GROWTH OF BACTERIA IN ENTERAL FEEDING SOLUTIONS

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SUMMARY. Solutions of Clinifeed ISO, Triosorbon, Vivonex Standard (full- and half-strength) and Vivonex HN were experimentally contaminated with two strains each of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Escherichia coli* and *Enterobacter cloacae* at concentrations of 10^2 – 10^3 organisms/ml. Samples were incubated at 4, 25 or 37°C and viable counts were made at 0, 4, 8 and 24 h.

No increase in numbers of any of the organisms was observed in any of the feeds during 24 h at 4°C. All organisms multiplied rapidly in Clinifeed ISO and in Triosorbon at 25 and 37°C. There was less rapid growth in half-strength Vivonex Standard at 25°C, although at 37°C all strains multiplied rapidly except for the two *S. aureus* strains, the growth of which was inhibited in half-strength Vivonex Standard at both 25 and 37°C. In full-strength Vivonex Standard at 25°C, only *P. aeruginosa* showed any increase in numbers during 24 h, whereas *P. aeruginosa*, *K. aerogenes* and *E. cloacae* all multiplied at 37°C. None of the test organisms multiplied in full strength Vivonex HN at any of the temperatures studied.

The results of the study show that bacteria survive and may multiply even in feeds with low pH and high osmolality, and emphasise the importance of strict hygiene during the preparation and handling of all enteral feeds.

INTRODUCTION

The rapid multiplication of microorganisms in enteral feeds has been reported by several authors (White *et al.*, 1979, Furtado *et al.*, 1980; Simmons, 1981; Bastow *et al.*, 1982; Gibbs, 1983; Anderton, 1984). However, both Furtado *et al.* (1980) and Stanek *et al.* (1983) demonstrated that not all enteral feeds support microbial growth. Stanek *et al.* (1983) reported that when samples of an elemental diet were artificially contaminated with *Staphylococcus aureus*, *Salmonella enteritidis*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Candida albicans* or *C. tropicalis* (all at a concentration of 10^2 organisms/ml) and incubated at 22°C for 24 h, only *P.*

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aeruginosa and the *Candida* spp. showed any growth; the numbers of the other species either remained constant or, in the case of *Camp. jejuni* was reduced. Furtado *et al.* (1980) also reported that in full strength Vivonex HN, *S. aureus* and *S. epidermidis* remained viable but did not proliferate and that neither *P. aeruginosa* nor group-B streptococci survived when incubated at 37°C for 24 h.

This study was undertaken to compare the rates of growth of various common bacterial contaminants (Anderton, 1983) in several commercially available enteral feeds.

MATERIALS AND METHODS

Feeds. The feeds used were: Clinifed ISO, a UHT-processed, low residue, milk-based, complete liquid feed (Roussel Laboratories Ltd, Wembley Park, London); Triosorbon, a dried, low residue, whole protein, lactose-free feed (E. Merck Ltd, Alton, Hants); Vivonex Standard, a dried, elemental, minimal residue preparation (Eaton Laboratories Ltd, Woking, Surrey); and Vivonex HN, a dried, elemental, high nitrogen, minimal residue preparation.

Dried feeds were reconstituted with sterile water. To ensure that there was no variation in the composition of the feed samples used for different bacteria, bulk samples of each feed were thoroughly mixed in separate sterile 2000-ml conical flasks for each experiment; a 20-ml sample removed aseptically and the pH measured. Portions of the feeds (100 ml) were then transferred to sterile 250-ml conical flasks. The composition, pH and osmolarity of each of the feeds are shown in the table.

Bacterial strains. Two strains of each of the following species were used: *S. aureus* (NCTC nos. 10652 and 10657), *P. aeruginosa* (NCTC nos. 6750 and 10332), *Enterobacter cloacae* (NCTC nos. 10005 and 8155), *Escherichia coli* (NCTC nos. 8007 and 8603) and *Klebsiella aerogenes* (NCTC nos. 8172 and 9997). All strains were grown in Nutrient Broth No. 2 (Oxoid) for 18–24 h at 37°C. The bacterial suspensions were then diluted in 0.1% peptone water (Oxoid) to give a concentration of approximately 10^4 cfu/ml. This provided the inoculum.

Procedure. Each bacterial inoculum (1.0 ml) was transferred to each of three separate flasks containing 100 ml of freshly prepared feed, to give an initial count of 10^2 – 10^3 cfu/ml of feed (Bastow *et al.*, 1982). After careful mixing a viable count was made on a sample of feed from each flask. Flasks were then incubated at 4, 25 or 37°C, and samples taken for viable counts at 4, 8 and 24 h. These temperatures were selected to represent the temperatures at which the feeds would be held in normal practice, i.e., refrigerator temperature, ambient (ward) temperature and the patients' body temperature.

Viable counts were made on serial tenfold dilutions of each sample in 0.1% peptone water;

TABLE I

Composition, pH and osmolarity of enteral feeds diluted to strengths suitable for administration to patients (Values given are those quoted by the manufacturer except for pH, which was determined experimentally)

Feed	Composition (g/100 ml)				Osmolarity (mosm/L)	pH	Preparation of feed
	Protein	Amino acids	Carbohydrate	Fat			
Clinifed ISO	2.8	...	13.1	4.1	270	6.8	Liquid, no dilution
Triosorbon	4.0	...	11.9	4.0	238	6.9	1 sachet of powder and water to volume of 400 ml
Vivonex Standard (full-strength)	...	2.06	23.0	0.15	550	5.4	1 sachet of powder and water to volume of 300 ml
Vivonex Standard (half-strength)	...	1.03	11.5	0.08	265	5.4	1 sachet of powder and water to volume of 600 ml
Vivonex HN	...	4.44	21.10	0.09	800	4.4	1 sachet of powder and water to volume of 300 ml

0.1 ml of each dilution was spread over the surface of pre-dried plates of Plate Count Agar (PCA; Oxoid). The plates were then incubated aerobically at 37°C for 24 h. Counts were made in triplicate and the mean count expressed as cfu/ml. The experiment was repeated three times.

Control samples of uninoculated feeds were examined simultaneously.

RESULTS

The two strains of each bacterial species grew at similar rates in all experiments and the results given are the average counts obtained. The results for all feeds except Triosorbon are presented in the figure. No organisms were detected in the control flasks of Clinifed ISO, Vivonex Standard (full- and half-strength) or Vivonex HN. In each of the three experiments, the control flasks of Triosorbon were found to be contaminated with aerobic sporeforming bacilli; the counts increased during 8 h from undetectable to 10^1 cfu/ml at 25°C and 10^2 cfu/ml at 37°C. After 24 h counts had risen to 10^7 and 10^9 cfu/ml at 25 and 37°C respectively.

All test organisms multiplied rapidly in Clinifed ISO and Triosorbon; the counts increased during 8 h from 10^2 – 10^3 to 10^4 – 10^5 cfu/ml at 25°C and to 10^5 – 10^6 cfu/ml at

Fig. 1.—Growth of various species of bacteria in Clinifed ISO, Vivonex and Vivonex HN at 4, 25 and 37°C. *E. cloacae* ■—■; *P. aeruginosa* △—△; *K. aerogenes* ○—○; *S. aureus* □—□; and *Esch. coli* ●—●.

37°C, although it was difficult to make accurate counts of the numbers of test organisms in Triosorbon because of the growth of contaminants and 24-h viable counts were not made in this feed. After 24 h, counts in Clinifeed ISO were 10^7 – 10^9 and 10^8 – 10^{10} cfu/ml at 25 and 37°C respectively.

At 37°C, the rate of growth of *E. cloacae* and *K. aerogenes* strains in half-strength Vivonex Standard was similar to that in Clinifeed ISO. Growth of *Esch. coli* and *P. aeruginosa* in half-strength Vivonex was slightly inhibited initially, only reaching 10^4 cfu/ml after 8 h. However, growth was then more rapid, reaching 10^8 and 10^9 cfu/ml respectively after 24 h. Half-strength Vivonex inhibited the growth of both strains of *S. aureus* at 25 and 37°C; there was very little increase in numbers after 8 h and the final count at both temperatures was only 10^3 cfu/ml. Growth of the remaining test organisms was slower at 25°C in half-strength Vivonex than in Clinifeed ISO; counts after 8 h were in the range 10^3 – 10^4 cfu/ml and those at 24 h in the range 10^6 – 10^8 cfu/ml.

In full-strength Vivonex Standard the growth of all the test organisms was inhibited at 25°C; there was no increase in numbers after 8 h or 24 h with the exception of *P. aeruginosa* which showed an increase in numbers from 10^3 to 10^6 cfu/ml between 8 and 24 h. *S. aureus* and *Esch. coli* were also inhibited at 37°C; numbers only increased from 10^2 to 10^3 – 10^5 cfu/ml in 24 h. However, *P. aeruginosa*, *K. aerogenes* and *E. cloacae* multiplied to give counts of 10^4 – 10^5 after 8 h and 10^8 – 10^9 cfu/ml after 24 h.

None of the test organisms multiplied in full strength Vivonex HN. Counts of both strains of *P. aeruginosa* decreased from 10^2 to 10^1 cfu/ml at all three temperatures after 4 h. Counts remained at this level after 8 h at 4°C but after 8 h at 25°C and 37°C and after 24 h at 4°C, no organisms were detectable. There was no change in the numbers of *S. aureus* during 24 h, counts remaining at 10^2 cfu/ml at 4, 25 and 37°C. *Esch. coli* survived for 24 h at 4°C but numbers decreased from 10^2 to 10^1 cfu/ml after 8 h and to undetectable levels after 24 h at 25 and 37°C. Strains of *K. aerogenes* and *E. cloacae* survived for 24 h at 4° and 25°C, but numbers decreased from 10^2 to 10^1 cfu/ml after 24 h.

DISCUSSION

The susceptibility of patients fed by the naso-gastric or naso-enteric route to colonisation and infection by microorganisms present in the feeds has been discussed by Casewell (1982) and Anderton (1983). The number of organisms that must be ingested by a patient for colonisation of the intestine to occur will vary according to the patient's treatment and the underlying disease. Pottecher *et al.* (1979) suggested that 10^4 organisms/ml of feed are sufficient to colonise the digestive tract of hospital patients but, more recently, in a discussion of the role of salads as a source of potential pathogens, Remington and Schimpff (1981) stated that 'for the patient with reduced resistance to colonisation due to the systemic administration of antimicrobials, 1 g of salad with 10^3 gram-negative rods is probably sufficient to lead to persistent colonisation of the intestinal tract'.

Multiplication of all the test organisms to numbers in excess of 10^4 cfu/ml of feed within 8 h was observed at 25 and 37°C in Clinifeed ISO and Triosorbon. Similar results have been reported by Furtado *et al.* (1980) and Simmons (1981) in studies on the multiplication of bacteria in Osmolite and a Complan-based home-made feed.

The counts of all the test strains in all the feeds remained fairly constant at 10^2 – 10^3

cfu/ml during 24 h at 4°C, and comparison of these figures with those recorded at 25°C demonstrate the importance of storing prepared feeds in a refrigerator.

Half-strength Vivonex was also an excellent growth medium for the test strains, with the exception of *S. aureus*; counts after 8 and 24 h at 25 and 37°C were only slightly less than those in Clinifed ISO, possibly because of the lower pH (5.5) of Vivonex. The noticeable inhibition of *S. aureus* might be explained by the low pH, the high concentration of glucose solids and the presence of sorbate and acetate in the formulation. Schmidt *et al.* (1969) found that sorbate 1 mg/g prevented the growth of *S. aureus* in artificial cream pies acidified to pH 4.5–5.0. Vivonex has a pH of 5.5 and at half-strength contains sorbate 0.25 mg/ml. Zacherle and Charache (1970), in a study of peritoneal dialysis solutions, found that the factors associated with the inhibition of *S. aureus* were low pH and the presence of acetate and dextrose; maximum inhibition was caused by a combination of all three factors.

The lack of growth of all the test organisms at 25°C in full-strength Vivonex during the first 8 h of the study was probably due to a combination of low pH, high osmolarity and an incubation temperature below the optimum for these organisms. The increase in numbers of *P. aeruginosa* from 10³ to 10⁶ cfu/ml between 8 and 24 h reflects this organism's tolerance of inhospitable growth conditions. At 37°C, *P. aeruginosa*, *K. aerogenes* and *E. cloacae* grew well in full-strength Vivonex despite the low pH and high osmolarity of the product, whereas *S. aureus* and *E. coli* showed little growth.

The failure of all organisms to grow in full strength Vivonex HN can be attributed to the high osmolarity of this feed (800 mosm/L). Similar results were reported by Furtado *et al.* (1980) and Hostetler *et al.* (1982). Stanek *et al.* (1983) showed that the growth of various bacterial strains was inhibited in an elemental diet with an osmolarity of 580 mosm/L.

The presence of aerobic sporeforming bacilli as contaminants in Triosorbon and their rapid multiplication at 25 and 37°C is of concern since Gilbert *et al.* (1981) suggested that several *Bacillus* spp. can cause opportunistic infections in compromised hosts and that some may also be capable of causing food poisoning. Bastow *et al.* (1982) reported the presence of aerobic sporeforming organisms (including *B. cereus*) in Complian, another dried product used in enteral feeds. Therefore, it should not be assumed that all the proprietary products available for use in the formulation of enteral feeds are sterile and it would be advisable to ascertain the microbiological quality of all batches of dried products before use.

The results of this study emphasise the importance of strict microbiological quality control of ingredients and stringent hygienic precautions during the preparation and handling of all enteral feeds because bacteria can survive and multiply in feeds with low pH and high osmolarity, as well as in neutral, isotonic feeds.

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