OCCASIONAL REVIEW

Coagulase-negative staphylococci*

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Laboratory assessment in the epidemiology of infections caused by coagulase-negative staphylococci

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One increasingly important group of diseases is that caused by organisms normally living in harmony with man, either within the normal flora or in the immediate environment. Disease caused by these organisms nearly always is associated with an abnormal circumstance such as surgical implantation of a foreign body, medical interference with host defences or an overwhelming dose of the organism bypassing the defences, often through failure of a mechanical device. Coagulase-negative staphylococci cause infections when foreign bodies are implanted (Marples, 1984) or when there are massive medical insults to host defences, as in the outbreak of coagulase-negative staphylococcal infection associated with extracorporeal-circulation machinery described by Lathrop *et al.* (1978). When a species that appears to be the cause of an infection is a frequent or universal member of the normal flora or of the environment, simple epidemiological methods are unrewarding in tracing sources but may be valuable in identifying susceptible targets. The laboratory-based sub-division of the species is essential to trace potential sources and routes of transmission of the infecting strain.

Coagulase-negative staphylococci are universally found on human skin and usually several biotypes can be detected on any individual. Infections and epidemics, however, are usually caused by a single

strain. The characteristics of this single strain must be demonstrated and contrasted with those of other strains with the discrimination or repeatability that confirms the belief that an infection exists or persists. The characters that are at present available for such discrimination include conventional species identification, biotyping, phage-susceptibility typing, investigation of carried phages and detailed antibiotic-resistance typing.

Identification and biotyping

Classification of staphylococci and micrococci has always been controversial. From the minimum scheme comprising only *Staphylococcus aureus* and *S. albus*, Baird-Parker (1965) produced a scheme suggesting six biotypes of *Staphylococcus*, with some sub-division, and eight biotypes of *Micrococcus*. Further schemes have been proposed (Kloos *et al.*, 1974; Kloos and Schleifer, 1975) and extended to a 47-biotype scheme (Marples, 1981). The distinction between *Staphylococcus* and *Micrococcus* (Baird Parker, 1974) changed to include most of the original Baird-Parker biotypes within the genus *Staphylococcus*. Regrouping of the biotype schemes into species groups has been started (Kloos *et al.*, 1976) and a firmer basis for epidemiological biotyping has been laid.

The value of these classifications was seen quickly in the understanding of urinary infections with coagulase-negative staphylococci. Biotype SII (*S. epidermidis*) was associated with hospital-acquired post-instrumentation infections and a new disease caused by biotype M3; later described as *S. saprophyticus*, was recognised in young women outside hospitals (Mitchell, 1968; Pead and Maskell, 1977).

The epidemiological value of biotyping was determined by a series of studies that were mostly concerned with the pathogenicity of SII (*S. epidermidis*). Noble (1969) believed that SII was found excessively frequently on damaged skin and we demonstrated similar findings in acne lesions (Izumi

Received 7 Jan. 1986; accepted 30 Jan. 1986.

* A review based upon papers presented in "Coagulase-negative staphylococci", a symposium at the 151st meeting of the Pathological Society of Great Britain and Ireland held at the University of Wales College of Medicine, Cardiff on 3 Jul. 1985.

et al., 1970). Studies of strains from serious infections submitted for a collaborative phage-typing study demonstrated that biotype SII was so frequent (75%) that further typing methods for this biotype or species were needed (Marples and Richardson, 1981). However, the other biotypes were sufficiently infrequent for biotyping to be of epidemiological value. For example, nine isolates from the blood and one isolate from the CSF shunt of one patient were all of biotype SVI (equivalent to *S. capitis*). Despite equivocal phage-typing results and sensitivity to all the antibiotics tested, the identification and the repetition strongly suggested a local infection (Marples, 1984).

Bacteriophage typing

Subdivision of the commonest biotype, SII (*S. epidermidis*), has been possible by phage-typing methods. The scheme used at Colindale is that developed by Dean *et al.* (1973) from the original Dutch phages. Alternative schemes have been developed, notably by the team in Cologne (Pulverer *et al.*, 1975). Several of these schemes were compared in an international collaborative study in which 400 isolates from serious infections, representing a minimum of 271 independent strains, were tested by 12 laboratories for susceptibility in their phage-typing schemes (de Saxe *et al.*, 1981). The results demonstrated inadequacies in all the typing schemes and reproducibility has since been reassessed. Further developments in the field are likely.

Because most strains of staphylococci are lysogenic it is possible to supplement direct phage typing with studies of the host range of the carried phages to form an additional typing system, termed reverse typing (de Saxe and Notley, 1978). Although direct phage typing remains useful, typability is not as high as would be preferred in multiresistant strains, even in strains from endocarditis (Richardson *et al.*, 1984), and reverse typing can be invaluable in discriminating between very similar strains. In the study of an epidemic of endocarditis infections (Marples *et al.*, 1978) the epidemic strain could be clearly distinguished from a similar normal flora strain by reverse typing, in which indicator strains for the carried phage and cross-spotting of induced supernates were both used (de Saxe and Notley, 1978).

Antibiotic-resistance typing

The reputation of antibiotic-resistance typing is, perhaps, lower than can now be justified, although this method is widely applied by clinical microbiolo-

gists. The poor reputation was acquired when few antibiotics were in use and cross-resistance was not understood. Now that a wide variety of antibiotics is available and many specific mechanisms of resistance can be defined in genetic terms, resistance typing can be re-assessed. *S. epidermidis* (SII) may show multiple resistances and the potential for typing has been studied (Richardson and Marples, 1982). For example, one strain in the endocarditis study mentioned above (Marples *et al.*, 1978) was of biotype SII but novobiocin-resistant, a combination sufficiently infrequent to be of epidemiological value. Further studies to determine reproducibility and discrimination are needed but may require international collaboration to accumulate suitable sets of test strains.

Practical applications of typing methods

The techniques of typing coagulase-negative staphylococci are inefficient (phage typing and resistance typing) or slow (biotyping). As with all applications of typing methods, clear questions must be asked and must be answerable. When an apparent outbreak occurs it should be studied to determine the source of the strain, the means of transmission and the effect of preventive and curative therapy. The first priority must be to confirm that an outbreak has occurred. Characterisation of strains related directly to clinically significant infection is the first step (Marples, 1985). When an outbreak is confirmed a search for sources can often be justified, but if the work-load is not to become excessive, some selection of strains for characterisation may be required. The source may be in an operating theatre (Lathrop *et al.*, 1978), or in an intensive care unit (Marples *et al.*, 1978; Houang *et al.*, in press) or may be sporadic. Routes of transmission may be difficult to determine by a retrospective study and prospective studies may be too labour-intensive to contemplate. However, the combination of detailed typing of isolates and epidemiological studies has clearly demonstrated the occurrence of single source outbreaks and of multiple infections. The findings of such studies may have real effects on hospital practice and certainly enhance our understanding of such infections. The efficiency of treatment and prophylaxis is very difficult to quantify but this needs to be done. Coagulase-negative staphylococci are ubiquitous, have the capacity to accumulate antibiotic resistance which may be transmissible to more aggressive species, and may be spread with every staff-patient contact in hospital. Perhaps greater awareness of, and concern for, infections associated with these organisms is needed.

Coagulase-negative staphylococci as skin commensals

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Together with the coryneforms, the coagulase-negative staphylococci form the major part of the skin flora of man and other mammals. The principal site of growth is not known but cocci are seen growing in the mouths of hair follicles and also on the skin surface where they form microcolonies, the size of which varies on the different skin areas. In general *S. epidermidis* (*sensu* Kloos and Schleifer) is the predominant coccus on the head and trunk whereas *S. hominis* predominates on the arms and legs (Kloos *et al.*, 1970). This may be due to the smaller amount of sebum, or to the generally drier nature of the skin, on the arms and legs, or to some unknown factor. There are also differences in the colonisation of individuals in separate geographical areas. Kloos and Noble (Noble, 1981) reported that about 10% of individuals in North Carolina (USA) carried *S. xylosus* but that this species was not isolated from a similar population in London (UK). Coagulase-negative staphylococci are acquired at birth but the carriage rates for all species continue to rise for at least the first 30 weeks (Carr and Kloos, 1977). Unlike *S. aureus* there seems to be no predilection of *S. epidermidis*, or the other staphylococci, for skin damaged by underlying dermatoses, e.g., psoriasis, eczema, rosacea (Noble, 1981).

The exchange of antibiotic-resistance plasmids between coagulase-negative and coagulase-positive staphylococci is now well documented (Jaffe *et al.*, 1980; Naidoo and Noble, 1981; McDonnell *et al.*, 1983; Naidoo, 1984). Naidoo (unpublished data) has shown that 12 of 35 epidemiologically independent gentamicin-resistant coagulase-negative staphylococci could transfer apparently conjugative plasmids to *S. aureus*. All of six *S. hominis* strains, four of 20 *S. epidermidis*, two of six *S. haemolyticus* but none of three *S. saprophyticus* strains could transfer genes. In most instances transfer occurs at a faster rate on human skin or mouse skin than in broth culture but filter experiments achieve rates comparable to those of skin. The conjugative mechanism of these plasmids is not known but seems to require cell to cell contact; it is independent of calcium ions or the presence of DNAase. Bacteriophage activity has not been demonstrated. In the systems studied in detail (Naidoo, 1984) these plasmids are able to mobilise other resistance plasmids (governing resistance to tetracycline, erythromycin or chloramphenicol) that exist as

distinct plasmids in both the donor and recipient strains.

The most recent studies (Naidoo, unpublished data) have shown that *S. epidermidis*, *S. haemolyticus* and *S. hominis* each transfer their own distinct gentamicin-resistance plasmids to *S. capitis*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. simulans* and *S. warneri* fairly readily. Transfer to *S. saprophyticus* also occurs regularly but with less ease, whereas transfer to *S. cohnii* and *S. xylosus*, although it occurs, is a much rarer event.

Virulence characteristics of *Staphylococcus epidermidis*

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In 1950, Elek and Levy failed to detect either α -, β - or δ -haemolysins in a collection of 77 coagulase-negative skin strains of staphylococci but did report the presence of a hitherto uncharacterised haemolysin designated ϵ -toxin in most of the strains examined. More recently Heczko *et al.* (1974) examined 423 strains of *S. epidermidis* isolated from the skin, nose and urine of healthy human volunteers and some 55 coagulase-negative staphylococci which had been isolated from various body fluids of children with either septicaemia, purulent skin lesions, urinary-tract infections or implanted ventriculo-atrial shunts for the relief of hydrocephalus. A greater percentage of the strains isolated from the infected patients produced δ -haemolysin, DNAase and staphylokinase (fibrinolysin) than did strains isolated from healthy individuals. Similarly the examination of 118 isolates from cases of subacute bacterial endocarditis, septicaemia, skin or urinary tract infections (Gemmell and Roberts, 1973) revealed that many of the strains produced up to six distinct toxins and enzymes including both α - and δ -haemolysins, which were clearly distinguishable from each other in tests for specific neutralisation of haemolysis of rabbit and human erythrocytes respectively with rabbit antisera prepared against each purified toxin. Biotypes 1, 2, 3 and 4, as designated by Baird-Parker (1974), showed differences in frequency and toxigenicity amongst a group of isolates from various clinical sources (Males *et al.*, 1975). Of the 228 strains tested, 145 belonged to biotype 1 and > 50% produced DNAase and several proteases, as well as urease and lysozyme. Furthermore, almost 65% hydrolysed various synthetic lipids (Tween compounds) but only 24% produced any haemolysin. The other isolates fell mainly into

biotypes 3 and 4, which had a rather lower incidence of production of enzymes and haemolysins.

Kleck and Donahue (1968) found that δ -haemolysin was produced by the majority of strains isolated either from the noses of healthy carriers or from the blood of patients with endocarditis. All the nasal strains were capable of elaborating the haemolysin when tested by the cellophane overlay technique on agar but only 83% of the clinically significant blood-culture isolates did so. However even these latter strains were more prolific haemolysin producers when this method of culture was used. Culture of coagulase-negative staphylococci in aerated brain-heart infusion broth also can provide significant yields of the haemolysin (Wadstrom *et al.*, 1976). Within 5h, yields of haemolysin of between 16 and 32 HU could be obtained with several strains. Of a wide variety of erythrocyte species tested, those of guinea pig and human origin were most sensitive. This haemolysin has been separated as a protein of mol. wt $\geq 100\ 000$ with an isoelectric point of 4.25 and resembles the δ -haemolysin of *S. aureus* (Newman strain). It is neutralised by specific antiserum prepared in rabbits (Gemmell, 1983). Antibody to both the *S. epidermidis* haemolysin and the *S. aureus* δ -haemolysin neutralised the haemolytic activity and formed a precipitin line with the haemolysin preparation in agarose gels (Turner and Pickard, 1979). The preparation was stable to heating at 60°C and neutralised by egg-yolk lecithin, which indicates that it is almost synonymous with the δ -haemolysin of *S. aureus*.

Amongst a collection of coagulase-negative staphylococci, this haemolysin occurs mainly amongst strains of *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus*. Haemolysin-positive culture supernates from several strains of coagulase-negative staphylococci were subjected to isoelectric focussing in polyacrylamide gels over the pH range 3.5–10.0 and the gels were then overlaid with a 2.0% suspension of human erythrocytes suspended in agarose. Incubation in a moist environment for 2–4 h resulted in the development of several bands of haemolytic activity. With several strains, two distinct bands of haemolytic activity ($pI = 4.5$ and 6.2) were detected whereas in others only one band ($pI = 4.5$) was visible.

Concomitant analysis of the exoprotein profiles of the various strains of coagulase-negative staphylococci revealed that those strains with significant haemolytic activity also elaborated the greatest number of extracellular proteins (table I). In addition to its cytolytic action against human erythrocytes, this haemolysin has been examined for bio-

Table I. Extracellular proteins of different species of coagulase-negative staphylococci

Species*	Mean number of extracellular proteins	Expression of	
		heat-stable haemolysin	cytotoxin (fibroblasts)
<i>S. aureus</i>	22.7	+	+
<i>S. capitis</i>	10.7	—	—
<i>S. cohnii</i>	6.3	—	—
<i>S. epidermidis</i>	14.7	+	+
<i>S. haemolyticus</i>	12.7	+	+
<i>S. hominis</i>	7.0	—	—
<i>S. saprophyticus</i>	10.0	—	—
<i>S. simulans</i>	9.7	+	+
<i>S. warneri</i>	12.0	+(W)	—
<i>S. xyloso</i>	8.0	+(W)	—

+ = enzyme/toxin activity detected; +(W) = weak effect; — = not detected.

* Three strains of each species were tested.

logical activity against tissue-culture cells. An investigation of 50 clinical isolates of coagulase-negative staphylococci revealed that the majority of *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus* strains that produced significant amounts of haemolysin also caused the release of ^3H -uridine from human embryonic lung fibroblasts (Gemmell and Thelestam, 1981). In the same study it was shown that the haemolysin was responsible for this damage and for the cytopathic effect seen when various strains of coagulase-negative staphylococci were grown on semi-solid agar overlaying a monolayer of mouse skin fibroblasts. Significantly, all of the isolates whose culture supernates damaged the integrity of human embryonic lung fibroblasts were also cytopathic in the colony overlay test (C.O.T.). Overall good correlation was obtained between a positive C.O.T. assay, leakage of ^3H -uridine from lung fibroblasts and haemolytic activity against human erythrocytes. Whether such biological activity demonstrated *in vitro* can be reproduced *in vivo* in clinical infections caused by coagulase-negative staphylococci remains to be seen.

Notwithstanding the ability of several strains of coagulase-negative staphylococci to elaborate a biologically-active cytotoxin, there is evidence that such strains also produce *in vitro* other exoenzymes that might play a part in the pathogenicity of these bacteria. Among such recognisable exoproducts are DNAase, fibrinolysin, proteinase and lipase-esterase. Menzies (1977) examined 728 strains of coagulase-positive staphylococci and found that heat-stable DNAase was produced by all of them

whereas in a collection of 307 coagulase-negative staphylococci only one produced heat-stable DNAase. Nevertheless 56 out of 307 (18.2%) of the coagulase-negative staphylococci exhibited some DNAase activity. This DNAase has been fractionated by isoelectric focussing in polyacrylamide gels (Gemmell *et al.*, 1981) and shown to comprise a major enzyme with a *pI* of 10.0 but other protein species are also present. Marked differences in the ability of various coagulase-negative staphylococci to hydrolyse various lipids and esters was recognised (Gemmell and Roberts, 1973). As with haemolysin production, lipase-esterase activity was found more often among the isolates from bacteraemias, endocarditis, abscesses and skin lesions. The nature of the enzyme that was capable of hydrolysing an emulsion of glycerol tributyrates has been studied by isoelectric focussing techniques and two distinct hydrolytic enzymes are distributed among the strains, one of *pI* 6.2 and the other of *pI* 5.1; no strain possessing both enzymes has been found. The *pI* 6.2 enzyme corresponds to a similar enzyme elaborated by *S. aureus* (Brunner *et al.*, 1981) which is a protein of mol. wt 44 000 with biological activity against a wide range of lipid substrates.

We have tried to evaluate the contribution of exoprotein biosynthesis by the various species of coagulase-negative staphylococci to our understanding of their pathogenicity for man and other animals. In this respect we have used the polytoxic *S. aureus* as a model with which to compare the toxigenicity of coagulase-negative staphylococci. In addition to the biological measurement of the various toxins, isoelectric focussing has been used to demonstrate the spectrum of exoproteins elaborated by various staphylococcal species. There are strong similarities between the products of *S. aureus* and *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus*. Immunological relationships have also been recognised between these exoproteins by crossed immunoelectrophoresis (Wadstrom, 1974) and between 15 and 30 protein components (depending on the strain being tested) have been detected in this manner.

In conclusion, there is good evidence that coagulase-negative staphylococci owe at least part of their virulence for man to their ability to elaborate some of the exoproteins produced by *S. aureus*. Some of these are recognised virulence factors and may play a similar role in the pathogenicity of the various coagulase-negative species. In infections with coagulase-negative staphylococci in man, it would be important to recognise whether antibodies to these exoproducts are elaborated before any pathogenic role was attributed to them. Whether coagulase-

negative staphylococci change their virulence characteristics during growth *in vivo*, like *S. aureus* (Beining and Kennedy, 1963), has not yet been investigated although their virulence for experimental animals has been proven (Gemmell *et al.*, 1976; Namavaar *et al.*, 1976).

Adherence of *Staphylococcus epidermidis* to plastic devices

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Infection is a major complication associated with the use of plastic prosthetic devices and intravenous catheters. Coagulase-negative staphylococci are the major causative micro-organisms in these "plastic infections" (Callaghan *et al.*, 1961; Duma *et al.*, 1977; Archer, 1978; Sitges-Serra *et al.*, 1980; Choo *et al.*, 1981; Peters *et al.*, 1981). Some aspects of the pathogenesis of this special type of staphylococcal infection have already been described (Christensen *et al.*, 1982; Hogt *et al.*, 1983) and reviewed (Peters and Pulverer, 1984). The species of coagulase-negative staphylococci involved reflect the species distribution commonly found in human clinical specimens and in the microflora of skin and mucous membranes. Strains isolated from various infected plastic devices revealed *S. epidermidis* to be the leading coagulase-negative staphylococcal species (table II). Some strains were not identifiable by the Kloos-Schleifer scheme but showed physiological properties close to those of *S. epidermidis*. There was no evidence for a selected group of coagulase-negative staphylococci with the ability to cause "plastic infections".

Table II. Features of 59 coagulase-negative staphylococci isolated from "plastic infections"

Source	Number of strains	Species*	Number (out of 59)
Intravenous catheters	47	<i>S. epidermidis</i>	44
CSF-shunts	4	<i>S. hominis</i>	1
Prosthetic heart valves	3	<i>S. haemolyticus</i>	1
Joint (hip, knee) prostheses	5	<i>S. cohnii</i>	1
		not identified	12

* According to scheme of Kloos and Schleifer (1975).

Scanning electronmicroscopy (SEM) of naturally and experimentally infected intravenous catheters was used to describe the features of *S. epidermidis* colonisation; the staphylococcal cell adhered to and grew on the surface of synthetic polymers (Peters *et al.*, 1982). Surface irregularities in the catheter lumen allowed preferential attachment when the catheters were perfused (Locci *et al.*, 1981). With longer incubation (>6 h) microcolonies were formed on the polymer surface, followed by a single and then multiple layers of staphylococcal cells (Peters *et al.*, 1982). After incubation for 12 h the adherent staphylococci began to produce an extracellular slime substance (ESS) (fig. 1), that finally covered the cells completely. In catheters from patients with a related *S. epidermidis* septicaemia, a similar slime matrix of embedded staphylococci up to 120 µm thick has been seen (Peters *et al.*, 1981). Similar results were obtained with transvenous endocardial pacemaker leads and various pure synthetic polymers (Ludwicka *et al.*, 1983, Peters *et al.*, 1984). The attachment of staphylococci to the polymer surface may be mediated via hydrophobic interaction and electrostatic bonding (Hogt *et al.*, 1983; Ludwicka *et al.*, 1984). However, the evalu-

ation of possible attachment *in vivo* is more difficult because the surface properties of the bacterium or the catheter may be altered.

The ESS produced by *S. epidermidis* is not a true capsule but is loosely adherent to the staphylococcal cell. The chemical structure of ESS is still unknown but it is most likely to be a glycoconjugate polymer. The water-soluble ESS is most probably identical with the "mucoid substance" described earlier during staphylococcal colonisation of Spitz-Holter shunts (Bayston and Penny, 1972). A specific marker for the presence of slime is not available. Therefore it is difficult to determine whether all *S. epidermidis* or other coagulase-negative strains can produce slime. Furthermore it remains unknown if the ESS is chemically identical in all strains.

The crude ESS of *S. epidermidis* shows some interesting properties. There is no doubt that slime or a component thereof is involved in adherence. The adherence of hydrophobic and hydrophilic *S. epidermidis* strains to polyethylene was inhibited when the polymer was precoated with crude slime substance (Ludwicka *et al.*, 1984). In-vitro investigations have revealed that ESS protects embedded staphylococci against natural host defences in dif-

Fig. 1. Scanning electronmicrograph showing the early stage (incubation for 12 h) of slime production by *S. epidermidis* adhering to a polyethylene surface.

ferent ways. Human polymorphonuclear leukocytes (PMNL) pre-incubated in increasing concentrations of ESS showed reduced migration towards the two chemotaxins—f.met.leu.phe. and zymosan-activated serum, C5a (Johnson *et al.*, 1984). Interference with a specific neutrophil receptor for C5a may be involved. Preliminary experiments on the uptake and killing of various bacterial strains with ESS, present during opsonisation or uptake, revealed some interference with normal opsonophagocytosis (Regelmann *et al.*, 1984). Bacterial opsonisation seemed to be inhibited in relation to the dose. ESS also inhibited blastogenesis of human peripheral mononuclear cells (Peters *et al.*, 1983; Gray *et al.*, 1984). The proliferative response to two different polyclonal modulators (PHA and BLA) was inhibited, the degree of inhibition depending on the dose of ESS used and the incubation time; the mechanism is still unknown. The significance of these findings *in vivo* is uncertain. ESS-induced protection against antibiotic action may also contribute to the pathogenesis and maintenance of *S. epidermidis* "plastic infections". The slime matrix may serve as a barrier to diffusion inwards of antibiotics and may thus protect the enclosed staphylococci. Furthermore, there is some evidence that ESS may inhibit specifically the action of some antibiotics. The present knowledge about the pathogenesis of *S. epidermidis* "plastic infections" provides some explanation of the clinical picture and course of this type of staphylococcal infection. The failure of the natural host response (inflammation) and antistaphylococcal chemotherapy to eradicate the organism (Archer, 1978) can be explained to some extent by the presence of ESS. It also emphasises the frequent necessity to remove the infected plastic device to limit the infection.

***Staphylococcus epidermidis* and the CAPD host**

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Continuous ambulatory peritoneal dialysis (CAPD) is increasingly used to treat end-stage renal disease in paediatric and adult patients. More than 19 000 patients have been treated by CAPD for periods of up to 10 years (Oreopoulos, 1984). Peritonitis, however, has been and remains the most important complication of CAPD. The majority of patients who enter a CAPD programme suffer from

at least one episode of peritonitis within 24 months; the overall incidence of peritonitis is rarely less than once per 12 patient-treatment months. Gram-positive bacteria are responsible for *c.* 70% of the peritonitis episodes and coagulase-negative staphylococci lead the list with 30–50% of the cases (Rubin *et al.*, 1980; Gokal *et al.*, 1982).

The species of coagulase-negative staphylococci isolated from CAPD peritonitis have not been studied in great detail. Speciation of a total of 34 isolates (table III) from Amsterdam showed that 76% were *S. epidermidis* and 6% were *S. haemolyticus* (Gruer *et al.*, 1984). Predominance of *S. epidermidis* amongst clinical isolates of coagulase-negative staphylococci has been noted before. One would, therefore, be tempted to ascribe a greater pathogenic potential to *S. epidermidis* than to the other species of coagulase-negative staphylococci. This hypothesis, however, needs further experimental support.

Table III. Species of coagulase-negative staphylococci from CAPD patients

Species*	Number of strains isolated (n = 34)
<i>S. epidermidis</i>	26
<i>S. haemolyticus</i>	4
<i>S. hominis</i>	2
<i>S. capitis</i>	1
Not identified	1

* Determined by the API STAPH system.

Little is known about the initial steps in the development of peritonitis. *S. epidermidis* is thought to enter the peritoneal cavity via the lumen of the CAPD catheter (the system is opened four times per day, and bags and tubing may leak) or *S. epidermidis* may grow along the outside of the CAPD catheter, cause tunnel infection, and in this way contaminate the peritoneal cavity (Oreopoulos *et al.*, 1983); scanning electronmicroscopy has demonstrated such growing biofilms on CAPD catheters *in vivo* (Marrie *et al.*, 1983). The ability of *S. epidermidis* to adhere to plastic is well established. Adherence of staphylococci is greatly enhanced if the bacterial cell surface is hydrophobic (Hogt *et al.*, 1983); 20 of 26 (77%) *S. epidermidis* isolates from the Amsterdam CAPD programme had hydrophobic cell surfaces (unpublished observation). Once adherent to a plastic surface *S. epidermidis* may be difficult to neutralise by the phagocytic defence mechanisms of the host. Phagocytosis of

plastic-adherent *S. aureus* was shown to proceed in the absence of opsonins (Lee *et al.*, 1983; Vandenberghe-Grauls *et al.*, 1984). In contrast, more recently phagocytosis of *S. epidermidis* adherent to plastic was shown to be opsonin-dependent; furthermore, hydrophobic strains of *S. epidermidis* are more resistant to opsonisation with serum than are hydrophilic strains.

The CAPD host

CAPD patients can be looked upon as hosts that are immunocompromised at a distinct site, i.e., the peritoneal cavity, only. There is no clear evidence of a generalised systemic reduction in host resistance against infection; however, resistance to infection of the peritoneal cavity is clearly reduced. Increased susceptibility is evidenced by the many episodes of peritonitis caused by micro-organisms that, historically, have been regarded to have a low virulence or, even, to be non-pathogenic in man. Also, the infective dose needed to produce CAPD peritonitis is probably low because gross contamination is rarely seen and bacterial counts of infected peritoneal fluid are usually $<10^3/100$ ml (Rubin *et al.*, 1980). However, contamination of the abdominal cavity probably occurs regularly without developing into peritonitis, indicating that many microbial insults are handled effectively by the peritoneal defence mechanisms (Vas, 1985). Patients can be retrospectively classified as either those who are peritonitis-resistant (no episodes or $<1/\text{year}$) and others who experience frequent bouts of peritonitis. Although *S. epidermidis* peritonitis is usually mild, clinically, some infections persist or recur despite apparently adequate antimicrobial therapy.

Elimination of invading staphylococci is the domain of opsonic proteins, such as immunoglobulins (IgG) and complement factor C3, and phagocytic cells. Normally the peritoneal cavity contains <50 ml of fluid with about 3×10^6 cells/ml. Effluent fluids from the peritoneal cavity of uninfected CAPD patients amount to >2 L with approximately 1×10^4 cells/ml; 70–80% of these cells have the characteristics of peritoneal macrophages (Verbrugh *et al.*, 1983). Further subtyping of the macrophages by cytochemical electronmicroscopy revealed that exudate-type macrophages predominate; this corresponds with the macrophage population found in chronically inflamed peritoneal cavities of experimental animals (Van der Meulen *et al.*, 1984). The ability of CAPD macrophages to phagocytose and kill opsonised *S. epidermidis* appeared intact *in vitro*; the majority of staphylococci are rapidly taken up by the macrophages and 80–90%

Fig. 2. Intercellular growth of *S. epidermidis* in peritoneal macrophages and monocytes from CAPD patients. Opsonised bacteria were incubated with PMNL (---), peripheral blood monocytes (....) or peritoneal macrophages (—) for 1 h and then washed. The cells were reincubated in RPMI-medium containing cefamandole $0.1 \mu\text{g/ml}$. Controls (—) were opsonised bacteria incubated without cells.

of the internalised bacteria are killed after 60 min (Verbrugh *et al.*, 1983). Recent experiments with prolonged incubation revealed, however, that some intracellular *S. epidermidis* may survive and grow within CAPD macrophages even in the presence of extracellular antibiotics at cidal concentrations (fig. 2). Macrophages then serve as sanctuaries for the surviving population of *S. epidermidis* from which infection may recur or persist; such intraleukocytic sequestration may be important in clinical CAPD peritonitis (Buggy *et al.*, 1984). Furthermore, insufficient numbers of peritoneal macrophages may be responsible for the reduced peritoneal resistance to infection. Growth of *S. epidermidis* in CAPD fluids *in vitro* was aborted only when $>5 \times 10^5$ macrophages/ml were added (Dunn *et al.*, 1984; Verbrugh *et al.*, 1984). Also, opsonins needed for phagocytosis of *S. epidermidis* may be absent or reduced in the peritoneal effluents of approximately half the patients studied. Patients without *S. epidermidis* opsonins were subsequently shown to have an almost 10-fold increased incidence of *S. epidermidis* peritonitis (Keane *et al.*, 1984). The peritoneal cavity of CAPD patients can thus be regarded as a true "*locus minoris resistentiae*" because of a relative deficiency of phagocytic cells and opsonins. Current CAPD solutions are lactate-buffered salt

solutions made hypertonic by the addition of 1.5–4.25% glucose; acid is added (final pH 5.0–5.5) to prevent glucose breakdown during sterilisation and storage. Macrophages and blood PMNL perform less well in these solutions and the complement system does not operate at pH values below 6.0 (Verbrugh *et al.*, 1984). We also found that the activity of some antibiotics is severely impaired in CAPD fluids. The ability of the peritoneal cavity to

handle invading *S. epidermidis* may thus be further compromised. Future efforts to prevent *S. epidermidis* peritonitis in CAPD patients should be directed towards correcting CAPD techniques to preserve or to augment the host defences of the peritoneal cavity. These efforts should include the development of catheters that prevent the adherence of *S. epidermidis*.

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