surrounding the water molecule. The lowering in compressibility[4-5] ($\Delta\beta = \beta_0 - \beta_s$) has been directly proportional to the number of ions in the solution. When a non-electrolyte is added to such an electrolyte solution, it shows a nonlinear variation which indicates the maximum complex formation at the corresponding point. Similar observations have been recorded for D-mannose solution. Thus ultrasonic velocity and compressibility lowering predict the complex formation between Na^+, K^+, Ca^{+2} and Mg^{+2} with D-glucose and D-mannose in aqueous solution. The composition of the complex can also be assessed from such studies.

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BACTERIOPHAGE BURST SIZE AS A FUNCTION OF MULTIPLICITY OF INFECTION

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THE last few decades have witnessed phenomenal advances in our understanding of the detailed mechanism of phage growth and reproduction. Studies on the burst size of bacterial viruses was reported earlier by a few workers[1-3]. The burst size of a particular phage may be determined by (a) the nutritional and physiological status of the host bacterium,[4] (b) phage-coded functions such as polymerases and regulatory proteins essential for phage production[5-7]. The inherently low efficiency of polymerases and regulatory proteins may not permit the phage to make full use of bacterial resources and consequently the burst size may be limited. In the present investigation the burst size of bacteriophage PIK in *Pseudomonas aeruginosa* PAO1 has been measured under conditions of varying multiplicity of infection (MOI).

The host *P. aeruginosa* PAO1 (gift from Dr B. W. Holloway, Monash University, Victoria, Australia) and the virulent bacteriophage PIK[8] were mixed in trypticase soy broth. The number of bacteria in each experiment was added in such a way that the final concentration of bacteria remained 1×10^7 cells/ml. The number of phage particles varied in each experiment to get the desired MOI. Phage adsorption was carried out using the procedure of Adams[9]. Various factors like MOI, burst size, effective MOI and effective burst size were calculated using the following equations:

(i) B/A = MOI, where B and A represent the number of phage and the number of bacteria respectively.
(ii) C–$D = X$, the number of unadsorbed free phage particles. Where C represents the number of infected bacteria plus the number of free phage particles and D, the number of infected bacteria.
(iii) $B - X = Y$, the number of phages adsorbed.
(iv) Y/D = effective MOI (mean number of phages adsorbed per infected bacterium).
(v) E/D = burst size (mean number of phages liberated per infected bacterium), where E represents the total number of phages liberated at the end of one cycle of phage growth.
(vi) E/Y = effective burst size (mean number of phages liberated per phage adsorbed).

The experiments were repeated four to five times at each MOI. The data were statistically analysed by the student's t test and the level of significance is indicated.

When MOI and the burst size were computed, a significant positive correlation was obtained (figure 1). The total production of phage particles was limited to certain MOI (MOI = 5). This cannot be a result of increased number of infected bacteria because the burst size tends to decrease if there would be any further increase in the MOI. At lower MOI, however, it may be possible that phages could not utilize the bacterial machinery completely and thus leading to lower burst size.

Although it is known that the burst size increases

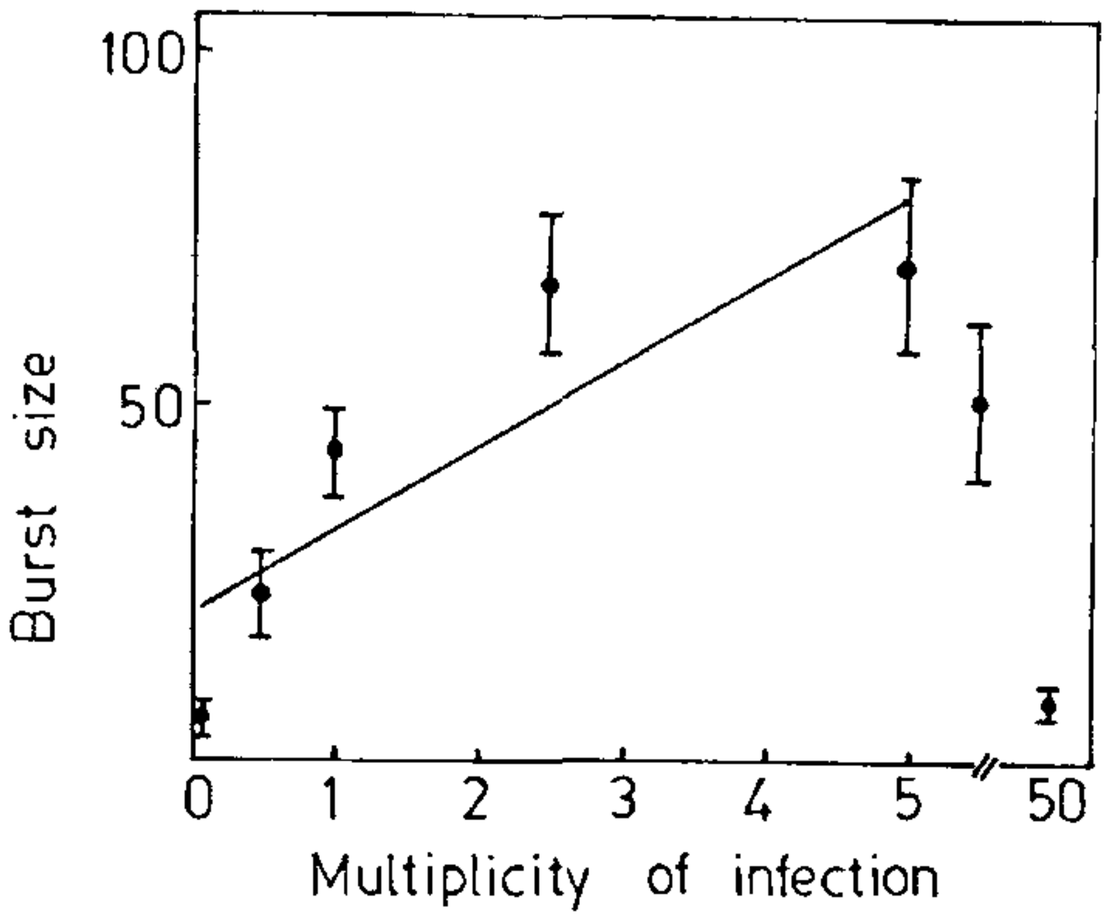

Figure 1. Correlation between MOI and burst size. Mean $\pm$ S. D. from 4 experiments for each point are shown. The solid line is the least squares fit. Correlation coefficient $= +0.84$, significant at $P < 0.01$.

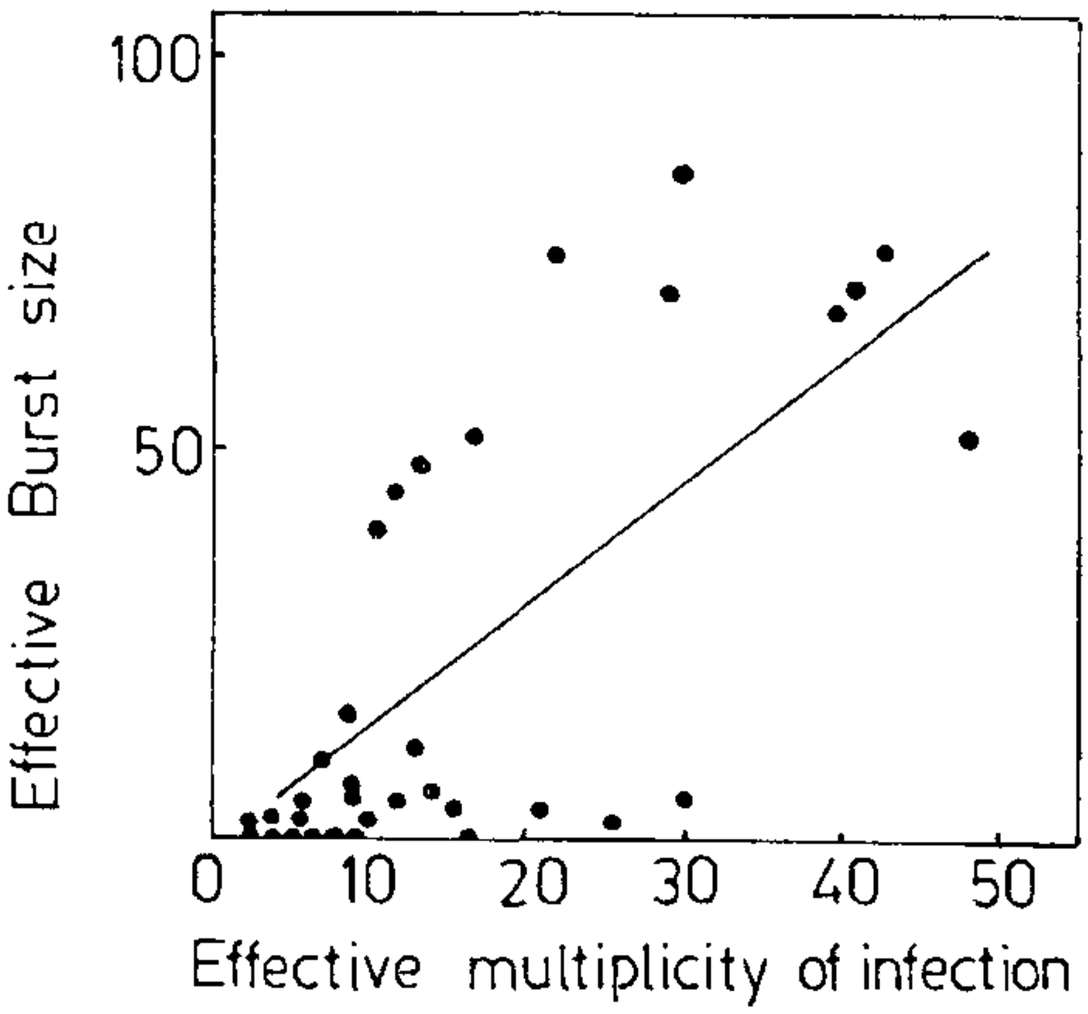

Figure 2. Scatter diagram showing correlation between effective MOI and effective burst size. The solid line is the least squares fit. Correlation coefficient $= +0.71$, significant at $P < 0.001$.

with increasing MOI but why at very high MOI the burst size decreases instead of increasing? Dulbacco showed earlier that burst size of a particular phage is limited and it decreases at high MOI probably because of the exclusion of most of the phages by the earlier adsorbed phages[10]. However, it is interesting to study how phages interacted with one another and contributed to the total phage production. For this reason the effective burst size is computed. A significant positive correlation between the effective MOI and effective burst size was obtained (figure 2). A similar observation was made by Hutchinson and Sinsheimer[11], who observed a 10-fold increase in phage production in a lysis defective mutant of $\emptyset \times 174$ as compared with the wild type phagé. This suggests that maturation of phage in the wild type is not a synchronous process and that lysozyme is produced much before all progeny particles mature. It may be reasonable to conclude in our case that increased MOI results in a situation similar to that of lysis inhibition and increase in burst size observed under these conditions may be due to similar mechanisms. For example, if maturation of phage is controlled by limiting concentration of one or more phage-coded catalytic factors, increased MOI can lead to increased phage production before the onset of lysis even in wild type condition. However, during higher MOI, the threshold concentration of lysozyme needed to lyse the cell, also might reach somewhat earlier, so that each phage produces less progeny than in single infection and yet the total phage production in one bacterium can be higher than in single infection.

Thus it could be a general property of phages that they utilize a small proportion of the bacterial resources in the course of their reproductive cycle and lyse the bacterium as quickly as possible. Phage production is determined by phage-coded regulatory factors and it has to adjust to such factors like availability of host, the growth rate of bacterium and the time and energy to channel for their production. However, more detailed investigation on the molecular mechanisms involved in resource utilization by a bacteriophage is needed.

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COMMENTS ON ESTIMATION OF SULPHATE REDUCING BACTERIA IN SEAWATER

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THE pollution of natural waters eventually leads to the multiplication of sulphate-reducing bacteria. These anaerobes thriving under rapidly reduced oxygen tension and Eh values have many undesirable consequences. One of the major problems has been an accelerated metallic corrosion of the water-front structures in industrially growing coastal towns[1].

The identification and enumeration of sulphate-reducing bacteria are of considerable interest to both pollution and corrosion scientists. One of the methods[2], commonly used for enumerating the sulphate reducers and to measure hydrogen sulphide generated by these anaerobes has been inadequate. A relationship between the amount of H_2S formed and the population of sulphate reducers quantified in the water sample has been found to be indifferent.

The American Standard Test Method (ASTM)[2] is based upon the iodometric determination of the hydrogen sulphide produced by sulphate-reducing bacteria in a suitable culture medium. Chaudhuri[3] found that the formula recommended by ASTM for calculating H_2S value was "incorrect" and stated that "such values of H_2S will still be valid provided they are multiplied ten times". The sulphate-reducing bacteria according to this method are considered to be absent if the calculated value of H_2S is less than 50 ppm or the same amount as in control bottle. They are considered as present if the value exceeds 50 ppm and further, the growth is termed as heavy if the H_2S value is as high as 350 ppm.

In our studies related to the monitoring of sulphate reducers in coastal waters, the use of ASTM method giving H_2S value as well as the extent of anaerobes in the water samples was employed. After repeated samplings monitored for one year, it was realised that the H_2S values obtained were very low even after multiplying the values by a factor of ten as suggested by Chaudhuri[3]. After a short incubation period, the culture bottles in reality showed a copious amount of black to gray flocculent precipitate of iron sulphide and heavy cloudiness of the medium accompanied by a very strong smell of H_2S. Higher values of H_2S were also reckoned by the intense blackening of wet lead acetate paper. The bacterial count executed simultaneously also gave very high values. In this study H_2S was estimated according to ASTM method and the sulphate-reducing bacteria were enumerated by adopting the 'shake tube' technique[4].

Table 1 gives the values of bacterial count and the amount of H_2S generated for various volumes of seawater samples incubated. Hydrogen sulphide values corrected according to Chaudhuri's suggestion are also mentioned. It is seen that even at a corrected concentration value of 37.3 ppm of H_2S, a bacterial count is 1.2×10^4 and at a concentration of 46.2 ppm, the count is as high as 13×10^4. Postgate[5] considered that a count of bacteria between 10^4 and 10^6 was adequate to cause serious pollution. Therefore, ASTM grading suggesting the absence of the organisms below 50 ppm of H_2S does not seem to be correct. Sasaki *et al*[6] observed that a concentration of 40 ppm of H_2S rendered the seawater corrosive. In any case, the H_2S values obtained by the ASTM method are very low.

Table 1 *Relationship between the number of sulphate reducers and the amount of H_2S formed in seawater samples*

Seawater sample (ml)	H_2S (ppm) ASTM method	H_2S (ppm) after correction	Bacterial count
0.5	2.31	23.1	4×10^2
1.0	3.73	37.3	1.2×10^4
5.0	4.62	46.2	3×10^4
10.0	5.84	58.4	13×10^4
Control	0.37	–	Nil

A communication received from ASTM conveys that "method D 993–58 part 31 (1977) will be replaced by a completely new method" and that "the new method will not give the user any indication on the amount of