DISINFECTION OF A CENTRALISED DIALYSATE SYSTEM WITH DIETHYL PYROCARBONATE

H.-G. SIEBERTH*, H. GENTH** and G. SIEMON*

*Medizinische Universitätsklinik, Köln and **Farbenfabriken Bayer, Werk Krefeld Uerdingen, Federal Republic of Germany

Heavy growth of microorganisms usually occurs in a dialyzing bath. According to Sherris et al. (1961) the growth should not exceed a level of $10^3$ germs/ml. It can be inhibited by cooling of the dialyzing solution to 10 to 15°C (Pendras et al., 1961; Cole et al., 1962), but at lower temperatures the dialysance decreases significantly. Bacterial growth is enhanced by nitrogen containing substances removed from the blood during dialysis. The single pass (Fry and Hoover, 1964) prevents accumulation of these substances in dialyzing solution. Growth of fungi and bacteria particularly occurs in the long tubing of centralised dialysate systems. Therefore, cleaning and disinfection with formalin or ZephironR, which usually is quite fussy, is required. Because of the high toxicity of the substances, the dialysate system must be sufficiently washed before dialysis.

Diethylpyrocarbonate (BaycovinR, Bayer-Leverkusen), a water clear, fruit-like smelling liquid, is a highly effective germ killing agent. When used in a concentration of 300 to 500 mg/l practically all fungi and bacteria will be destroyed (Genth, 1964). Diethylpyrocarbonate can be easily dissolved in alcohol and poorly in water. In order to disinfect watery media the substance must be previously dissolved in alcohol or directly sprayed into water. In water, within a few hours, hydrolysis to alcohol and CO₂ of this substance takes place, being dependent on temperature and pH.

\[ C_2H_5O \ce{O} + \ce{H2O} \rightarrow 2C_2H_5OH + 2\ce{CO2} \]

At pH 7 and 15°C Baycovin concentration decreases to less than 1% of the initial concentration within 7 to 10 hours. Because of these favourable properties diethylpyrocarbonate has been used in cold sterilisation of beverages (Pauli and Genth, 1966). It therefore appeared as well suited for disinfection of centralised dialysate systems.

METHODS

The dialysate system of our hospital consists, as shown in Fig. 1, of a proportion pump which mixes an electrolyte-glucose concentration and softened water in a proportion of 1 : 35. The dialyzing solution is then delivered into a 100 l tank. The pump is controlled by a float switch. The dialyzing solution flows out of this tank through tubings to the rheothermostats and to the dialyzers. At the end of each dialysis 600 mg of diethylpyrocarbonate per litre of dialyzing solution will be automatically added by means of a delivery pump and a spraying jet behind the proportion pump (STM-Gerät, Orllta, Giessen). The complete system can be filled with Baycovin-containing dialyzing solution within 10 minutes. The next dialysis can be started without cleaning the system 7 to 10 hours later.

Before introduction of this kind of disinfection, the system was cleaned mechanically and
chemically and disinfected with Zephirol once a week. Samples of dialyzing solution were taken from the inlet of the Kill dialyzer several days a week, before starting dialysis. These examinations were carried out before and after the introduction of diethylpyrocarbonate. In vitro experiments were carried out to determine the bacterial activity of diethylpyrocarbonate in relation to time of exposure and concentration of diethylpyrocarbonate.

Bacteria of 17 different agar slant mixed cultures were suspended in Ringer’s solution at a final concentration of $10^{11}$ germs/ml. To tubes containing 20 ml of this solution various amounts of a 20% alcoholic diethylpyrocarbonate stamp solution were added, so that the final concentration in Ringer’s solution was in the range of 100 to 500 μl/l. After storage for 2, 5 and 24 hours at 37°C, Ringer’s solution was suctioned through a membrane filter (type col). The membranes were then incubated on standard bouillon agar (Oxoid M221).
Fig. 2. Germ count in dialyzing fluid before and after disinfection with diethylpyrocarbonate.

RESULTS

During weekly disinfection with Zephirol dialysate germ count ranged between 800 and 700,000 germs per ml. The following germs were found: fungi of the candida group, gram-positive and gram-negative bacteria, e.g. *Aerobacter aerogenes*, *Staphylococcus aureus*, *Staphylococcus albus*, *Pseudomonas pyocyanea*, or *Escherichia coli*. After disinfection with Baycovin germ counts in the dialyzing fluid decreased to 0-80 germs/ml (Fig. 2). Sedimentation of fungi within the tubing system was no longer observed. The results of the *in vitro* experiments are shown in Table I. After a 2 to 5 hours' exposure of the bacterial suspension to diethylpyrocarbonate in a concentration of 500 μg/l Ringer's solution, a considerable decrease in the germ count was recognised. After 24 hours and a concentration of 300 μg/l all germs were destroyed. The effectivity of diethylpyrocarbonate is dependent on the number of bacteria/ml (Pauli and Genth, 1966). In our experiments germ counts of $10^{11}$ were used. These are significantly higher germ counts than those in dialyzing fluid which are in the range $10^5$.

**TABLE I**

<table>
<thead>
<tr>
<th>Diethylpyrocarbonate μg/l</th>
<th>2 hours</th>
<th>5 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>200</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>300</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>400</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>500</td>
<td>(+)</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Ko+</td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Ko−</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Bacterial growth: strong ++++, sterile —
Start concentration $10^{11}$ bacteria
The disinfection with diethylpyrocarbonate results in an almost complete elimination of germs from the dialyzing fluid. The procedure of disinfection is automatic and results in a significant diminution of work. The substance can be directly added to the dialyzing fluid because of the hydrolysis of diethylpyrocarbonate to alcohol and CO₂; rinsing of the system after disinfection is no longer necessary.

ACKNOWLEDGEMENT

We thank Prof. Pulverer of the Institute of Bacteriology, University of Cologne, for kindly determining germ counts in the dialyzing solution.

REFERENCES


