Letters to the Editor

Isolation of Bacillus circulans from a wound infection

A 78 year old woman underwent bilateral salpingo-oophorectomy and omentectomy for bilateral ovarian carcinoma with spread. Wound dehiscence had occurred by the ninth postoperative day, a swab was taken, and treatment was begun with co-trimoxazole. The wound was resutured and it subsequently healed normally.

Microscopy showed many round ended, Gram positive rods but no polymorphonuclear leucocytes. Cultivation overnight at 37°C on blood agar yielded a pure growth of creamy grey spreading colonies and motile microcolonies, which rotated to the right or left (Fig. 1); growth had covered the whole surface of the medium within 48 h, but spreading was limited on thoroughly dried media. When tested on Iso-Sensitest agar (Oxoid) the organism was sensitive to discs containing cefuroxime (30 μg), co-trimoxazole (25 μg), and gentamicin (10 μg) and resistant to amoxycillin (2 μg) and cephalexin (5 μg). Phase contrast microscopy at 48 h showed motile vegetative cells and sporangia distended by ellipsoidal, subterminal endospores (Fig. 2). The organism was identified as a strain of *Bacillus circulans* using the method developed by Logan and Berkeley,[1] which is based on tests in the API system.

Eight of 61 strains of *B circulans* that we studied produced motile microcolonies but only five of these showed spreading growth; this latter phenomenon is more characteristic of *B alvei*. *B circulans* is a heterogeneous species,[1] strains of which are common in the environment; three of the five spreading strains mentioned were isolated from soil, one from grass, and one from infant bile.

We are aware of only one previous report of infection, a fatal case of meningitis,[2] by *B circulans*, but it is now recognised that several species of *Bacillus* other than *B cereus* may be opportunistic pathogens in a range of conditions[3] and that the identification of all *Bacillus* isolates from clinical specimens is desirable.

We thank Professor PW Howie and Mr PE Preece of the Ninewells Hospital, Dundee, for permission to report on their patient.

NA LOGAN
DC OLD*
HM DICK*
Department of Biological Sciences, Glasgow College of Technology, Glasgow and
**Microbiology Department, University of Dundee Medical School, Ninewells Hospital, Dundee*

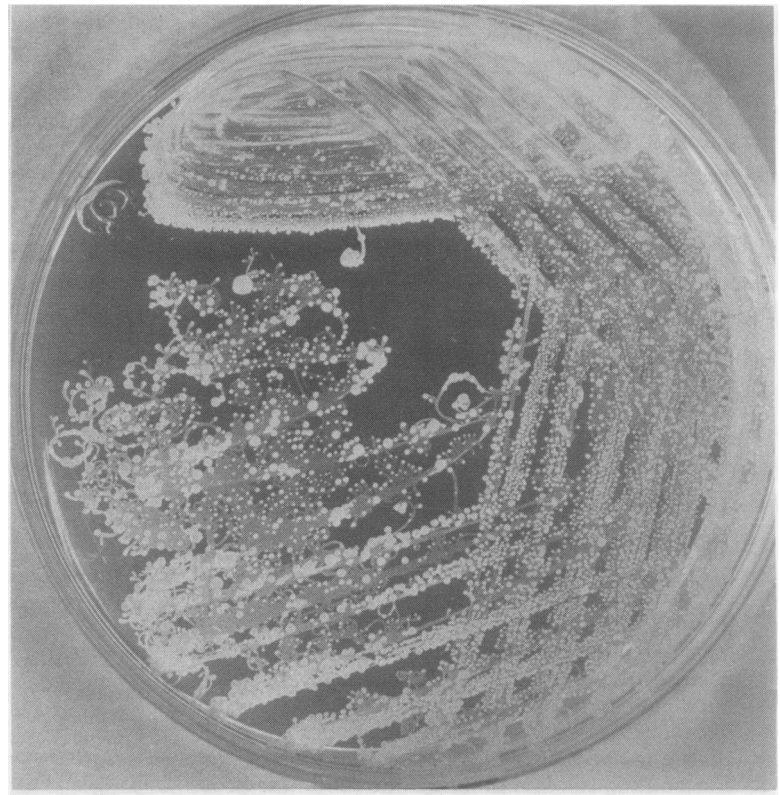

Fig. 1 *Spreading growth of motile microcolonies of Bacillus circulans on nutrient agar.*

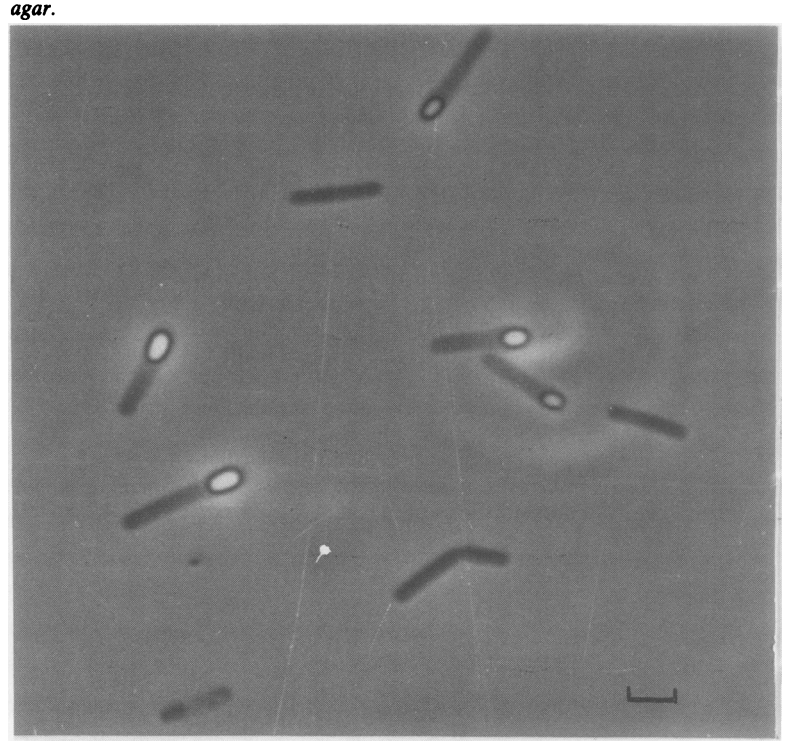

Fig. 2 *Vegetative cells and sporangia of Bacillus circulans. The spores are ellipsoidal, lie subterminally, and they distend the sporangia slightly. Bar marker represents 2 μm.*

References

[1] Logan NA, Berkeley RCW. Identification of bacillus strains using the API system. *J Gen Microbiol* 1984;**130**:1871–82.

[2] Boyette DP, Rights FL. Heretofore undescribed aerobic sporeforming bacillus in child with meningitis. *JAMA* 1952; **148**:1223–4.

[3] Norris JR, Berkeley RCW, Logan NA, O'Donnell AG. The genera bacillus and lactobacillus. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, eds. *The prokaryotes*, Berlin Heidelberg: Springer-Verlag, 1981:1711–42.

Detection of antibodies to Staphylococcus epidermidis in infected total hip replacements by an enzyme linked immunosorbent assay

Infection is the most serious complication of total hip replacement operations, with reported rates of between 0·7%[1] and 11%.[2] Coagulase negative staphylococci are often implicated in these prosthetic infections, but as they tend to be chronic and low grade, a positive diagnosis may, in some cases, be difficult. In these cases the ability to detect the infection by showing increased concentrations of antibodies to the organism would be a useful adjunct to the diagnosis. The use of an agglutination test to detect antibodies to *Staphylococcus epidermidis* in patients with infected cerebrospinal fluid shunts has been described.[3,4] Although a good discrimination between results from infected and uninfected patients was obtained in this generally younger population, this technique has proved less successful in cases of infected total hip replacements, where most patients are over the age of 60.

The use of cell wall teichoic acids from *Staphylococcus aureus* to detect the serological response to infections by these organisms has been successful,[5,6] and we thought that a similar cell wall antigen from a coagulase negative staphylococcus might be used for the detection of prosthetic infection. *Staphylococcus epidermidis* sensu stricto, which contains the cell wall polysaccharide B,[7] is the most common biotype of coagulase negative staphylococcus isolated from clinical sources. An antigen was therefore prepared from an organism known to contain cell wall polysaccharide B, and its potential for use in the serological diagnosis of infection of total hip replacements was investigated.

Material and methods

ANTIGEN PREPARATION

The organism used was isolated from an infected total hip replacement. It was biotyped as *Staphylococcus epidermidis* using API Staph. (API Laboratories Ltd) and was shown to contain cell wall polysaccharide B. The antigen was prepared as described previously.[8] Briefly, this consisted of extracting 20 g of organisms with 0·07M phosphate buffer at pH 6·5; precipitating with ethanol; and, after dialysing against distilled water, lyophilising the resulting polysaccharide.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

An indirect ELISA described previously[9] was used and the method was based on that detailed for viral antigens[10] with the following exceptions: the serum was incubated for 90 min at 37°C and the substrate, at a concentration of 0·25 g/l, was incubated for 1 h at room temperature. The antigen was used at a dilution of 10 mg/l and the samples of serum were diluted 1/400 for use in the test.

The antibody concentration in each sample of serum was expressed as a fraction of the optical density obtained with a positive control serum tested on the same microtitre plate.

Serum samples from 88 patients about to undergo surgery at the Royal National Orthopaedic Hospital were tested using the method described to assess the concentration of antibody in an uninfected population. The ages of the patients ranged from 4 to 84 years with average age of 56·9 years. In 95% of cases the serum from these patients gave an ELISA result of less than 0·6, and this was therefore taken to be the upper limit of normal.

Case report

A 73 year old woman underwent a right total hip replacement for osteoarthritis in May 1976. Her immediate postoperative course was uneventful, but later in the same year she noticed that her hip was clicking when she walked. In May 1977 she complained of pain in her hip and a radiograph showed changes that were thought to indicate infection. At this time, she had an erythrocyte sedimentation rate of 48 mm in the first hour (Westergren). From this stage onwards the hip began to deteriorate clinically, and it was removed in May 1979. At operation, there was no pus present, although much granulation tissue was seen. Both the acetabular and the femoral components were loose. Immediately after removal the prosthesis was sent to the laboratory, where it was examined as described previously.[11] A heavy growth of coagulase negative staphylococci was obtained from the broth used to wash the prosthesis, and this was defined by API Staph as *Staphylococcus epidermidis* (API code 6606113). The Table shows the results of sequential serological studies on this patient.

Discussion

The increase in erythrocyte sedimentation rate in patients with infected total hip replacements has been described previously.[12,13] Although this test on its own is simple to perform and a fairly reliable indication of infection, it is non-specific with regard to the causative organism. Therefore, the availability of an additional test such as the ELISA described here, which allows the detection of specific antibody concentrations, may offer an advantage. In this particular case, the antibody concentrations were considerably higher than the

Table *Results of sequential serological studies on patient described in case report*

Months after total hip replacement	*ELISA (OD)*	*Erythrocyte sedimentation rate (mm in the first hour)*
0	0·205	4
12	1·19	48
13	1·19	50
14	1·01	42
16	1·14	23
18	1·4	55
22	1·11	38
26	1·37	49
38	0·82	15
41	0·75	5
43	0·54	4
49	0·45	3
61	0·37	8

The prosthesis was removed in month 36.