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Kinetics of growth characteristics of micro-organisms in dextrose infusion solutions

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Abstract

The patterns of growth kinetics of micro-organisms introduced into dextrose IV infusion solutions have been studied using different microbiological approaches. The study was prompted by the need to explore the behavioral patterns of the numerous reported incidences of microbial contamination, their survival and patient infection, arising from the use of infusion solutions. The various factors, including microbial types, dextrose concentrations, solution agitation and temperature which may influence the survival and growth of these contaminants in IV solutions were critically assessed and quantitatively analysed. The results showed that the apparent generation rate constants K_{app}, for different organisms which include *Staph. aureus Bacillus subtilis*, *Escherichia coli*, *KLebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans* decreased with increasing dextrose concentrations, thus indicating suppression of growths. The extent of growth suppression (inhibition) was dependent on dextrose content, incubating conditions (static or turbulent) and pH of dextrose solutions. The different growths of all the micro-organisms examined in the dextrose solutions under varying conditions appeared to be due to inhibition or saturation of enzymes involved in dextrose metabolism; and the observed changes at any given condition of incubation may be due to a modification of overall enzyme activity.

Keywords: Growth characteristics of micro-organisms; Dextrose infusion solutions; Microbial growth in non-nutritive solutions; Factors influencing growth kinetics of micro-organisms

1. Introduction

There are numerous reports concerning the contamination of, and survival in dextrose infusion solutions by micro-organisms (Maki et al.,

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1974; Holmes and Allwood, 1979; Hugbo and Akpan, 1989).

Whilst many of these contaminants are reported to be capable of growth in certain dextrose concentrations, (Gelbart et al., 1973; Maki, 1976) others have been shown to progressively loose viability in these solutions (Wilkinson et al., 1973; Hugbo and Imanlanhimi, 1983). These conflicting reports may be due to the fact that most workers often focused their studies on the survival states of these organisms in the solutions as an attempt towards improved IV fluid therapy practice. Factors which could decisively influence survival/growth patterns are not often considered.

The survival and growth of micro-organisms in a given milieu involves several factors interacting together at the same or different levels. Factors such as pH, osmolality, dextrose concentration, oxygen requirements, type of micro-organisms, inoculum size and cultural history of the organism are important considerations which affect microbial growth status in non-nutritive solutions.

This paper describes the influence of some of these various factors, including B-lactamase presence in the organisms in relation to survival and growth in dextrose infusion fluids.

2. Materials and methods

2.1. Selection and standardisation of test organisms

The organisms were selected so as to reflect the spectrum of those organisms most frequently found as contaminants of intravenous fluids, particularly during clinical use. The choice of both B-lac$^+$ and B-lac$^-$ isolates of each species of organisms was guided by an earlier observation (Akpan et al., 1989) that these two types of isolates often behaved differently as regards their growth characteristics in non-nutritive solutions.

Thus B-lac$^+$ and B-lac$^-$ isolates of *Staph. aureus*, *B. subtilis*, *Ps. aeruginosa*, *E. coli*, and *Kleb. pheumoniae* were obtained, identified to species level and employed. A yeast, *Candida albicans* was also included in the study. The bromocresol purple acidometric methods for betalactamase detection was used to determine betalactamase status of all bacterial isolates used.

Each micro-organism was maintained on blood agar slope at 4°C and transferred to fresh tryptone soya slant (Oxoid), 24 h. prior to use for experiment. A colony of the test organisms was carefully removed from the slant, inoculated into 20 ml sterile distilled water and maintained at 30°C for 15 h. to exhaust endogenous metabolic pool; thus the cells were starved. Vigorous shaking was done to obtain a homogeneous suspension, and viable counting was carried out (Miles and Misra, 1938) to yield a standard suspension, of which, when 1 ml was added to 39 ml of dextrose solution, 100 ± 10 colony-forming units (cfu/ml) would be obtained as inoculum size. A 100 cfu/ml concentration of micro-organisms is recognised by the 1978 National Co-ordinating Committee on Large Parenterals as the size of inoculum that might result from accidental touch contamination.

2.2. Survival of micro-organisms in dextrose solutions

Dextrose solutions were prepared and sterilised to contain 2.5, 5.0, 10 and 20.0% w/v dextrose in water. Sterile water was used as controls. These concentrations were chosen to reflect closely those that are normally encountered in clinical practice. For each bacterial isolate, two sets of flasks were employed to study the survival/growth patterns of B-lac$^+$ and B-lac$^-$ species, and one set of *C. albicans*. Each set then consisted of five replicates per concentration of dextrose. Each flask, containing 39 ml of dextrose solution was inoculated with 1 ml of bacterial suspension and maintained as a stationary culture at 27 ± 0.5°C for 72 h.

Samples (0.2 ml) were taken in quadruplicates at intervals of 0, 3, 6, 9, 12, 24, 48 and 72 h, diluted and plated out for viability determination (Miles and Misra, 1938).

Incubation was maintained at 37°C for 24 h.

The effect of aeration by turbulence was studied by holding another set of culture flask at 150 rev/min. in a G-10 gyratory New Brunswick shaker.

Results were analysed statistically using the Student's t-test for paired data in case of:

1. Differences in growths between B-lac$^-$ and B-lac$^+$ isolates for individual species;
2. Differences in growth between different species;
3. Whilst changes within individual isolate population were analysed using standard deviation and coefficient of variation (CV).

A value of $P < 0.05$ or $P < 0.01$ was then chosen as a priori level of significance.

3. Results

The results show that when a small inoculum, ($100 \pm$ cfu) of starved bacterial cells is added to increasing concentration of dextrose solution, there is a steady decrease in the generation rates of the organisms until a steady state is attained. Apparent first order generation rate constant, K_{app} was calculated from slopes of the linear portion of the log percent survivor/time curve from the expression.

$$\log N = \log N_0 + \frac{K_{app} t}{2.303} \quad (1)$$

where N = number of surviving cells after time, t and N_0 = Initial number of cells.

Plots of calculated K_{app} values against dextrose concentrations produced linear regressions (Figs. 1–5) in accordance with the equation,

$$K_{app} = K_0 + K_c C \quad (2)$$

where K_0 in h^{-1} is the extrapolated value for K_{app} at zero dextrose concentration: the slope K_c, in ml/g^{-h} is the specific metabolic constant for dextrose.

A positive slope indicates survival, while a negative value indicates target site saturation, or inhibition of dextrose metabolising enzymes or sites. Hence, at saturation, K_{app} becomes less than K_0, and C is the dextrose concentration.

The influence of the kinetic parameters in Eq. (2) above on the different organisms were to depend on the organisms in question and also on whether it produced B-lactamase or not. Thus, for *Staph. aureus*, monophasic death curves were demonstrated by the B-lac$^-$ species. The K_{app} values for B-lac$^+$ *Staph. aureus* in dextrose were found to be generally lower than those obtained for the same organism in distilled water, indicating some degree of inhibition of cell generation by dextrose. When the K_{app} values were plotted against their corresponding dextrose concentrations, a curvilinear relationship (Fig. 1) was obtained. Under turbulent conditions of aeration, the K_{app} vs dextrose concentration plots 'dipped' downwards denoting lower rates of survival.

Figs. 2–5 are the plots of K_{app} vs dextrose concentration for *B. subtilis*, *E. coli*, *Ps. aeruginosa* and *Kleb. pneumoniae* respectively.

Essentially, they are all in agreement with the general observation. In presence of dextrose K_{app} values are lower than those in water alone, both for B-lac$^+$ and B-lac$^-$. However, plots of K_{app} vs dextrose concentration for the two types of *B. subtilis* were different from those obtained with *Staph. aureus* as the test organism.

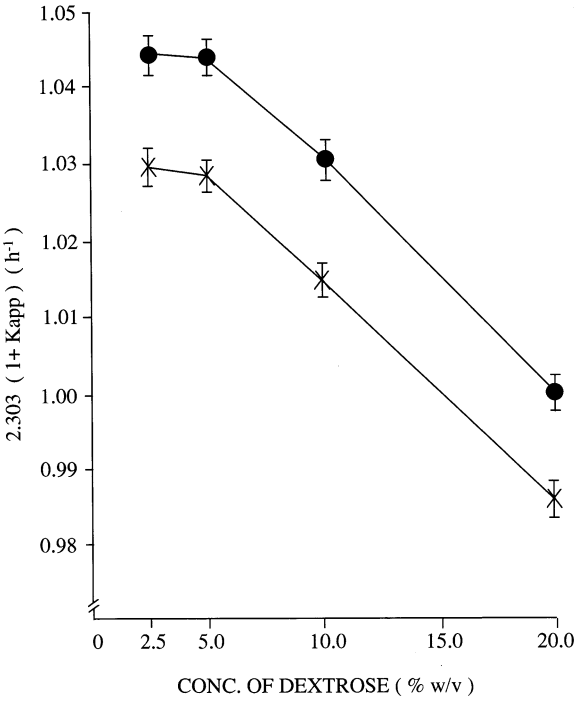

Fig. 1. Influence of Dextrose Concentration on Survival Rate Constants of B$^+$ Strain of *S. aureus* under Static – ∘ – and aerated – × – incubation.

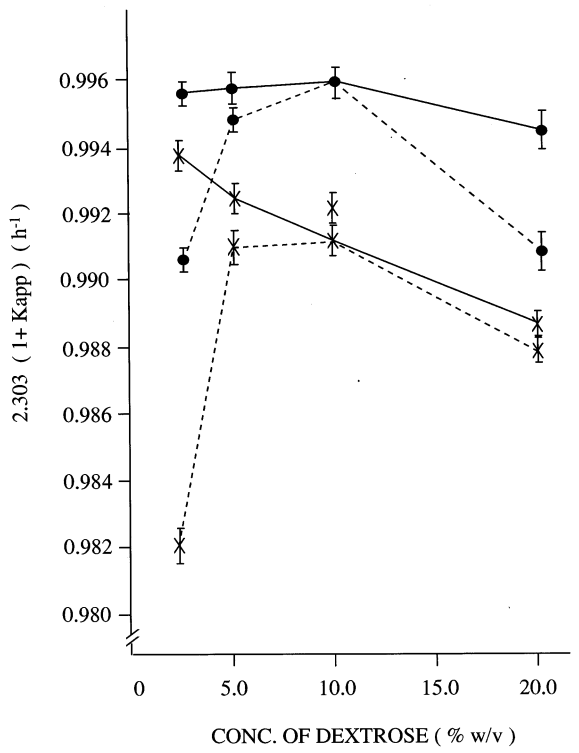

Fig. 2. Influence of Dextrose Concentration on Survival Rate Constants for B^+ (——) and B^- (— — —) of *B. subtilis* under Static – ◦ – and aerated – × – incubation.

For example, there was an initial increase in K_{app} followed by a decrease as the dextrose concentration increased up to and beyond 10% w/v. This resulted in, first, a positive and then a negative value for K_c, the specific metabolic constant. When the cultures were agitated, K_c values were negative, being -0.092 and -0.065 in ml/g^{-h} for the B-lac$^+$ and B-lac$^-$ cells respectively.

The same profile of results was obtained for *E. coli*, *Kleb. pneumoniae* and *Ps. aeruginosa*.

With the last organism however, there were surprisingly, no survivors in all dextrose solutions studied at the 72 h period of incubation. Furthermore, the organism progressively declined in viable numbers until the cultures became sterile. Values of K_c obtained from relevant calculations were markedly negative, these being -31.32 and -32.24 ml/g^{-h} for the B-lac$^-$ isolate and -16.12 and -18.89 ml/g^{-h} for the B-lac$^+$ isolates under static and turbulent conditions of incubation respectively.

Candida albicans, the only yeast specie studied neither increased nor decreased in viable number over 72 h.

4. Discussion

Many studies which focused on the viability of micro-organisms in non-nutritive infusion solutions have reported the inability of most organisms to grow in simple IV solutions containing dextrose (Wilkinson et al., 1973; Hugbo and Imanlanhimi, 1983; Bleach et al., 1988). But the reasons why these organisms are unable to grow are not often explicit.

Often, low pH, high osmolality and lack of essential nutrients have been implicated (Wilkinson et al., 1973; Maki, 1976; Akpan et al., 1989) without offering any mechanistic explanation as to why they cause inhibition of growth.

In the present studies, we have used dextrose concentrations which are considerably higher than those normally employed in IV infusion therapies.

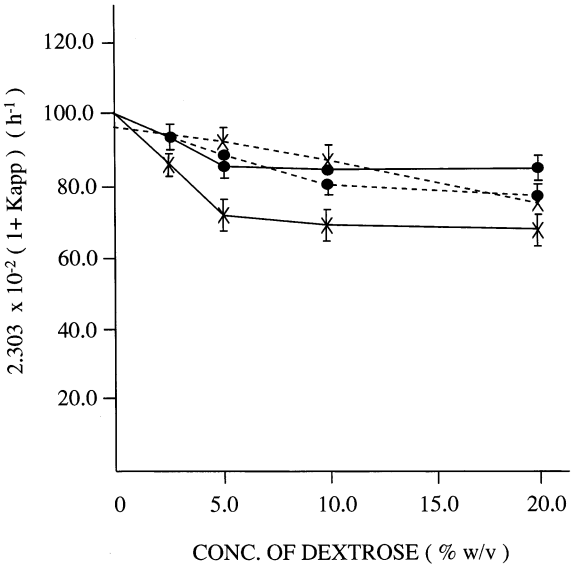

Fig. 3. Influence of Dextrose Concentration on Survival Rate Constants for B^+ (——) and B^- (— — —) of *E. coli* under Static – ◦ – and aerated – × – incubation.

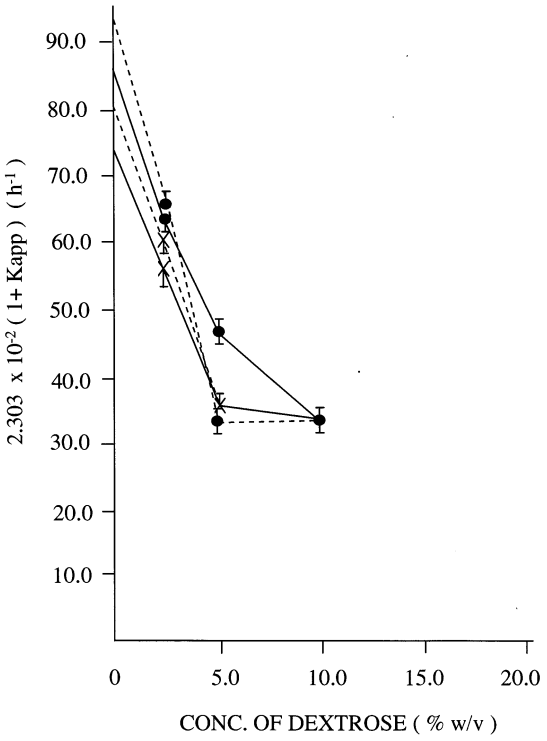

Fig. 4. Influence of Dextrose Concentration on Survival Rate Constants for B^+ (——) and B^- (— — —) of *Ps. aeruginosa* under Static – ◦ – and aerated – × – incubation.

This became necessary in order to highlight, more truly the mechanisms by which dextrose solutions suppress bacterial growths. These have shown that dextrose can significantly inhibit the generation rates of many species of bacteria, notably at high dextrose concentrations. The data appear to fit a theory of inhibition of enzyme-catalysed reaction in which the substrate is present in excess. For example, the plots of K_{app} vs dextrose concentrations for B-lac$^+$ *Staph. aureus*, *Kleb. pneumoniae* and for *B. subtilis* are very identical to the typical enzyme-catalysed degradation plots with attendant saturation phenomenon. Therefore, the loss of viability through generation rate inhibition may arise from saturation of the enzyme systems associated with dextrose metabolism in these organisms. The overall extent of growth-rate inhibition is therefore, a reflection of the degree of saturation attained in the enzyme(s) involved.

The inability of *Ps. aeruginosa* and to some extent, *E. coli* to grow in dextrose solutions greater than 5% w/v even though they grew in distilled water must thereby be due, to the ease which the relevant enzymes are saturated by dextrose molecules in these organisms.

Therefore, the death of Gram-negative bacteria, other than Klebsiellae tribe which has often been associated with low pH of solution, may in fact, be due to an effect of pH on an enzyme-dextrose complex. Trinci and Thurston (1976) for example, proposed that, the rate of growth of a microbial population may decelerate and even cease before the advent of nutrient exhaustion, an effect attributed to changes in pH of medium and/or accumulation of secondary metabolic products.

With Gram-positive bacteria, loss of viability was often more pronounced with the B-lac$^-$ than with B-lac$^+$ isolate, and also when the cell culture was agitated. This was clearly the case in respect of the two types of *Staph. aureus* and *B. subtilis* isolates employed.

This may well be indicative of a smaller number of enzymes sites available for dextrose metabolism in the B-lac$^-$ cell than its B-lac$^+$ counterpart. Such an hypothesis is supported by the fact that

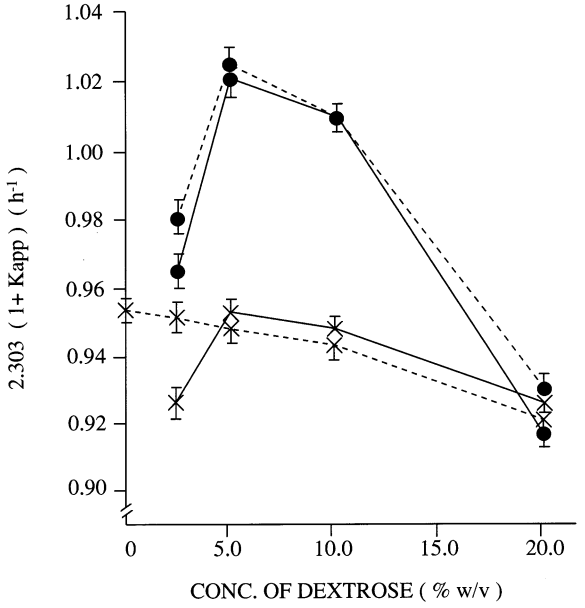

Fig. 5. Influence of Dextrose Concentration on Survival Rate Constants for B^+ (——) and B^- (— — —) of *K. pneumoniae* under Static – ◦ – and aerated – × – incubation.

higher dextrose concentrations were required to inhibit the B-lac$^+$ cell, implying the presence of dextrose metabolising enzymes, inactivated only, at high dextrose levels. With B-lac$^-$ cells, low levels of dextrose were sufficient to inhibit growth.

The growth and survival of *C. albicans* was not in any way affected by dextrose at any concentration under all conditions of incubation.

It thus appears from the results obtained in this study that the inability of many bacteria to grow in dextrose solutions of high concentrations is largely due to inhibition of dextrose- metabolising enzymes due to saturation of relevant enzyme sites. This contrasts with frequently held views that nutritional deficiency, pH and osmolality of solution are the main factors responsible for observed growths supression on bacteria. It further shows that greater danger from contamination may be posed by moulds since the survival of these are not affected by quality of the IV solution.

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