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THE GROWTH OF MICRO-ORGANISMS IN PARENTERAL NUTRITION SOLUTIONS CONTAINING AMINO ACIDS AND SUGARS

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SUMMARY

The viability of various micro-organisms in solutions containing crystalline amino acids with and without the inclusion of a carbohydrate was studied. The fungus, *Candida albicans*, proliferated in all solutions examined. Addition of dextrose in several concentrations or sorbitol did not influence the growth of the fungus. Bacterial growth was, in comparison, considerably inhibited in solutions containing crystalline amino acids alone or in combination with dextrose. Previous publications have indicated that solutions containing crystalline amino acids do not permit bacterial multiplication. In contrast, strains of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* employed for this study were observed to multiply in solutions containing 4.25% w/v crystalline amino acid and 25% w/v dextrose. The substitution of dextrose with sorbitol resulted in further inhibition of the majority of bacteria.

INTRODUCTION

Total Parenteral Nutrition (T.P.N.) is an established clinical procedure. During the first years of T.P.N. use, reports of associated sepsis were widespread (Ashcroft and Leape, 1970; Curry and Quie, 1971; Sanderson and Deitel, 1973; Copeland et al., 1974). Although culprit micro-organisms were typical of nosocomial infections, the incidence of fungal septicaemia, and in particular due to Candida species, was unusually high. Several investigators have examined the viability of micro-organisms in conventional fluids and solutions employed for T.P.N. (see Maki, 1976). They have reported that, in general,

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growth in simple infusion solutions, such as 5% w/v dextrose and 0.9% w/v sodium chloride solutions, was limited to bacteria of either the tribe Klebsielleae or Pseudomonas species. Published data has indicated that the type of micro-organism which may be permitted to survive and multiply in T.P.N. solutions is influenced by the choice of amino acid source, whether it be a protein hydrolysate or crystalline amino acid solution. For instance, several investigators have found that T.P.N. solutions comprising a mixture of 5% w/v protein (usually casein or fibrin) hydrolysates and 25% w/v dextrose, permitted the proliferation of the fungus Candida (Brennan et al., 1971; Wilkinson et al., 1973; Pierpaoli et al., 1973; Gelbart et al., 1973). Little consistency exists between reports on the viability of bacteria in solutions containing protein hydrolysate and dextrose. Some workers have observed the proliferation of several species of bacteria in such solutions (Goldmann et al., 1973; Pierpaoli et al., 1973), while contrasting reports have been made in which bacterial populations of the same species have remained constant (Wilkinson et al., 1973; Gelbart et al., 1973). Mixtures of crystalline amino acids and dextrose permitted the multiplication of Candida, but rarely as rapidly as in solutions containing protein hydrolysates. Such mixtures have been found unable to permit the multiplication of any of the bacteria species yet examined (Gelbart et al., 1973; Goldmann et al., 1973; Wilkinson et al., 1973). However, little information is presently available on the influence that the type and concentration of carbohydrate has on the survival and multiplication of micro-organisms. This study, therefore, has examined the effect of varying the concentration of dextrose in solution on the viability of different species of micro-organisms. This effect was observed in aqueous solutions of dextrose alone and when in combination with crystalline amino acid solutions. The viability of various micro-organisms was also studied in crystalline amino acid solution alone and in combination with sorbitol. The viability of various micro-organisms in distilled water and 0.9% w/v sodium chloride solution was also assessed.

MATERIALS AND METHODS

Preparation of bacterial and fungal suspensions

The bacteria employed were *Staphylococcus epidermidis* NCTC 7944, *Escherichia coli* NCTC 9001, *Enterobacter aerogenes* NCTC 10006 and *Pseudomonas aeruginosa* NCTC 10662. The fungus employed was *Candida albicans*, obtained from the Bacteriology Department, Manchester Royal Infirmary, Manchester, England. After growth in Tryptone Soya Broth (Oxoid) at 37°C for 18 h, cells were harvested by centrifugation at 6000 rpm for 10 min, washed twice and resuspended in sterile distilled water. The resulting suspension was then serially diluted with sterile distilled water to produce two dilutions containing about 5×10^4 cells/ml and 5×10^2 cells/ml respectively.

Solutions employed

Water was obtained by distillation in a glass still and sterilized by autoclaving at 115°C for 30 min. 50% w/v dextrose, 8.5% w/v crystalline amino acid (Synthamin '14') and 4.0% w/v crystalline amino acid + 15% w/v sorbitol solutions were obtained from Travenol Labs. Ltd. (Thetford, Norfolk, England).

Preparation of test solutions

All manipulations were performed using strict aseptic technique. 50% w/v dextrose solution was diluted with sterile distilled water to prepare 5, 15 and 25% w/v dextrose solutions. Solutions containing 4.25% w/v crystalline amino acids alone or in combination with either 5, 15 or 25% w/v dextrose were prepared by mixing appropriate volumes of 8.5% w/v crystalline amino acid solution, 50% w/v dextrose solution and sterile distilled water. Of each test solution, 49 ml was transferred to 60 ml containers (No. 125C, Sterilin Products, Teddington, Middlesex, England).

Inoculation of test solutions

One ml of each diluted bacterial and fungal suspension was inoculated into 49 ml of test solution and mixed by shaking. The resulting suspension contained either about 10^3 or 10 viable cells/ml.

Sampling of test solutions

The first sample was taken immediately after inoculation of the test solution. From the solution containing about 10 cells/ml, two 1-ml volumes were removed and plated out separately in Tryptone Soya Agar (Oxoid). From the solution containing about 10^3 cells/ml, 1 ml was transferred to 9 ml of the same solution and mixed by shaking. From the resulting suspension two 1-ml volumes were removed and plated out separately in Tryptone Soya Agar. The pour plate technique was employed throughout the study, all plates being incubated at 37°C for 48 h. Sampling was repeated at suitable intervals. Multiplication of micro-organisms was accommodated by appropriate serial dilution of the test solution. All inoculated solutions were protected from light between sampling times and stored at room temperature (24 ± 2°C).

pH measurements

The pH of each test solution was measured using a pH meter 7020 (Electronic Instruments Ltd., Richmond, Surrey, England).

RESULTS

In all solutions, growth of test micro-organisms in solutions containing an initial concentration of about 10 cells/ml virtually parallelled that of solutions containing 10^3 cells/ml; that is, the first evidence of cell multiplication was detected at the same sampling times and the rates of multiplication were similar. Thus, the number of cells at the smaller concentration remained about 2 log cycles less than that of the larger concentration. For these reasons, results refer only to solutions inoculated with about 10^3 viable cells/ml.

The viability of micro-organisms stored in freshly distilled water and 0.9% w/v saline solution

The three Gram-negative bacilli, *E. coli*, *Ps. aeruginosa* and *E. aerogenes*, multiplied in both these solutions. The population of viable *Ps. aeruginosa* cells increased by about

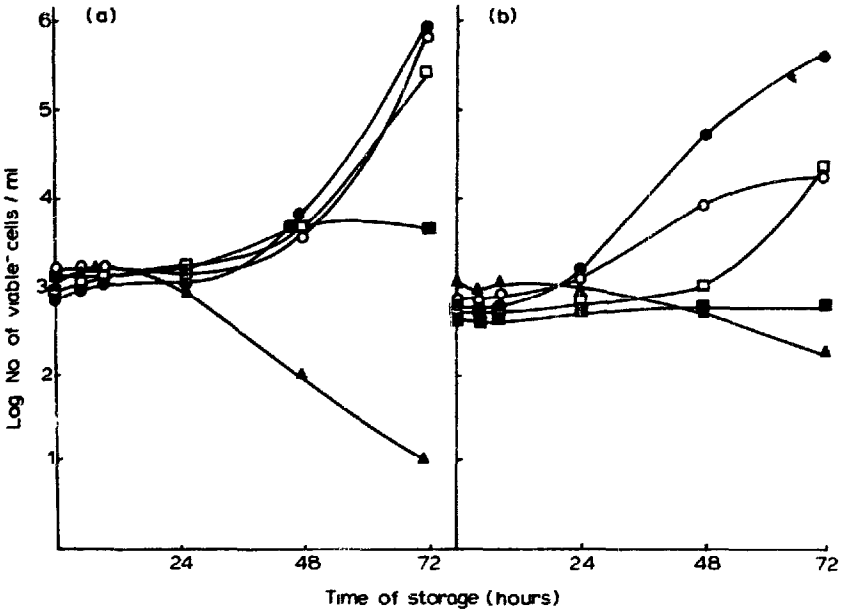

Fig. 1. The viability of micro-organisms when stored in (a) freshly distilled water and (b) 0.9% w/v sodium chloride solution. ●———●, *E. aerogenes;* ▲———▲, *Staph. epidermidis;* □———□, *E. coli;* ○———○, *Ps. aeruginosa;* ■———■, *C. albicans.*

3 log cycles when stored in distilled water for 72 h in comparison to 1 log cycle increase of the other two bacilli (Fig. 1a). In 0.9% w/v saline solution, however, the population of each species of Gram-negative bacteria increased by about 2 log cycles within 72 h (Fig. 1b).

The number of viable *Staph. epidermidis* cells slowly decreased over the study period in both distilled water and 0.9% w/v saline solution. *C. albicans* slowly multiplied after 24 h storage in 0.9% w/v saline solution, whereas its concentration remained approximately constant in distilled water during the 72 h study period.

The viability of micro-organisms stored in dextrose solution

Solutions containing 5, 15, 25 and 50% w/v dextrose in distilled water were employed to study the effect of increasing the concentration of dextrose in solution on the survival and multiplication of various micro-organisms. *Staph. epidermidis* was unable to survive in any of the dextrose solutions, complete loss of viability being observed in each solution within 24 h of inoculation (Fig. 2). *Ps. aeruginosa,* likewise, showed complete loss of viability within 24 h in all except 5% w/v dextrose solution. After 48 h storage in this solution, however, no viable cells were detectable. The population of viable *E. coli* cells also decreased within the study period in each dextrose solution, the rate of loss of viability of cells increasing as the concentration of dextrose was increased from 5 to 25% w/v (Fig. 2). Little difference between the 25 and 50% w/v dextrose solutions was

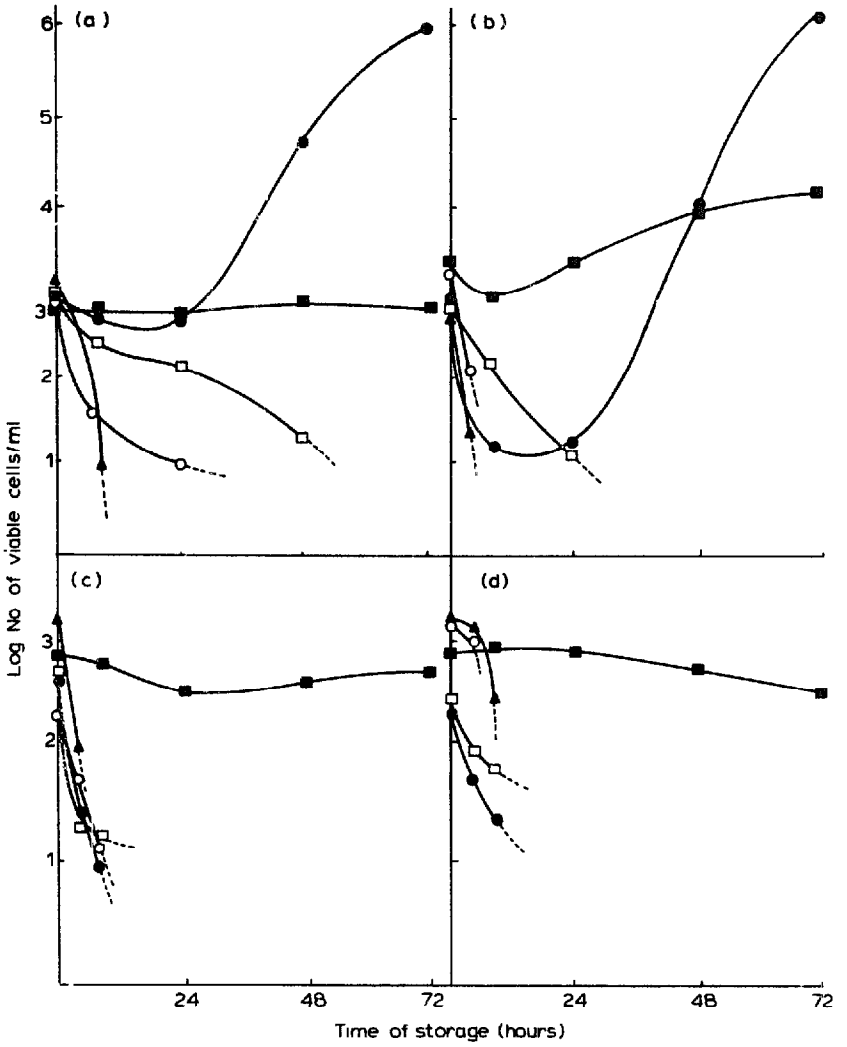

Fig. 2. The viability of micro-organisms when stored in (a) 5% w/v dextrose solution; (b) 15% w/v dextrose solution; (c) 25% w/v dextrose solution, and (d) 50% w/v dextrose solution. ●——●, *E. aerogenes;* ▲——▲, *Staph. epidermidis;* □——□, *E. coli;* ○——○, *Ps. aeruginosa;* ■——■, *C. albicans.*

observed, the number of viable cells rapidly decreasing within 24 h after inoculation of the solutions. *E. aerogenes* also rapidly lost viability in 25 and 50% w/v dextrose solution, (Fig. 2c, d). In 5 and 15% w/v dextrose solution, however, after an initial reduction in concentration, rapid multiplication was observed to a level in excess of 10^6 cells/ml within 72 h (Fig. 2a, b). Several bacteria belonging to the tribe Klebsielleae have previously been reported to proliferate in dextrose solutions (Felts et al., 1972; Maki and Martin, 1975), probably because such bacteria are capable of nitrogen-fixation and

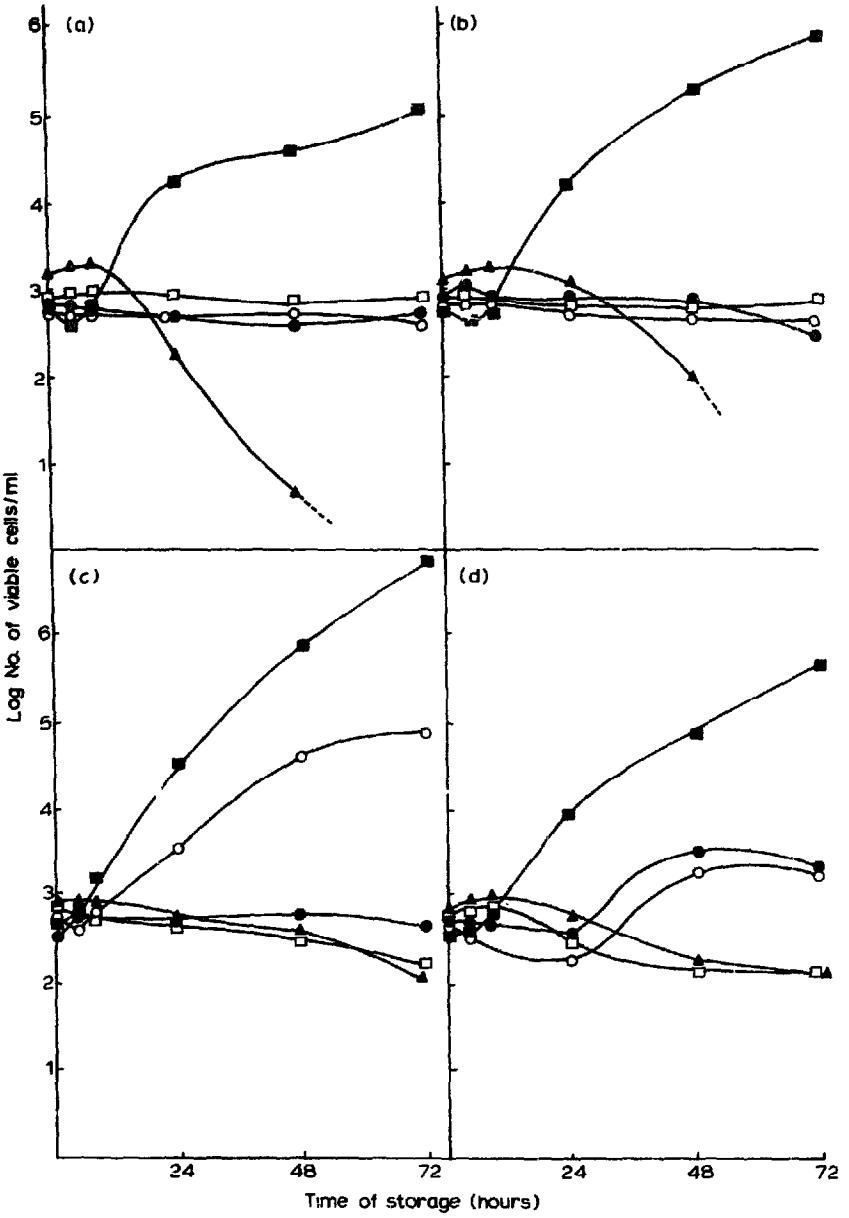

Fig. 3. The viability of micro-organisms when stored in (a) 4.25% w/v crystalline amino acid solution; (b) 4.25% w/v crystalline amino acid + 5% w/v dextrose solution; (c) 4.25% w/v crystalline amino acid + 15% w/v dextrose solution, and (d) 4.25% w/v crystalline amino acid + 25% w/v dextrose solution. ●——●, *E. aerogenes*; ▲——▲, *Staph. epidermidis*; □——□, *E. coli*; ○——○, *Ps. aeruginosa*; ■——■, *C. albicans*.

possess the ability to utilize trace amounts of inorganic elements present in commercial infusion fluids (Maki et al., 1976).

The concentration of *C. albicans* remained constant in the 5, 25 and 50% w/v dextrose solutions. An increase of 1 log cycle was observed in 15% w/v dextrose solution within 72 h (Fig. 2).

The viability of micro-organisms stored in crystalline amino acid solution alone and in combination with dextrose solutions

C. albicans proliferated in all solutions containing crystalline amino acids. The population of viable *C. albicans* cells increased by about 2 log cycles within 72 h when stored in 4.25% w/v crystalline amino acid solution (Fig. 3a). Addition of 5, 15 or 25% w/v dextrose to the amino acid solution permitted the number of viable cells to increase by about 3 log cycles in each case within 72 h (Fig. 3b–d). In comparison to the fungus, the survival and multiplication of the bacteria examined was inhibited, and in many instances loss of cell viability was recorded within 72 h. The viable population of *Ps. aeruginosa* remained approximately constant when stored in 4.25% w/v crystalline amino acid solution alone or in the presence of 5% w/v dextrose solution. Addition of 15% w/v dextrose to the amino acid solution, however, permitted an increase of nearly 2 log cycles in the viable cell concentration within 72 h (Fig. 3c), but only 4-fold increase was noted on addition of 25% w/v dextrose to the amino acid solution (Fig. 3d).

The population of viable *E. aerogenes* cells remained constant in crystalline amino acid solution alone and in combination with 5 and 15% w/v dextrose (Fig. 3a–c). In contrast, an increase of about 1 log cycle was observed within 72 h when stored in 4.25% w/v crystalline amino acid + 25% w/v dextrose solution (Fig. 3d). The viable population of

TABLE 1

pH AND OSMOLARITY OF TEST SOLUTIONS

Solution	pH	Calculated osmolarity (mOsmol/litre)
Distilled water	5.82	–
0.9% w/v sodium chloride	5.44	300
5% w/v dextrose	4.73	250
15% w/v dextrose	4.38	750
25% w/v dextrose	4.12	1250
50% w/v dextrose	3.74	2500
4.25% w/v crystalline amino acid (c.a.a.)	5.83	580
4.25% w/v c.a.a. + 5% dextrose	5.79	830
4.25% w/v c.a.a. + 15% w/v dextrose	5.75	1330
4.25% w/v c.a.a. + 25% w/v dextrose	5.67	1830
4.0% w/v c.a.a. + 15% w/v sorbitol	5.66	1380

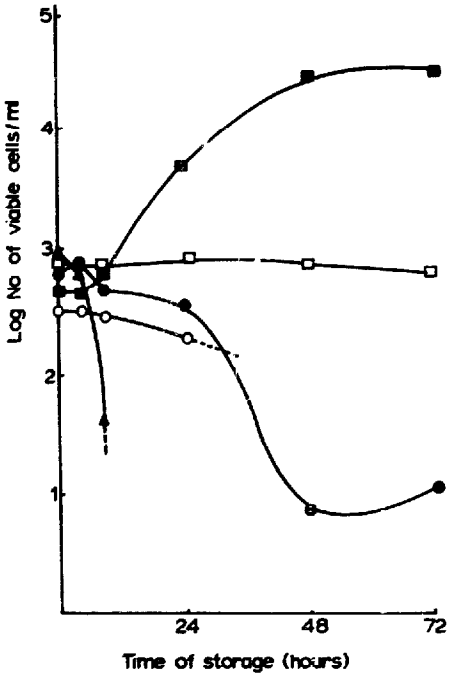

Fig. 4. The viability of micro-organisms when stored in 4.0% w/v crystalline amino acid + 15% w/v sorbitol solution. ●———●, *E. aerogenes;* ▲———▲, *Staph. epidermidis;* □–·–·–□, *E. coli;* ○———○, *Ps. aeruginosa;* ■———■, *C. Albicans.*

E. coli remained approximately constant over the study period when stored in 4.25% w/v crystalline amino acid solution, and was unaffected by the addition of 5% w/v dextrose. However, addition of 15 or 25% w/v dextrose caused a gradual decrease in the viable cell concentration over the study period (Fig. 3c, d).

Staph. epidermidis showed complete loss of cell viability within 72 h when stored in 4.25% w/v crystalline amino acid solution alone and when combined with 5% w/v dextrose. Addition of 15 and 25% w/v dextrose considerably reduced the rate of cell death (Fig. 3c, d).

The viability of the Staphylococcus, Pseudomonas and Enterobacter species was noticeably inhibited when stored in a T.P.N. solution consisting of 4.0% w/v crystalline amino acid + 15% w/v sorbitol when compared to a 4.25% w/v crystalline amino acid + 15% w/v dextrose, each species losing complete viability within 8, 48 and 72 h, respectively (Fig. 4). The viability of *E. coli* and *C. albicans* was, however, similar in both solutions.

The pH and osmolarity of test solutions are shown in Table 1. In particular, it is clear that dextrose lowers pH, but has much less influence on the pH of amino acid solutions.

DISCUSSION

The viable population of *C. albicans* remained approximately constant when stored in infusion fluids of simple composition, but rapidly increased in solutions containing crystalline amino acids with or without dextrose. This observation indicated that such solutions probably provide nutrients essential for the multiplication of the fungus. The high osmotic pressure of these solutions did not inhibit fungal multiplication. Although multiplication of *C. albicans* was not observed in dextrose solutions, addition of dextrose to amino acid solutions did not inhibit multiplication of the fungus. In contrast to these observations, it was found that the growth of all bacteria examined was inhibited when stored in crystalline amino acid solutions alone or in combination with dextrose, in comparison to storage in distilled water or 0.9% w/v sodium chloride solution. Possible reasons for such behaviour may be due to adverse conditions of pH and osmolarity exhibited by the crystalline amino acid solutions or the presence of inhibitory substances. *Staph. epidermidis* steadily lost cell viability in all concentrations of dextrose or crystalline amino acid solutions. When combined, however, the rate of loss of viability was considerably reduced. Staphylococcal species are sensitive to adverse pH conditions (Gelbart et al., 1971) and prefer a growth environment of neutral pH. The pH of solutions containing crystalline amino acid solution and dextrose was always lower than the amino acid solution alone, indicating that pH was not the major factor influencing the survival of this bacterium, but rather the presence of certain amino acids and a utilisable carbon source such as dextrose. It is known that staphylococcal species require many extrinsic amino acids for growth (Gelbart et al., 1971). Loss of viability of *Staph. epidermidis* was rapid in solutions containing sorbitol. This effect was also observed with *E. aerogenes* and *Ps. aeruginosa*. Clearly sorbitol is deleterious to cell survival.

The viable population of *E. coli* and *E. aerogenes* remained approximately constant when stored in crystalline amino acid solutions with or without low concentrations of dextrose. *E. aerogenes* grew in the presence of 25% w/v dextrose. Comparison of the viability of *E. aerogenes* in 5 and 15% w/v dextrose solutions alone and in combination with 4.25% crystalline amino acid solution indicates that the presence of the amino acids inhibited the multiplication of this bacterium. The high osmolarity of the amino acid solution is not responsible since growth occurs in the presence of higher dextrose concentrations. In contrast, the presence of the amino acid solution appeared to prevent the loss of viability of *E. coli* which occurred when stored in dextrose solutions alone. This may have been due to the provision of certain nutrients and the more favourable pH of the amino acid–dextrose mixture.

Ps. aeruginosa exhibited cell multiplication only in solutions containing amino acids with high concentrations of dextrose. Of the solutions examined, the mixture of 4.25% w/v crystalline amino acid and 25% w/v dextrose provided adequate nutrients in such proportions for growth to occur without subjecting the organisms to excessive adverse conditions of pH and osmolarity. The finding that multiplication of *Ps. aeruginosa* and *E. aerogenes* occurs in solutions containing crystalline amino acids and dextrose is in contrast to previous reports (Gelbart et al., 1973; Goldmann et al., 1973; Wilkinson et al.,

1973). The survival of various types of micro-organisms in different T.P.N. solutions cannot be predicted and depends on the nature of the solution as well as on the properties of the organism.

The possibility that the growth pattern of micro-organisms in the amino acid and dextrose mixtures examined was a feature peculiar to the strain of each organism studied cannot be excluded. However, this seems unlikely as behaviour of the micro-organisms in simple infusion solutions were comparable to other documented reports.

Deliberate contamination of infusion solutions with viable micro-organisms of concentrations of up to 10^3/ml may not yield results comparable to the smaller numbers introduced during accidental contamination during their use in the hospital environment (Arnold and Hepler, 1971; Deeb and Natsios, 1971; Letcher et al., 1972; Hansen and Hepler, 1973; Kundsin et al., 1973; Maki et al., 1974). However, experiments employing inoculum concentrations of 10^3 and 10 viable cells/ml gave similar results, indicating that growth of smaller populations of cells would be similar.

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