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Bubble scavenging of bacteria in freshwater quickly produces bacterial enrichment in airborne jet drops[1]

Abstract—Air bubbles rising through laboratory bacterial suspensions collect bacteria by interception, a collection mechanism that depends on the size of the bacteria. Many of the scavenged bacteria are transferred to the jet drops when the bubbles burst at the surface. The enrichment factor (EF) for bacteria in the top jet drops increases rapidly with the distance the bubbles rise through the water, reaching values of about 400 after only 3 cm of rise for a bubble of 380-μm diameter. Although these numbers vary with bubble size and other parameters, cautious extrapolation to natural waters suggests that bubble scavenging is an important factor in the water-to-air transfer of bacteria by jet drops.

The well known tingling of the face that occurs when one is drinking a carbonated beverage is produced by small droplets from the bursting of CO_2 bubbles. On a global scale, the salt residue from droplets from bursting air bubbles at the surface of the sea accounts for a cyclic atmosphere-ocean exchange of between 10^9 and 10^{10} tons of sea salt per year (Duce 1978). When these bubbles burst, a jet of water rises upward from the collapsing bubble cavity (Blanchard 1963). The jet breaks up into several jet drops that, depending upon their size and the turbulence of the air, can remain airborne for minutes or days.

The top jet drop from a bubble that has risen through a bacterial suspension can have a concentration of bacteria that may be hundreds of times that in the suspension (Blanchard and Syzdek 1970, 1978; Bezdek and Carlucci 1972), but the origin of the bacteria that produce such large enrichment factors (EF) is not clear. Bubble scavenging has been suggested, but some investigators feel that this is unlikely since neither inertial impaction nor diffusion calculations can account for any significant number of bacteria scavenged by a bubble (Carlucci and Bezdek 1972; Quinn et al. 1975). Consequently, they believe that the high EF values for jet drop bacteria are produced when the bubble bursts and strips bacteria from a concentrated microlayer at the bulk air–

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water interface (Crow et al. 1975; Norkrans and Sörensson 1977). We present here experiments which show that such microlayers are of little importance in this regard, and that bacterial enrichments in jet drops are indeed caused by the scavenging of bacteria by bubbles rising through bacterial suspensions. When the bubble bursts, it acts like a microtome (MacIntyre 1972) that strips and transfers the bacteria from its surface into the jet drops. With this mechanism, a bubble need move only a few centimeters through a bacterial suspension to collect sufficient bacteria to produce an EF of several hundred in the top jet drop.

Our experiments were performed by letting air bubbles of about 380-μm diameter, produced from a fine, glass capillary tip, rise one at a time a known distance through a suspension of *Serratia marcescens*. The top jet drops (34-μm diam) from the bursting bubbles were collected on inverted nutrient-agar plates and the number of bacteria determined by the spread-plate method. The total number of bacteria (viable plus nonviable) was obtained by catching the jet drops on glass slides and making a direct count under a microscope. Details of the preparation of bubble-producing capillary tips, bacterial suspensions, and the determination of drop size can be found elsewhere (Blanchard and Syzdek 1975, 1977, 1978).

The EF for the top jet drop as a function of bubble rise distance (BRD) is shown in Fig. 1 for three experiments with a suspension of *S. marcescens* in pond water. Over the range of BRD covered, from about 0.1 cm to nearly 11 cm, the top drop EF increases with BRD. Initially, the increase is very rapid; an EF of 400 is attained with a BRD of only 3 cm. The EF increases more slowly after 3 cm, attaining only 600 after 10 cm of BRD. Other experiments with bubbles of equal size indicated that an EF of $\geq$1,000 was reached with a BRD of about 25 cm (Blanchard and Syzdek 1978).

A bubble of the size used here ejects up to six jet drops. The top drop of the jet set (the entire set of jet drops produced

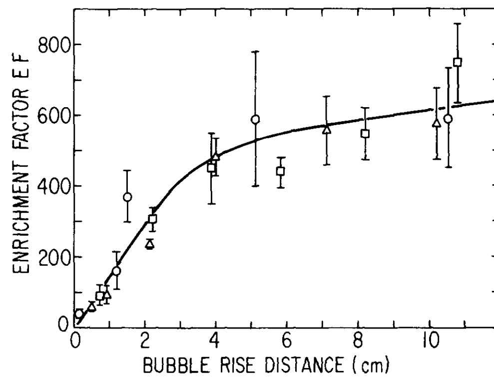

Fig. 1. Effect of distance a bubble rises through a bacterial suspension of *S. marcescens* in pond water on enrichment factor (EF) for bacteria in top jet drop. Bubble diameter = 380 μm. EF is ratio of bacterial concentration in drop to that in bulk suspension. Each point shows EF as calculated from average number of bacteria found on five nutrient-agar plates. Vertical bar—SD. For two experiments (○, △) bacterial suspension was prepared by adding 4 ml of a 20-h culture of *S. marcescens* in Difco nutrient broth to 1,200 ml of unsterilized pond water and mixing with a magnetic stirrer. For one experiment (□), cells were spun down in a centrifuge and washed twice before they were added to pond water. In this case, nutrient broth concentration in bulk suspension was estimated to be <0.01 ppm, as opposed to about 30 ppm for other two experiments. Bulk concentration of bacteria in all three experiments was $2 \times 10^6 \cdot ml^{-1}$. Experiments were done at room temperature (about 22°C). Bubbles were produced from a glass capillary tip inserted through a polyethylene stopper in the bottom of a glass tube (about 3-cm i.d. and 30 cm long).

by a bubble) rises to a height of about 3 cm, but the bottom drop barely reaches 0.1 cm. The bacterial enrichment factor is by far the highest in the top drop and decreases rapidly with drop position in the jet set; the bottom drop EF may be only two or three (Blanchard and Syzdek 1978). We wondered how the average jet set EF would vary with a BRD that extended out to a meter or more. Such experiments are not easily done in a conventional way, since it is awkward to use bacterial suspensions a meter deep; but they are easily done with an aging tube, a device that uses a downward-moving stream of water to keep a bubble beneath the surface for any desired time (Blanchard and Syzdek 1972). Multiplying the time by the bubble rise speed gives us

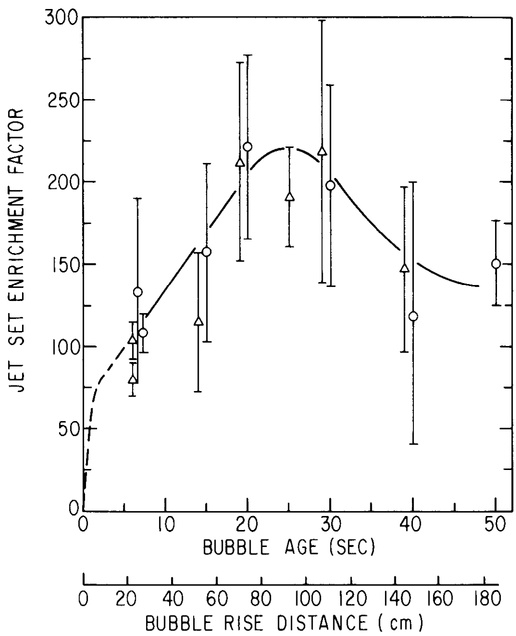

Fig. 2. Effect of distance a bubble rises through a bacterial suspension of *S. marcescens* in pond water on enrichment factor (EF) for bacteria in jet set. Bubble diameter = 380 μm. In both experiments, bacteria were grown and washed as described in legend to Fig. 1. In one experiment (△) concentration in suspension was $1.6 \times 10^6 \cdot ml^{-1}$, and in the other (○) $1.2 \times 10^6 \cdot ml^{-1}$. As in Fig. 1, each point shows average EF and SD. Work was done at 21°–22°C. Dashed line extending to zero BRD is an extrapolation based in part on data of Fig. 1.

the BRD. The collection of all the drops of the jet set on an inverted agar plate is done by electrostatic induction (Blanchard and Syzdek 1975).

The results of two experiments (Fig. 2) show that the jet set EF increases rapidly with BRD for the first several centimeters, paralleling that of the top drop (Fig. 1) but less rapidly after that, reaching over 200 at a BRD of about 100 cm. From there on out to about 180 cm, where the experiment ended, the jet set EF shows a marked decrease. There are no data for a BRD <20 cm, since the geometry of the aging tube prevents this. However, a reasonable extrapolation can be made down to 12 cm, about the upper limit for the top jet drop EF (Fig. 1). There, the jet set EF of 85 is about one-seventh that of the top drop, 600. This dominance of EF in the top drop confirms earlier work (Blanchard and Syzdek 1978).

There seems little doubt that the bacterial EF values in the jet drops are a result of bubble scavenging. First, since the EF has a near-negligible value at a BRD of zero but increases with BRD, it is hard to imagine any other hypothesis, much less one involving the surface microlayer. Second, direct measurements show that the numbers of bacteria scavenged by a bubble can account for the numbers found on the jet drops (Blanchard and Syzdek 1974). Third, although diffusion and inertial effects cannot account for the bacteria scavenged by a bubble, they can be accounted for by interception (Weber 1981). Interception is a collection mechanism which comes into play because the bacteria have a finite size. Because inertial effects are negligible, the bacteria follow the fluid streamlines past the bubble. But due to their finite size, bacteria following streamlines very close to the bubble surface make contact with the bubble and are scavenged. Quantitative comparisons between EF data and predications of scavenging by interception will be published separately.

During the first few centimeters of rise, a bubble of the size used here moves as a fluid sphere with a mobile surface (Tedesco and Blanchard 1979). In addition to bacteria, it collects surface-active material during its ascent. As a result, the mobility of the bubble surface decreases with rise distance until enough surfactant has been accumulated to render the surface immobile (Clift et al. 1978). It then rises as a solid sphere. The rate of collection of bacteria by a bubble in the early period of rise when its surface is mobile is much higher than the rate later on when its surface is immobile. This change from a fluid to a solid sphere accounts for the marked decrease in the rate of increase of EF at a BRD of about 4 cm. We cannot account for the apparent decrease of the EF with increasing BRD beyond about 100 cm; our earlier experiments also suggested this (Blanchard and Syzdek 1972). Perhaps there is a species of surfactant in the water that adsorbs more slowly, yet is much more strongly attached to a bubble than bacteria, with the result that bacteria are displaced from the bubble as the BRD exceeds 100 cm. An analogous and still unexplained decrease in the electrical charge of saline jet drops has been observed (Blanchard 1963; Medrow and Chao 1971).

The quantitative relationship between EF and BRD shown here will not hold for all experiments for it depends on many factors, including jet drop and bubble size, species of bacteria (Blanchard and Syzdek 1978; Blanchard 1978), and perhaps their physiological condition. However, we believe that the rapid increase of bacterial enrichment factors with the first few centimeters of bubble scavenging is a general phenomenon in all laboratory experiments. If these experiments can be extrapolated to natural bodies of water, and we suspect that they can, it then appears that only a few centimeters of bubble scavenging accounts for a significant fraction of the bacteria that are carried by jet drops into the atmosphere.

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