ANTIBODIES TO *CANDIDA* AFTER OPERATIONS ON THE HEART

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SYSTEMIC forms of candidiasis have been seen with increasing frequency with the advent of advanced therapeutic procedures such as open operations on the heart and organ-transplantation. They are serious infections, difficult to treat and have a poor prognosis unless diagnosed early (Seelig *et al.*, 1973). Clinical and laboratory diagnoses are, however, difficult. Cultures are often negative in the presence of infection and serological tests are unreliable. A high agglutination titre to *Candida* is of little diagnostic significance (Winner, 1955). The precipitin test with extracts of yeast somatic antigen was originally claimed to be the most reliable and specific guide to infection (Taschdjian *et al.*, 1969), but precipitating antibodies have since been demonstrated in patients undergoing open-heart surgery in the absence of systemic disease (Murray, Buckley and Turner 1969; Parsons and Nassau, 1974). It was originally assumed that these precipitating antibodies were indicative of inapparent infection (Murray *et al.*, 1969) but more recently it has been postulated that an increase in the number of yeasts in the alimentary tract may be responsible (Parsons and Nassau, 1974). It has been confirmed that the frequency of yeasts among these patients is high and that numbers of yeasts increase post-operatively (Evans, 1975). Precipitins may be related to transient candidaemias resulting from persorption of yeasts across intact intestinal mucosa (Krause, Matheis and Wulf 1969; Stone *et al.*, 1974).

To elucidate the mechanism of antibody formation, the frequency of yeasts and of antibodies to yeasts were compared in a "normal" group of open-heart surgery patients and a group receiving pre- and post-operative antifungal prophylaxis to reduce their commensal yeast population.

MATERIALS AND METHODS

Patients

The 137 patients included in this study were an unselected series undergoing open-heart surgery, mainly for valve replacement with fascia lata homografts. Patients were admitted 4–6 days before surgery, kept in the intensive care unit for 2–4 days after surgery, returned to the thoracic surgery ward and discharged 2–3 weeks after operation. Eighty-seven patients received orthodox surgery (untreated group) and 50 patients received, in addition, antifungal prophylaxis (antifungal prophylaxis group).

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Antifungal prophylaxis

Prophylactic treatment began 12 days before admission to hospital and continued, apart from the period in the intensive care unit, until the patient's discharge from hospital. It consisted of nystatin tablets (Nystan, E. R. Squibb: 500 000 units, four times daily), amphotericin B lozenges (Fungilin, E. R. Squibb: 10 mg, six times daily) and nystatin vaginal pessaries (Nystan, E. R. Squibb: 100 000 units, twice daily) for the women.

Investigations

Mouth swabs and blood samples were taken from patients on admission to hospital and 1, 2 and 3 weeks after operation. The mouth swabs were taken by the same person and cultured immediately on petri dishes of Sabouraud's dextrose agar containing chloramphenicol 0.05 mg per ml. Yeast colonies were counted after incubation for 48 h at 37°C. The yeasts isolated were tested for germ-tube production in serum and positive isolates were recorded as *Candida albicans*. Germ-tube-negative isolates were identified by morphological and physiological criteria according to Lodder (1970).

Sera were examined for the presence of agglutinins and precipitins to *Candida albicans* serotype A and *Candida parapsilosis* by the methods of Murray *et al.* (1969). Whole cells were used for the agglutination tests and an extract of yeast somatic antigen obtained by disrupting cells with a Braun homogeniser (Evans *et al.*, 1973) for the agar double-diffusion precipitin tests. When insufficient serum was available for both serological tests to be done preference was given to the precipitin test.

Results were analysed statistically by means of the χ^2 test.

RESULTS

The frequency of yeasts, agglutinins and precipitins was significantly higher in the untreated group of patients than in the group receiving antifungal prophylaxis. The prophylactic treatment greatly reduced the percentage of patients harbouring yeasts. In the untreated group, 76 of the 87 patients (87%) had yeasts in the mouth at some time during their stay in hospital, in comparison with 23 of the 50 patients (46%) treated prophylactically ($P < 0.005$); most isolates (93%) were *Candida* spp., usually *C. albicans* (83%). There was, however, a distinct pattern of colonisation in both categories (see table). On admission, 39% of patients in the untreated group harboured yeasts in comparison with 9% in the antifungal-prophylaxis group, but by 1 week after the operation the number had increased in both groups to 72% and 34% respectively. This was followed by a gradual reduction over the next 2 weeks in the number of patients harbouring yeasts. The increased frequency of yeasts in the immediate post-operative period was accompanied by a massive increase in the numbers of yeasts isolated from the patients, although this was not as pronounced in the patients receiving antifungal antibiotics. In the untreated group, the mean number of yeast colonies (expressed as a geometric mean because of the logarithmic distribution of yeast numbers) isolated from the mouth on admission was one: this rose to 27 one week after the operation, and fell to 4 after a further 2 weeks. Cultures from 25 of these patients (29%) showed confluent yeast growth (in excess of 1000 colonies) 1 week after surgery. In the antifungal-prophylaxis group the mean number of colonies was less than one on admission, rising to only one after operation; only two patients yielded cultures with confluent growth 1 week after operation.

FIGURE.—Agglutination titres to *Candida albicans* in patients before and after open-heart surgery.

In both groups of patients, agglutination titres to *C. albicans* were low on admission (agglutination titres to *C. parapsilosis* were consistently lower than to *C. albicans* and were discounted) and none of the patients had precipitating antibodies to extracts of somatic antigens of *C. albicans* or *C. parapsilosis*.

TABLE

The occurrence of yeasts and yeast antibodies in patients before and after open-heart surgery

Sampling times in "untreated", and "antifungal-prophylaxis" groups	Number of patients	Patients tested for yeasts in mouth	Number (and percentage) of patients with yeasts in mouth	Mean number of yeast colonies per sample	Patients tested for agglutinins	Mean agglutinin titre	Patients tested for precipitins	Number (and percentage) positive
<i>Untreated</i>								
On admission	87	87	34 (39)	1	80	3	84	0 (0)
1 week a.o.	87	87	63 (72)	27	76	23	76	23 (30)
2 weeks a.o.	84*	83	48 (58)	5	75	31	78	31 (40)
3 weeks a.o.	50*	49	28 (57)	4	42	20	46	12 (26)
<i>Antifungal prophylaxis</i>								
On admission	50	46	4 (9)	<1	45	1	48	0 (0)
1 week a.o.	50	50	17 (34)	1	45	10	49	4 (8)
2 weeks a.o.	50	49	10 (20)	<1	41	11	47	7 (15)
3 weeks a.o.	22*	22	5 (23)	<1	19	12	20	3 (15)

a.o. = After operation.

* Number reduced by early discharge of patients.

Agglutination titres rose in both groups after operation, reaching a peak after 2 weeks (see figure). This rise was, however, more pronounced in the untreated group where the geometric mean of the agglutination titres rose from 3 on admission to 31 two weeks after operation (see table) at which time 25% of patients had titres of 256 or above (see figure). In the treated group the mean agglutination titres rose from 1 on admission to only 12 after operation, and 2 weeks after operation only three patients (7%) had titres of 256 or more. In all, 56 patients (64%) in the untreated group developed an agglutination titre to *C. albicans* of 64 or above, whereas only 12 (24%) had similar titres in the antifungal-prophylaxis group ($P < 0.025$). Similarly, precipitins to *Candida* developed in a larger percentage of the patients in the untreated group (47%) than in the other group (18%) ($P < 0.005$) and, as with the agglutinins, there was a peak 2 weeks after operation in the number of people with precipitins. Moreover, the precipitin reactions were considered subjectively to be weaker in the antifungal-prophylaxis group, the majority being detectable only after staining.

When 52% of the 137 patients were examined 1 and 3 months after surgery, members of both groups showed that the commensal yeast flora of the mouth had returned to the levels seen on admission and invariably agglutinins and precipitins to *Candida* had disappeared.

DISCUSSION

There has not previously been a combined cultural and serological study of patients undergoing open-heart surgery to explain the appearance of antibodies to yeasts in these patients.

None of the patients in this survey developed an overt systemic or superficial yeast infection, yet many produced yeast antibodies. The antibody response seen in the untreated group had similar proportions and followed a similar pattern, with a peak 2 weeks after operation, to that found in a previous serological survey of open-heart surgery patients (Murray *et al.*, 1969). The

time of occurrence of the peak suggested an antigenic stimulus near the time of operation. Although it is possible that in some patients the stimulus may have been an inapparent and transient yeast infection, it seems more likely that in most instances the antibody response resulted from the increase in the commensal yeast population in the immediate post-operative period. This is borne out by the fact that when the commensal yeast population was suppressed by antifungal antibiotics, the antibody response was also reduced. Moreover, when yeast population-levels fell 1-3 months after operation the antibodies in both groups disappeared.

The precise way in which yeasts in the intestinal tract induce the formation of antibodies is not clear. Soluble antigenic substances may cross the gut wall into the lymphatics and bloodstream but it is more likely that the yeast cells themselves pass across intact intestinal mucosa into the bloodstream by a persorption mechanism. This has been shown to occur from the small intestine of animals (Stone *et al.*, 1974) and there is one account of a similar event in man (Krause *et al.*, 1969). Such a phenomenon would also explain the transient occurrence of yeasts that has been observed in the blood of some patients (Ellis and Spivack, 1967; Kozinn *et al.*, 1969) and that is apparently without clinical effect.

Clearly there is an association in this survey between the occurrence of yeasts and the appearance of antibodies. In a few instances, however, antibodies appeared in patients from whom no yeast was grown. In such instances, it is possible that yeasts were present at sites not sampled. Conversely, the presence of yeasts was not invariably associated with detectable antibody formation, but this may be explained by the suggestion that a minimal threshold concentration of yeast is necessary before persorption occurs (Stone *et al.*, 1973).

If persorption of yeasts occurs in the way postulated, there are clear implications with regard to the development of systemic forms of yeast infection in open-heart and related groups of patients. These are discussed elsewhere (Evans, 1975). The findings also have an important bearing on our interpretation of serological tests for the diagnosis of systemic yeast infections. The results confirm the unreliability of existing serological tests, since rising yeast agglutinin titres and demonstrable precipitins appear in open-heart surgery patients in the absence of overt systemic infection. We further illustrate here that in the majority of patients a positive precipitin result probably reflects nothing more than an immunological response to a heavy commensal yeast population.

The precipitin test, despite its lack of specificity, is still the best available serological aid to diagnosis of systemic yeast infections. However, until the test is improved, probably by the development of better antigenic extracts more specific to the invasive forms of the organism, we should modify our attitude to the interpretation of its results. For diagnosis of systemic infection we should look for a continued rise in antibody levels after operation rather than for the mere presence of yeast antibodies.

SUMMARY

The occurrence of yeasts and antibodies to yeasts was studied in patients undergoing open-heart surgery without antifungal prophylaxis, and in a

similar group receiving antifungal prophylaxis. An association was demonstrated between the occurrence of commensal yeasts and the appearance of antibodies. None of the patients developed overt systemic or superficial yeast infection. The antigenic stimulus for the post-operative production of antibodies appeared to be the increase in the yeast flora that occurred shortly after operation. When the commensal yeast population was suppressed by antifungal antibiotics, the antibody response was also reduced. The implications of these findings in the interpretation of serological tests for diagnosis of systemic yeast infections are discussed.

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