	3	4	...	
	L	0	...	...	...	...	...	...	...	
<i>C. mycoderma</i>	O	23	3	16	2	2	...	...	...	
	L	8	7	...	1	...	...	...	...	
<i>T. glabrata</i>	O	24	...	...	...	16	4	4	...	
	L	21	4	7	...	7	3	...	...	
<i>R. rubra</i>	O	19	11	4	4	...	...	...	...	
	L	1	...	1	...	...	...	...	...	

Abbreviations as in table III.

Sensitivity tests

The results of the sensitivity tests with the four antifungal agents are shown in tables II–VI. The MICs of all the isolates to nystatin (table III) ranged from 1 to 30 units/ml. The highest MICs were recorded for *C. krusei* and *C. tropicalis*, whilst the *C. albicans* strains were all sensitive in the range 1–20 units/ml. On 23 occasions, separate isolates from the same site were found to differ by at least threefold in sensitivity to nystatin.

All the isolates were very sensitive to amphotericin B (table IV), the highest MIC being 5 µg/ml; more than 90% of the isolates had an MIC of 1 µg/ml or less.

TABLE V
Minimum inhibitory concentration of miconazole for yeast isolates

Species	Group derivation	Number tested	Number of isolates inhibited by miconazole at concentration (μg/ml) of													
			0·1	0·5	1·0	2·0	4·0	5·0	10	15	20	25	30	40	50	
<i>C. albicans</i>	O	671	94	63	111	67	185	95	54	...	1	1	...	...	...	
	L	256	85	42	48	61	19	...	1	...	...	...	...	...	...	
<i>C. tropicalis</i>	O	164	8	4	9	23	11	4	8	42	31	18	5	1	...	
	L	74	...	9	...	7	14	1	21	18	1	1	2	...	...	
<i>C. pseudotropicalis</i>	O	29	1	1	3	6	4	...	14	...	...	...	...	...	...	
	L	7	...	1	2	...	4	...	...	...	...	...	...	...	...	
<i>C. guilliermondi</i>	O	14	...	2	2	2	4	1	3	...	...	...	...	...	...	
	L	18	1	1	3	4	6	3	...	...	...	...	...	...	...	
<i>C. parapsilosis</i>	O	11	...	4	4	3	...	...	...	...	...	...	...	...	...	
	L	8	2	4	1	1	...	...	...	...	...	...	...	...	...	
<i>C. krusei</i>	O	19	2	10	4	3	...	...	...	...	...	...	...	...	...	
	L	0	...	...	...	...	...	...	...	...	...	...	...	...	...	
<i>C. mycoderma</i>	O	23	4	4	11	4	...	...	...	...	...	...	...	...	...	
	L	8	...	...	2	4	2	...	...	...	...	...	...	...	...	
<i>T. glabrata</i>	O	24	...	...	...	...	...	3	11	8	1	...	...	1	...	
	L	21	...	...	...	...	4	1	6	3	7	...	...	...	...	
<i>R. rubra</i>	O	19	...	4	7	6	2	...	...	...	...	...	...	...	...	
	L	1	...	...	1	...	...	...	...	...	...	...	...	...	...	

Abbreviations as in table III.

The MICs for miconazole (table V) were more difficult to determine. Endpoints were less reproducible and the values shown are the highest obtained after three tests of the difficult isolates. One hundred and thirty-four determinations were repeated, with final MICs ranging from 4 to 25 µg/ml. All the isolates were sensitive in the range 0·1–40 µg/ml, the highest MICs being obtained from *T. glabrata* and *C. tropicalis*.

The MICs for ketoconazole (table VI) were also difficult to determine. In 76 tests on *C. albicans*, growth was not present at one concentration only to reappear at a higher concentration; this effect was reproducible for 73 of the 76 isolates. The MIC in these cases was taken as the highest concentration that

TABLE VI
Minimum inhibitory concentration of ketoconazole for yeast isolates

Species	Group derivation	Number tested	Number of isolates inhibited by ketoconazole at concentrations ($\mu\text{g/ml}$) of												
			0.1	0.5	1.0	2.0	4.0	5.0	10	15	20	25	30	40	50
<i>C. albicans</i>	O	671	...	1	...	5	28	24	111	87	123	143	106	43	...
	L	256	...	1	...	2	...	3	87	93	41	21	8	...	...
<i>C. tropicalis</i>	O	164	...	...	...	...	5	...	42	5	78	11	23	...	...
	L	74	1	1	2	1	1	1	...	16	10	31	7	3	...
<i>C. pseudotropicalis</i>	O	29	...	...	8	...	1	1	...	4	7	4	4	...	...
	L	7	...	...	1	...	2	...	2	...	2	...	...	...	...
<i>C. guilliermondi</i>	O	14	...	1	...	...	2	...	...	11	...	...	...	...	...
	L	18	...	...	4	6	2	6	...	...	...	...	...	...	...
<i>C. parapsilosis</i>	O	11	...	...	...	...	...	...	...	...	...	4	4	3	...
	L	8	...	...	...	...	...	...	...	...	2	3	2	1	...
<i>C. krusei</i>	O	21	...	...	...	...	1	7	...	4	3	6	...	...	...
	L	0	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>C. mycoderma</i>	O	23	...	...	...	...	...	...	...	8	7	4	2	2	...
	L	8	...	...	...	...	...	1	...	3	3	1	...	...	...
<i>T. glabrata</i>	O	24	...	...	...	...	...	...	...	...	...	4	14	...	6
	L	21	...	...	3	...	...	8	...	...	...	8	...	1	1
<i>R. rubra</i>	O	19	...	...	...	11	4	...	...	4	...	...	...	...	...
	L	1	...	...	...	...	...	...	...	1	...	...	...	...	...

Abbreviations as in table III.

inhibited growth. As a check, the test was repeated with ketoconazole at three times the MIC, to ensure that no more growth was possible; in no case was further growth seen. All isolates were sensitive to ketoconazole in the range 0.1–50 $\mu\text{g/ml}$.

DISCUSSION

The two groups of patients studied were those with an oral malignancy in whom irradiation would directly affect the oral mucous membranes as well as the overlying skin of the lesion itself, and those who were treated for laryngeal carcinoma in whom the skin and the lesions would be directly irradiated but the oral mucous membrane would receive only peripheral irradiation.

The oral flora of both groups was sampled with cottonwool swabs. This technique is at best semi-quantitative and compares unfavourably with the more accurate imprint technique of Arendorf and Walker (1980). However, it is the most commonly used clinical method for sampling these sites and it was found that many of the patients in both groups had yeasts in their oral cavity before radiation. These may have been part of the normal commensal flora or may have become established as a consequence of neoplastic disease. However, immediately after the start of therapy there was a rapid increase in the isolation of yeasts from all sites in both groups. This increase continued throughout treatment and persisted at least for 6 months beyond the treatment period. The reason for this prolonged increase is not known, because the oral

mucosa in both groups appears clinically to return almost to normal after radiotherapy with no signs of fungal infection (Al-Tikriti, 1980). This reservoir of fungi could be a source for further oral or disseminated fungal infection, and in such debilitated patients must be considered a significant clinical hazard. A similar increase in fungal isolations was seen at the skin sites of irradiation in both groups; it persisted for at least 6 months in the oral group but showed slight evidence of diminution at this time in the laryngeal group. The increased isolation of fungi was coincident with a predominantly moist desquamation of the skin surface of the type described by Moss *et al.* (1973). The fungi isolated from the skin were probably responsible wholly or in part for the reactions seen. It is also possible that the radiation had altered the normal barrier properties of the skin and its secretions, making it more susceptible to infection. These latter effects of radiation have been noted previously (Walter *et al.*, 1979) but the possibility that they could be exacerbated by fungi has not.

It is interesting to speculate on the source of the fungi found on the skin after the start of radiation because these organisms are usually confined to moist skin folds in the non-debilitated patient (Somerville, 1969; Odds, 1979). One possible source is the mouth, from which the fungi might be spread on the fingers; many of the oral isolates of *C. albicans* had MICs identical to those of isolates from the skin. However, to establish such a link firmly it would be necessary to type oral and skin strains by a suitable method, for example that of Warnock *et al.* (1979).

C. albicans was the most numerous isolate, confirming the results of Rosenthal and Wilkie (1965) and Bodey (1975). The increase in growth of this organism was found in both groups of patients despite the fact that the laryngeal group had not orally directed irradiation. One explanation for this increase may be that peripheral radiation affected the submandibular and parotid salivary glands, causing a change in the composition and quality of saliva (Al-Tikriti, 1980); such a change of salivary-gland function and increase in yeast infection was noted by Robinson (1976), who suggested that the antimicrobial substances in saliva regulate the number of yeasts in the mouth.

In addition to *C. albicans*, six other species of *Candida* were isolated, of which *C. tropicalis* was the most numerous. This latter organism is often isolated from clinical specimens, accounting for 18% of laboratory growths in one survey (Walker and Luecke, 1974). The isolation of *T. glabrata* and *R. rubra* from oral sites was noted by Budtz-Jorgensen, Stenderup and Grabowski (1975). However, the significance of these organisms in mixed infections is not known. Similarly, the significance of *Klebsiella* spp. is not known, although these organisms are often isolated from patients with neoplastic disease (Singer, Kaplan and Armstrong, 1977). The isolation of *Klebsiella* did not follow any definite pattern or distribution among the patients sampled, this result being in agreement with the work of Holbrook and Russell (1979) and Martin (1981). *Klebsiella* probably becomes established as a transitory opportunist.

Organisms resistant to any of the antifungal agents were not found. The

MICs for most of the species tested were considerably higher than those recorded by Bannatyne and Cheung (1978) and Moody *et al.* (1980), but were similar to those of Dixon *et al.* (1978) and Odds *et al.* (1980). The in-vitro tests suggest that any of the antifungal drugs studied would be appropriate for treatment of the superficial fungal infections seen in these patients. Because the isolates tested were selected on the basis of colonial morphology, resistant strains may not have been included; however, the chances of this would have been decreased by the large number of isolates tested.

The MICs of nystatin and amphotericin B were low, except against *C. tropicalis* and *C. krusei*; this is in agreement with the results of Athar and Winner (1971).

However, on 23 occasions, the MIC of nystatin against *C. albicans* isolates from the same site differed by at least threefold. This may be evidence that two distinct strains of *C. albicans* had infected the same site. However, it will be necessary to examine these strains in more detail before such an assertion can be proved.

The MICs for miconazole and ketoconazole were not in agreement with those reported by Bannatyne and Cheung (1978) and Moody *et al.* (1980). Moody *et al.* (1980) suggested that their low MIC values for yeasts were a consequence of their use of fresh isolates, which may be more active metabolically. In our study fresh isolates have been used and therefore this explanation is probably not valid. The difference between our results and those of Moody *et al.* (1980) could reflect the different methods used. We used a heavy inoculum of approximately 1×10^6 cells/ml; initial studies (unpublished) had shown that this gave reproducible results. Despite the heavy inoculum, there were difficulties in obtaining MIC values for the imidazoles, particularly ketoconazole. Apparent disappearance and re-emergence of growth at higher concentrations of the drug were found, for which an explanation is still lacking. The lower MIC results for ketoconazole obtained by Moody *et al.* (1980) may have been a result of recording the first disappearance of growth rather than the second. The use of ketoconazole for the treatment of fungal infections is likely to increase because the preliminary tests have given encouraging results in animals (Odds *et al.*, 1980).

Our results show that many fungi can be isolated from the oral and skin sites of irradiation in patients receiving radiotherapy. This increase persists for a prolonged period after treatment. It remains to be determined which therapeutic regimes can significantly suppress the fungal flora during and after treatment. It is to be hoped that the treatment of these fungal infections will ameliorate some of the distressing effects of radiation experienced by these patients.

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