

The incidence of the serological groups of *Candida albicans* in southern England

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INTRODUCTION

It is now known that *Candida albicans* as a distinct species can be classified into groups A and B by means of a direct agglutination test with absorbed rabbit antisera (Hasenclever & Mitchell, 1961*a*). Although these workers attempted to employ double diffusion in agar-gel as an alternative method, the results were reported as being not consistent in showing the differences observed with agglutination tests. Nevertheless, double diffusion has demonstrated that two serological groups do in fact exist and that the so-called group A and group B strains as supplied by Dr Hasenclever can be differentiated by this means (Stallybrass, 1964).

An attempt was made by Hasenclever & Mitchell (1963) to correlate the *C. albicans* groups with the site of isolation in 242 patients, although unfortunately many of the sites were listed together and no figures are available for particular sources such as sputum. In one collection of 55 vaginal cultures from an unspecified number of pregnant Negro women 22 belonged to group A and 33 to group B, whereas in another collection of 653 cultures obtained from 242 patients 68% were A and 32% B. As the particular strains of *C. albicans* isolated from the several sources in individual patients may sometimes have been identical, the figures indicate neither the true incidence of group A and group B strains at the site of isolation, nor the total incidence in the 297 patients.

The present paper reports the results of grouping 234 strains of *C. albicans* obtained from 234 patients in southern England during 1962 and 1963, using the agar-gel double diffusion technique.

MATERIALS AND METHODS

Candida albicans test strains

These strains were obtained from primary cultures on Sabouraud dextrose medium or blood agar, from patients attending eight hospital centres situated in London, Berkshire, Surrey, and Hampshire. Of the 234 cultures, 48 were from sputa of patients at one hospital alone, 36 were from vaginal swabs collected at a second hospital, and 150 from various sites including sputum and vagina from patients attending six other well-separated hospital centres.

All the strains, after being checked for purity, were tested for the ability to ferment glucose, maltose, lactose and sucrose (3% in peptone water), and to form

chlamydospores on Taschdjian's medium (Taschdjian, 1957). The fermentation tests were read after 1 week at 37° C., while the Taschdjian plates were left at room temperature and examined daily for the development of chlamydospores. Those strains producing acid and gas from glucose and maltose only, and also producing chlamydospores within 5 days were accepted as *C. albicans*. After identification, these strains were maintained at room temperature by monthly subculture from the main growth on Sabouraud dextrose medium containing 20 and 40 µg./ml. respectively of penicillin and streptomycin.

Candida albicans control strains

One strain each of group A (no. 207/5/3) and group B (no. 792/5/3) were supplied through the courtesy of Dr Hasenclever.

Antigen preparations

(a) *Formamide extracts*. Between 45 and 60 mg. wet weight of *C. albicans* obtained from two 48 hr. slope cultures was extracted by Fuller's (1938) method with 0.5 ml. formamide at 160° C., treated with acid-alcohol and acetone, and the polysaccharide finally dissolved in 2.0 ml. saline at pH 7.6.

(b) *Mickle extracts*. The same amount of growth made as a 50% (v/v) suspension in peptone water was placed in a polythene tube (capacity 5 ml.) containing 75 stainless-steel balls of $\frac{3}{32}$ in. (2.4 mm.) diameter. After shaking for 15 min. in the Mickle disintegrator the contents of the tube were centrifuged and made up to 1 ml. in peptone water and stored frozen overnight. After thawing, the fluid was centrifuged again, when the supernatant was ready for use as the Mickle extract.

Extracts of both types were prepared from the test and control strains.

Antisera

Two rabbits each weighing 3.5 kg. were inoculated intravenously. Rabbit 'X' received five injections of a formalin-killed vaccine of the group A strain supplied by Dr Hasenclever, in doses ranging from 4 to 20 million cells over a period of 139 days. Rabbit 'T' received four injections of a similar vaccine prepared from another group A strain, in doses ranging from 4 to 40 million cells over a period of 36 days. Blood was taken from the marginal vein of the ear on days 39 and 150 from rabbit 'X' and on day 50 from rabbit 'T'. The samples of serum obtained from these bleedings were stored separately at -79° C. until required for use.

No vaccine of a group B strain was used because a group B antiserum will not differentiate the two groups (Stallybrass, 1964).

Double diffusion method

Tests were performed in flat-bottomed Petri-plates ('Anumbra') of 9 cm. diameter, containing 0.8% ion-agar (Oxoid) in saline with 1% sodium azide. Wells of 6 mm. diameter were cut and the bases of these were sealed with melted

medium; they were arranged round a central well so that their margins were separated from this by a distance of 5 mm. After filling the peripheral wells with appropriate extracts and the central one with the group A antiserum the plates were incubated at 37° C. and then inspected for precipitin lines after 24 and 48 hr.

Grouping procedure

The strains were differentiated by three separate precipitin tests. The first test was used simply to select extracts for further examination by the second, and in both of these formamide extracts were employed. The third test was intended as a means of checking the results previously obtained, and in this the Mickle extract was used.

Test 1. The formamide extracts were placed in the wells so that a maximum of four extracts were reacting with the group A antiserum. For the whole batch of tests one group A and one group B extract were included to demonstrate the differential pattern (Plate 1*a*). On the basis of this screening test, those extracts appearing to react as group B were selected for further testing.

Test 2. Each test extract was placed in a well immediately adjacent to the control group A extract (Plate 1*b*) and allowed to diffuse against the group A antiserum; the control group B extract was included in each batch. Those extracts which reacted as group B in this second test were recorded and the appropriate strains subjected to the final investigation.

Test 3. Mickle extracts prepared from fresh 48 hr. subcultures of the original strains were employed, and each extract was placed in a peripheral well adjacent to wells containing similar extracts of group A and group B controls. The group A antiserum was as usual placed in the central well, and the plates read after 24 and 48 hr.

RESULTS

Both in the individual hospital centres making up the series and in the series as a whole, group A strains have been found to predominate. Of the 234 cultures of *C. albicans* 75% were of group A and 25% of group B, figures which agree broadly with those of Hasenclever & Mitchell (1963). The figures here refer to isolations obtained from human sources in eight hospital centres, some of which provided specimens from a single site only (e.g. sputum, vagina, skin) while others provided specimens from various sites.

Table 1. *The incidence of Candida albicans groups A and B*

Site of isolation	Nos. of centres and hospitals supplying strains	Total isolations	Sputum isolations	Total group A	Total group B	Sputum group B
All	Nos. 1-8	234 (25)	91 (26)	174	60	24
Various	Nos. 1-4, 6, 8	150 (22)	43 (14)	117	33	6
Vagina only	No. 5	36 (25)	—	27	9	—
Sputum only	No. 7	48 (38)	48 (38)	30	18	18

The figures in parentheses represent percentage values for group B strains.

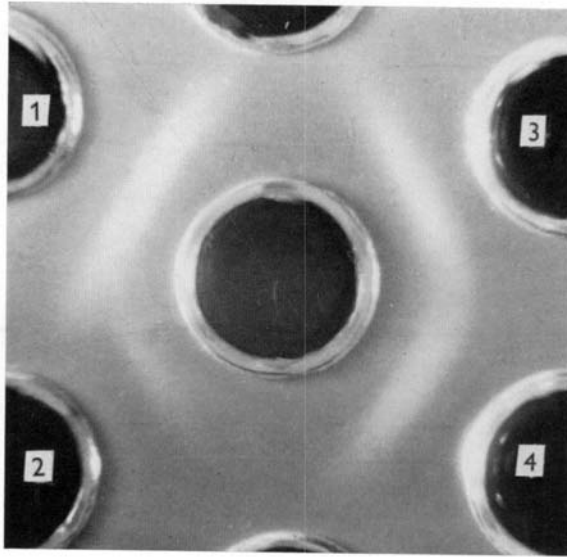
Of 36 isolations from vaginal swabs provided by one hospital, 9 were of group B, figures which contrast with those of Hasenclever & Mitchell (1963) who found group B strains to predominate in Negro women. The significance of race, if any, is as yet unknown. Of 150 isolations obtained from six hospital centres collecting from a variety of specimens 22% were of group B, but of 48 isolations obtained from the sputum of patients under treatment at one hospital 18 (38%) were of group B. These results are summarized in Table 1.

DISCUSSION

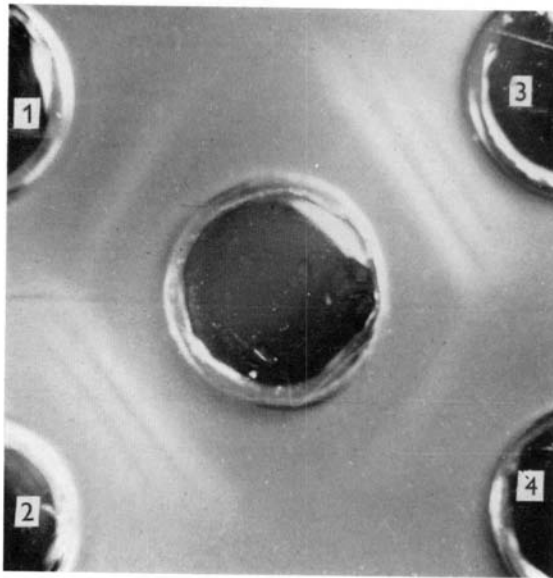
The group characteristics of *C. albicans* appear to be stable within the limits of the experimental conditions outlined in this report. Three group A strains were grown and extracted at monthly intervals for 3 months, and at all times the extracts gave the group A reaction.

The diffusion method itself has proved reliable for differentiating the two groups. One of the group A strains was at first thought to be of group B on the basis of diffusion tests with a formamide extract, but when the Mickle extract was tested the strain reacted as a member of group A, a finding which was confirmed by the preparation and testing of fresh formamide and Mickle extracts. Apart from this single exception, there was complete agreement in the three tests with all strains. It was found that the grouping was more easily achieved by use of the formamide extracts and that the ease with which the strains could be differentiated depended in part on the properties of the group A antiserum employed. In the present series two group A antisera were used and both were satisfactory for the purpose; however, one of them ('T') was found to give sharper differentiation at 24 hr. since the group B precipitin bands were consistently very faint at this time. If grouping is to be performed by one diffusion test only, it is essential to use an antiserum which differentiates clearly.

Little is known of the significance of the two serological groups of *C. albicans*. Apart from the data relating to Negro women all the evidence to date shows that group A strains predominate in man. It seems unreasonable to postulate that strains of the organism can be selected on the basis of their antigenic constitution by the host's tissues at different sites, and it is tentatively suggested that the antigenically deficient group B strains may be attenuated forms produced by environmental factors; these may include prolonged exposure to antibiotics such as tetracycline, or to influences related to survival outside the body in clothing, ward dust, etc. Although it is reasonable to postulate that group B strains may lack a capsular polysaccharide and are avirulent, this has not yet been proved experimentally. No difference between the groups in their virulence for mice was found by Hasenclever & Mitchell (1961*b*), and group B strains certainly proved lethal to rabbits. Unpublished experiments by the present author in which serial blood cultures were taken before and after intradermal inoculation of rabbits with strains of groups A and B have failed to show that group A strains are more invasive than the group B. It would be useful to know if group B strains are ever responsible for systemic candidosis in man.



(a)



(b)

Considering all the data available in the present series, no evidence has been found that sputum cultures in particular are associated with a high incidence of group B strains (Table 1). The unusually high incidence of group B in the sputum of long-term hospital patients from No. 7 hospital may be accounted for by a process of cross-infection, but there was in fact no epidemiological evidence to suggest that this cause was operating here. Nevertheless, it is possible that prolonged survival of *C. albicans* in a ward environment may lead to loss of some antigens, so that repeated reinfection with attenuated strains may occur.

SUMMARY

Cultures of *C. albicans* have been classified into two groups by means of a double diffusion precipitin test employing rabbit antisera and polysaccharide extracts. In the present series it has been found that 75 % of cultures belong to group A and 25 % to group B, and it is suggested that the higher incidence of group B strains occurring in the sputum of hospital in-patients with chest disease may be the result of reinfection by attenuated forms which persist in the environment.

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EXPLANATION OF PLATE 1

- (*a*) Preliminary screening test of formamide extracts. (1) Group A control, (2) group B control, (3) and (4) tests. The group A antiserum is in the central well.
- (*b*) Second test with formamide extracts of suspected group B strains. (1) and (4) test extracts of group B; (2) and (3) control extracts of group A. The group A antiserum is in the central well.