Multiplication of Nosocomial Pathogens in Intravenous Feeding Solutions

S. M. GELBART, G. F. REINHARDT, AND H. B. GREENLEE

Research Service and Surgical Service, Veterans Administration Hospital, Hines, Illinois 60141, and Department of Microbiology, Loyola University Dental School and Department of Surgery, Loyola University Stritch School of Medicine, Maywood, Illinois 60153

Received for publication 10 August 1973

A major problem in total parenteral nutrition is sepsis, particularly that caused by Candida. Studies of four solutions, a casein hydrolysate, a fibrin hydrolysate, and two crystalline amino acid solutions, show that the protein hydrolysate solutions appear to be highly selective for Candida over bacteria, whereas the crystalline amino acid solutions are not. These findings suggest that the crystalline amino acid preparations may offer a partial solution to the infection problem by minimizing the contribution of the solution as a reservoir for organism multiplication, because they retard the growth of both bacteria and Candida.

Total parenteral nutrition (TPN) can support and often salvage patients that would starve because of their inability to sustain themselves on oral nutrients (6). Although most reports dealing with problems encountered during the use of this technique have focused on its metabolic consequences, septicemia is generally regarded as its major complication (5). Various estimates of septicemia rates ranging from 6 to 27% (9) have been reported. The majority of these septicemias have been due to one organism, Candida. More disturbing was the observation that 18 of 22 patients that developed fungal septicemia in one report died, and that this complication was the primary cause of death in 15 (2). This high rate of septicemia has lead some physicians to believe that this risk of infection may outweigh the value of TPN therapy (7).

One obvious concern is that the nutrient solution serves as a reservoir for organism multiplication. Even if the solutions are sterile when they arrive from the pharmacy, several studies (4, 9) have demonstrated that they are prone to contamination from extrinsic sources. Deeb and Natsios (4) reported that fungi (Candida albicans) and bacteria (Staphylococcus aureus, Klebsiella-Enterobacter group, and most Pseudomonas aeruginosa strains) show growth in a 1:2 dilution of a 24-h thiglycolate broth culture of each organism in TPN solution (5% protein hydrolysate and 5% dextrose, 750 ml; 50% dextrose, 300 ml; electrolyte and vitamin supplement, 10 ml). However, they reported poorer growth of bacteria than fungi when using a 1:20 dilution, i.e., a smaller inoculum. Brennan et al. (1) studied the growth of C. albicans in protein hydrolysates (casein hydrolysate, Travenol Laboratories, Morton Grove, Ill.; fibrin hydrolysate, Abbott Laboratories, North Chicago, Ill.). They found that Candida multiplied significantly in both solutions at 30 and 37 C, but not at 4 C.

Our experiments were designed to compare multiplication of bacteria and Candida in various types of TPN solutions, utilizing the newer crystalline amino acid solutions as well as fibrin and casein hydrolysates. The solutions were formulated as recommended by the manufacturers for patient administration to simulate the clinical setting.

This paper was presented in part at the 73rd Annual Meeting of the American Society for Microbiology, 6–11 May 1973, Miami Beach, Fla.

MATERIALS AND METHODS

Organisms. All strains of bacteria and fungi utilized in these experiments were clinical isolates except for one strain of S. aureus (ATCC 12600, American Type Culture Collection, Rockville, Md.). These isolates were from blood cultures obtained from patients who had developed septicemia concomitant with intravenous (i.v.) therapy. They were identified by the Clinical Laboratories, Veterans Administration Hospital, Hines, Ill., and the Departments of Microbiology of Loyola University’s Schools of Medicine and Dentistry, Maywood. Ill. Strains of Erwinia herbicola, Enterobacter cloacae, and Klebsiella pneumoniae
were obtained from the Center for Disease Control, Atlanta, Ga. (We recognize that the taxonomy of Erwinia is still under debate and have chosen to keep the designation Erwinia over the newer designation Enterobacter agglomerans to remain consistent with earlier studies on this epidemic [8].) These three organisms were isolated from i.v. solutions during an outbreak of septicemia caused by contaminated i.v. solutions (8).

**Solutions tested.** TPN solutions utilized in this study were a casein hydrolysate (5% Amigen in 5% dextrose, Travenol Laboratories), a fibrin hydrolysate (5% Aminosol in 5% dextrose, Abbott Laboratories), crystalline amino acid mixture A (8.5% FreAmine, M McGaw Laboratories, Glendale, Calif., and crystalline amino acid mixture B (10% Travasol, Travenol Laboratories). The formulation for each solution is presented in Table 1. For comparison, we also tested 5 and 50% dextrose solutions (D5W and D50W, Abbott Laboratories) and nutrient broth (Difco Laboratories, Detroit, Mich.).

**Culture methods.** All organisms were grown in nutrient broth to a concentration of $10^8$ to $10^9$ colony-forming units (CFU)/ml. This starting culture was shaken on a Vortex mixer to break up clumps, diluted 1:100 in 0.9% saline, and incubated for 18 to 24 h at 37°C. This was done to deplete the organism's metabolic pool. The saline suspension was then diluted 1:100 in 0.9% saline, and 1.0 ml of the last dilution was added to 9.0 ml of each of the TPN solutions, 5

<table>
<thead>
<tr>
<th>Table 1. Total parenteral nutrition solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>TPN solutions</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
</tr>
<tr>
<td>800 ml of 5% casein hydrolysate in 5% dextrose; 300 ml of 50% dextrose</td>
</tr>
<tr>
<td>Fibrin hydrolysate</td>
</tr>
<tr>
<td>750 ml of fibrin hydrolysate in 5% dextrose; 250 ml of 50% dextrose</td>
</tr>
<tr>
<td>Crystalline amino acid (mixture A)</td>
</tr>
<tr>
<td>500 ml of solution A (8.5% amino acids); 250 ml of 50% dextrose</td>
</tr>
<tr>
<td>Crystalline amino acid (mixture B)</td>
</tr>
<tr>
<td>250 ml of solution B (10% amino acids); 250 ml of 50% dextrose</td>
</tr>
</tbody>
</table>

**Electrolytes (mEq)**

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>PO_4^-</th>
<th>Ca^2+</th>
<th>Mg^2+</th>
<th>Cl^-</th>
</tr>
</thead>
<tbody>
<tr>
<td>K^+</td>
<td>16</td>
<td>4</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Na^+</td>
<td>28</td>
<td>13</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>---</td>
<td>4.575</td>
<td>6.25</td>
<td>4.25</td>
<td>4.25</td>
</tr>
</tbody>
</table>

**Total nitrogen (g)**

<table>
<thead>
<tr>
<th>TPN solutions</th>
<th>Volume</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein hydrolysate</td>
<td>1.100 ml</td>
<td>40 g of amino acids; 190 g of dextrose</td>
</tr>
<tr>
<td>Fibrin hydrolysate</td>
<td>1.100 ml</td>
<td>35 g of amino acids; 225 g of dextrose</td>
</tr>
<tr>
<td>Crystalline amino acid (mixture A)</td>
<td>1.000 ml</td>
<td>42.5 g of amino acids; 250 g of dextrose</td>
</tr>
<tr>
<td>Crystalline amino acid (mixture B)</td>
<td>1.025 ml</td>
<td>26.5 g of amino acids; 250 g of dextrose</td>
</tr>
</tbody>
</table>

**Fig. 1.** Growth of Staphylococcus aureus in various solutions at 37°C.
and 50% dextrose, and nutrient broth. These solutions were then incubated at 37 C, and samples were taken hourly for the first 8 h and at 24 h. Incubation was at 37 C to obtain optimal multiplication of the organisms. Sampling time was based on the fact that the maximum administration time for a single container of fluid was 8 h. The 24-h sample was taken to reveal multiplication after a lag due to initial starvation. 

Viable counts from these samples were made by plating serial tenfold dilutions onto nutrient agar (Difco, 1.5%) in triplicate. After 48 h of incubation at 37 C, counts were made on a Quebec-type colony counter (American Optical Corp., Buffalo, N.Y.).

Interpretation of data. Triplicate counts of at least two separate experiments were made for each strain of each organism for each solution. The results were normalized to the lowest size inoculum, and the composite values of viable counts for t = 0 h (the time of initial inoculation into the test media) and t = 24 h were compared by Student's t test to see if they represented different populations, i.e., if a significant increase had occurred. The mean values of the composite of all viable counts for each species for each time were plotted against time.

RESULTS

The viability of all organisms was ascertained by luxuriant growth in nutrient broth within 24

Fig. 3. Growth of Enterobacter aerogenes in various solutions at 37 C.

Fig. 2. Growth of Escherichia coli in various solutions at 37 C.

Fig. 4. Growth of Enterobacter cloacea in various solutions at 37 C.
h. S. aureus strains ATCC 12600 and two clinical isolates all showed a significant decrease in viable count in all i.v. solutions within 24 h (Fig. 1). Two strains of Escherichia coli showed no significant multiplication in any of the i.v. fluids within 24 h (Fig. 2).

Members of the tribe Klebsiellae showed no significant multiplication in any of the TPN solutions or in 50% dextrose, although all strains of Enterobacter aerogenes (two strains), E. cloacae, E. herbicola, and Serratia marcescens (two strains) demonstrated significant multiplication in 5% dextrose (Figs. 3 to 6). K. pneumoniae, however, showed no significant multiplication in any of the i.v. solutions (Fig. 7).

P. aeruginosa revealed a pattern similar to that observed for E. coli. There was no significant increase in viable count in the composite of the three strains studied in any of the i.v. solutions (Fig. 8); however, one strain did show exponential growth in 5% dextrose. Pseudomonas cepacia, however, presented a similar pattern to that observed for most of the members of the tribe Klebsiellae. The two strains studied grew very well in 5% dextrose, but showed no significant multiplication in any of the other i.v. solutions (Fig. 9).

In summary, none of the bacteria tested displayed significant multiplication in any of the TPN solutions within 24 h, although many could grow in 5% dextrose.

In contrast, three strains of the fungus C. albicans showed significant multiplication in both casein hydrolysate and fibrin hydrolysate solutions within 24 h, but showed no significant increase in viable count in the newer crystalline amino acid preparations or in dextrose solutions (Fig. 10).

**DISCUSSION**

It is evident from our data that the protein hydrolysate solutions appear to be selective for Candida over bacteria. This apparent selectivity could be one possible explanation for the frequency of Candida sepsis as compared with bacterial sepsis when the hydrolysate solutions are employed in long term i.v. feeding. Our findings also demonstrate the possible efficacy of the crystalline amino acid preparations in minimizing the contribution of the solution as a reservoir for organism multiplication, because they retard the growth of both bacteria and Candida for 24 h.

Several investigators have reported that cer-
Figure 7. Growth of *Klebsiella pneumoniae* in various solutions at 37°C.

Figure 8. Growth of *Pseudomonas aeruginosa* in various solutions at 37°C.

Figure 9. Growth of *Pseudomonas cepacia* in various solutions at 37°C.

Tain bacteria will grow in certain TPN solutions (4, 9, 10). These data, which admittedly conflict with ours, are based on observations for longer time periods, i.e., greater than 24 h, and the discrepancy may in part result from methodological differences. Our major deviation in methodology was that we employed starved organisms in the stationary phase of growth. Nevertheless, the finding that all of the organisms showed luxuriant growth in a suitable nutrient broth which contained no blood, serum, or other special enrichment, and that many of the gram-negative bacteria demonstrated substantial growth in 5% dextrose should evince that the starvation did not overly impair the organisms' ability to multiply in a suitable medium. Almost all studies of this nature that give initial inoculum size employ initial viable counts of approximately $10^6$ colony-forming units/ml (8, 11), and we have followed their precedent. The high inoculum facilitates reproducible recovery of the organisms...
when they are transferred through the various media.

The finding that many gram-negative bacteria grow in 5% dextrose solutions confirms the work of Felts et al. (8). Moreover, our studies suggest that 5% dextrose solutions are selective for specific gram-negative bacteria, e.g., Enterobacter, Serratia, and Pseudomonas, over Staphylococcus, or Candida. The failure of Staphylococcus to grow in these solutions can most probably be attributed to their requirement for specific amino acids and vitamins (3); however, no specific amino acid or vitamin requirement has been demonstrated for Candida. In our studies, none of the TPN solutions was supplemented with vitamins or additional electrolytes. The possibility that these supplements influence bacterial growth in TPN solutions has not been investigated. Currently at our institution, vitamins and additional electrolytes are generally given through a separate parenteral route along with other i.v. fluids.

At this time, no well-substantiated reason can be given to explain why bacteria do not multiply in TPN solutions under our experimental conditions; however, we speculate that the inordinately high osmolality of these solutions may be a factor. It should be noted that our observations are based on studies of at most three strains of each species, and although no significant strain variation was observed, our data may not give an accurate picture for all strains. Nevertheless, we feel that employing crystalline amino acid solutions in place of protein hydrolysates should contribute to the control of the infection problem by minimizing the contribution of the solution as a reservoir for organism multiplication.

ACKNOWLEDGMENTS

This research was supported by the Research Service, Veterans Administration Hospital, Hines, Ill.

We thank Regina Adams for her excellent technical assistance. We also would like to thank Thomas Bird, Clinical Microbiologist, Veterans Administration Hospital, Hines, Ill., and James Kraiss, Loyola University's Medical and Dental Schools, Maywood, Ill., for their cooperation in providing several clinical isolates.

We also acknowledge the cooperation of the following companies who provided the TPN solutions and technical information: Travenol Laboratories, Morton Grove, Ill.; McGaw Laboratories, Glendale, Calif.; and Abbott Laboratories, North Chicago, Ill.

LITERATURE CITED


