Occurrence of yellow and blue-green fluorescence exhibited by renal tubular cells, red blood cells, and lymphocytes found in urinary sediments of renal allograft recipients immunosuppressed with cyclosporin A

Treatment with cyclosporin for immunosuppression in renal allograft recipients was started in this hospital at the beginning of the year. Cyclosporin is nephrotoxic, but the exact mechanism by which the nephrotoxic effect is excited is unknown. In this preliminary study three cases were examined in an attempt to recognise the effect of cyclosporin on a cytological basis.

Fresh preparations of urinary sediment were stained in the usual way by Giesma, methylgreen pyronin, and Papanicolaou. One preparation was also subjected to study under blue light fluorescence.

During the first two to three days after the allograft when the patients were receiving a high dose of cyclosporin most of the cells in the urinary deposits showed faint yellow fluorescence. By the third day cells had appeared that displayed a variable degree of blue-green fluorescence extending from the nucleus or periphery of the cell to the entire cell. The number of cells exhibiting blue-green fluorescence and the intensity of fluorescence continued to increase as the dosage of cyclosporin was maintained. The types of cells that displayed the blue-green fluorescence were initially tubular cells, renal tissue fragments, lymphocytes, and red cells, if these were present. The cells were identified by phase contrast microscopy. The blue-green fluorescence appeared only when high doses of cyclosporin were given, and on three occasions whole blood monitoring of cyclosporin by radioimmunoassay showed cyclosporin concentrations above 1000 ng/ml. When the dosage of cyclosporin was reduced fewer cells exhibited blue-green fluorescence, and the intensity of the fluorescence was reduced. The urinary deposits from recipients of renal allografts on low maintenance doses of cyclosporin were also examined and no blue-green fluorescence was observed.

The urinary sediments from patients other than recipients of renal allografts, and who were not receiving cyclosporin treatment but were receiving various other drugs were also examined, and no blue-green fluorescence was seen. Cell deposits from other body sites such as pleural effusions and breast cyst fluids, were also examined and blue-green fluorescence was not observed. In all of these samples many cells did show varying degrees of yellow fluorescence that were considered to be non-specific.

Various types of cells were bathed in vitro in solutions of cyclosporin for varying times both at 37°C and 22°C (room temperature) and then examined for evidence of fluorescence. No blue-green fluorescence was seen, which suggested that a metabolite of cyclosporin may have been responsible for the blue-green fluorescence seen in renal cells when high doses of the drug were being given.

We found that is was essential to examine fresh samples of urine as the blue-green fluorescence tended to fade and after two hours had sometimes disappeared altogether. Specimens of urinary sediment were examined on a Laborlux 12 microscope with an HBO 50 watt lamp, an excitation filter 450–490 nm, a beam splitter cut off at 510 nm, and a suppression filter above 515 nm. Cells exhibiting blue-green fluorescence were photographed using Kodak 400 daylight with an exposure time of two minutes.

The discovery of cells that exhibit blue-green fluorescence in the urinary sediments from recipients of renal allografts may be a simple indicator of cyclosporin toxicity. Further studies are in progress.

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Indifferent streptococci in sticky eyes of neonates

We read with interest the paper by Reeder et al:1 over the past 12 months we have attempted to speciate α haemolytic streptococci (Streptococcus viridans) isolated from "sticky" eyes of neonates in our maternity unit. We were prompted to do this because of the frequent isolation of this heterogeneous group of streptococci (15%) among a wide range of other organisms from eye swabs of our babies,2 and particularly because we were uncertain about their clinical importance. Most of the earlier reports had either not commented on the possible pathogenicity or had not speciated these α haemolytic streptococci (apart from pneumococci), and the advent of convenient rapid identification systems such as the API 20 Strept have enabled subdivision of this diverse group of streptococci. We agree with Bone3 that although S viridans is a convenient label for clinical purposes, this is merely a heterogeneous collection of streptococci. Our investigation concentrated on those neonates with sticky eyes whose swabs yielded only α haemolytic streptococci and no recognised bacterial pathogen. Sixty-two babies were identified in this way and because the sticky eye in each case cleared up quickly and without specific topical antimicrobial treatment further culture for chlamydia was not undertaken.

All 62 α haemolytic streptococci were from primary isolation on 7% horse blood agar layered plates and were speicated by the API 20 Strep system (Table). Most species were identified to the genus level at this time.

<table>
<thead>
<tr>
<th>Streptococcus sp</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S mitis (mitior)</td>
<td>30 (48.4)</td>
</tr>
<tr>
<td>S sanguis</td>
<td>14 (22.6)</td>
</tr>
<tr>
<td>S salivarius</td>
<td>7 (11.3)</td>
</tr>
<tr>
<td>S hoois</td>
<td>4 (6.4)</td>
</tr>
<tr>
<td>Unspeciated</td>
<td>7 (11.3)</td>
</tr>
</tbody>
</table>

From these figures it is clear that S viridans is the most commonly identified species, followed by S sanguis, with S hoois and S salivarius each representing only a small percentage of isolates. All 62 isolates were typed as α haemolytic streptococci.

Our experience has been that the API 20 Strep system is a useful tool for the rapid speciation of these bacteria and that the results are easily accepted by parents of neonates. However, we feel that further study is needed to determine the role that these streptococci play in neonatal conjunctivitis.

The source of these streptococci could be...
from the birth canal or the respiratory tract of those handling the baby after delivery, when colonisation would be favoured in the presence of poorly functioning nasolacrimal drainage, or both, or even overgrowth in the increased fluid formed due to any irritation—for example, excess antiseptic applied to the maternal perineum before or during delivery or inflammation caused by other well recognised pathogens.

In the spectrum of bacterial isolates the reported incidence of a haemolytic streptococci has varied between 11–62 3%, and this wide variation adds to the problem of establishing a direct pathogenic role. From our study, however, speciation does not seem to have had a direct bearing on clinical management as our isolates seem to be non-pathogenic or of a very limited pathogenicity, and treatment does not seem to be required.

**References**


**Clinical importance of production of slime by coagulase negative staphylococci in chronic ambulatory peritoneal dialysis**

Coagulase negative staphylococci are an important cause of infections associated with foreign bodies, but deciding whether a particular isolate is responsible for infection or is merely a contaminant can be difficult. Unsuccessful attempts have been made to find a laboratory marker which would correlate with the clinical importance. Recently, production of slime by *Staphylococcus epidermidis* was shown to promote adherence to prosthetic devices, and it has been postulated that the slime substance may protect the organism against host defences. To investigate the importance of production of the slime in vivo we examined episodes of peritonitis caused by coagulase negative staphylococci in patients undergoing chronic ambulatory peritoneal dialysis.

A retrospective analysis of the clinical records of 42 patients was made. All patients were undergoing chronic ambulatory peritoneal dialysis, and coagulase negative staphylococci were isolated from the peritoneal effluent on 115 occasions. Peritonitis was defined as pain or discomfort in the abdomen associated with cloudy peritoneal effluent (>100 white cells/mm³). When these criteria were applied 36 patients (mean age 52 years) were found to have had 91 episodes of peritonitis. The coagulase negative staphylococci were speciated using API Staph (appareils et procédés d’identification) and further characterised by phage type, bio-type, and antibiotic susceptibility pattern. Slime was detected using the method described by Christensen et al.

Species cultured from the 91 episodes of peritonitis included: *S. epidermidis* (73%), *S. haemolyticus* (11%), and *S. hominis* (7%). Slime was detected in 37 strains (41%), all of which were *S. epidermidis*. When peritonitis recurred caused by the same bacterial strain within three to four days after stopping appropriate treatment with antibiotics the peritonitis was labelled as “recurrent.”

Strains were considered to be identical if they had the same species, bio-type, phage type, and antibiotic susceptibility pattern. Eighteen strains were responsible for recurrent peritonitis (two to five episodes) and 45 for uncomplicated peritonitis. There was no increase in the length or severity of peritonitis when slime producing strains of coagulase negative staphylococci were isolated. Recurrent peritonitis, however, was more likely to occur if the strain produced slime (Table); this difference was significant ($\chi^2$ test and Yates’ correction = 6·08, p < 0·02).

The results of this preliminary survey, show that strains of coagulase negative staphylococci, which produce slime, are more likely to be associated with recurrent peritonitis than strains that do not produce slime. Isolating a productive strain from the peritoneal effluent was associated with a 50% chance of recurrence, compared with only a 17% chance when the isolate did not produce slime. This observation may be related to the superior adherence properties of the strains that produce slime and their ability to encase themselves in a protective matrix of slime substance on artificial surfaces.

Our results suggest that such production may be a useful prognostic marker. Laboratories concerned with the care of patients receiving chronic ambulatory peritoneal dialysis might usefully examine isolates of coagulase negative staphylococci from such patients for production of slime. Whether such advice should be extended to include isolates from infections associated with other prosthetic devices is unclear and requires further study.

We thank the Division of Hospital Infections, Central Public Health Laboratory, London, for performing the biotype and phage type investigations.

**References**

1. Christensen GD, Simpson WA, Bisno AL, *et al.* Adherence of slime producing strains of *Staphylococcus epidermidis* to an artificial surface.

**Table: Correlation of peritonitis episodes with presence or absence of slime**

<table>
<thead>
<tr>
<th>Peritonitis</th>
<th>Slime</th>
<th>No slime</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Not recurrent</td>
<td>11</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>41</td>
<td>63</td>
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