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Water-to-Air Transfer of Virus

Abstract. *Bubbles rising through suspensions of the bacteriophages T2 and T4 and of* Escherichia coli *adsorb and eject these particles in droplets that are formed when the bubbles burst. The concentration of the viruses in ejected droplets, determined from electron microscopy, exceeded the suspension concentration by 50 times. Similar results were obtained for* Escherichia coli. *The viability of some of the adsorbed particles was established by biological counts.*

This study concerns the possibility that bubbles adsorb viruses and propel them into the atmosphere when the bubble bursts. Bubbles bursting at a liquid surface eject a tiny jet of fluid into the air. The jet breaks up into a series of tiny drops with the diameter of the uppermost drop one tenth that of the bursting bubble (*1*) and with most of the lower drops having larger diameters. Such jet drops contain much of the material adsorbed to the bubble (*1*) as well as some of the material adsorbed to the surface of the liquid (*2*).

Transport into the atmosphere of red-tide toxins (*3*), organic matter (*4*), and bacteria (*1*) has previously been related to aerosol formation. Gruft *et al.* (*5*) postulated that aerosol of marine origin is a vector of *Mycobacterium intracellulare* (Battey) infection. Whether pathogenic viruses are similarly airborne is clearly of public health concern.

In the present study, the coliphages T2 and T4 were chosen as harmless indicator viruses which, after aerosol ejection, could be assayed biologically and morphologically. If these viruses, with their complex structure and sensitivity to drying and ultraviolet radiation, remained viable and became concentrated during aerosol formation, then it could be assumed that the simpler, less fragile viruses are similarly dispersed.

The jet droplets used in this study were formed by admitting air bubbles (0.03 cm in diameters) under pressure (1 to 2 kg/cm^2) (*6*) into the bottom of a liquid column (17 cm in depth) containing *Escherichia coli* and the bacteriophages suspended in buffer. The phage concentration was adjusted to 5×10^8 per milliliter and the bacteria to a concentration of 5×10^6 per milliliter.

Depending on the experiment, different suspending fluids were used in the column. Jet droplets containing phage were seen in the electron microscope when the phages were suspended in tris buffer (0.1*M*, *p*H 7.6, with 3 percent NaCl). The phage lysate and washed bacteria were added to distilled water. Lysate facilitated adhesion of jet droplets to the carbon film of the electron microscope grid, and upon drying left a distinct circular contour which we used for diameter measurement.

The T2 and T4 bacterial viruses were prepared in high titers from lysates of their host cell B/1,5 (a strain of *E. coli*) grown in synthetic medium (*7*) with heavy aeration. In some experiments the phages were centrifuged away from the lysate and resuspended in buffer before they were added to the column. *Escherichia coli* B/4, a strain that is resistant to T2 and T4, was grown overnight in 1 percent tryptone broth with aeration at 37°C to the stationary phase. The cells were centrifuged and the pellet was suspended in the same buffer as that used in the bubble chamber.

To collect jet droplets for electron microscopy, grids bearing carbon films were held inverted for 30 to 45 seconds about 1 cm above the surface where the bubbles were bursting. In this configuration of emitting bubbles and collecting surface we collected the uppermost two or three droplets of the jet (*8*). The collected droplets were dried in air for 1 minute to minimize subsequent loss of material, stained in saturated uranyl acetate for 2 minutes, rinsed in distilled water, drained, and dried.

The specimens were photographed at two magnifications (×3000 and ×10,000) by means of a Jeolco 100B electron microscope at 60 kv. Enlarged prints at the lower magnification were used for diameter measurements of the dried droplets and for counting the number of viruses and bacteria in measured areas. The smallest area used in the counts was a quadrant. Only particles showing both a head and an extended tail were counted as virus.

The fields photographed at the higher magnification were randomly selected and provided morphological evidence of intact virus in the droplets.

Viability assays were obtained by holding moist petri dishes containing tryptone agar above the surface of the bubble column for 10 to 40 seconds. Soft agar (0.7 percent) was immediately added to the surface of the dish and spread by vigorous shaking. In the case of the phage assays the soft agar contained the sensitive cell, B/1, 5. The dishes were incubated overnight at 37°C.

Electron microscopy showed that both phages and bacteria were transferred from bulk fluid into air by jet droplets. The mean volume calculated from 18 droplets was $2 \times 10^{-8} \pm 1 \times 10^{-8}$ ml (± standard deviation) with a range of 4×10^{-8} to 7×10^{-9} ml. Thus the range of diameters of drops ejected from bubbles that were 300 μm in diameter was 24 to 42 μm, the mean being 34 μm. These data corroborate Blanchard's (*1*) rule of thumb that the diameter of the top drop of the jet set is one-tenth the diameter of the bubble. The range of drop sizes results from collecting the uppermost three drops. The second and third drops of the set are smaller and larger, respectively, than the top drop.

The number of phages per droplet was determined from the electron micrograph by counting the number of particles having both a head and a tail per unit area of droplet. Bacteria were easily identified by shape and size. Eighteen separate droplets captured from a col-

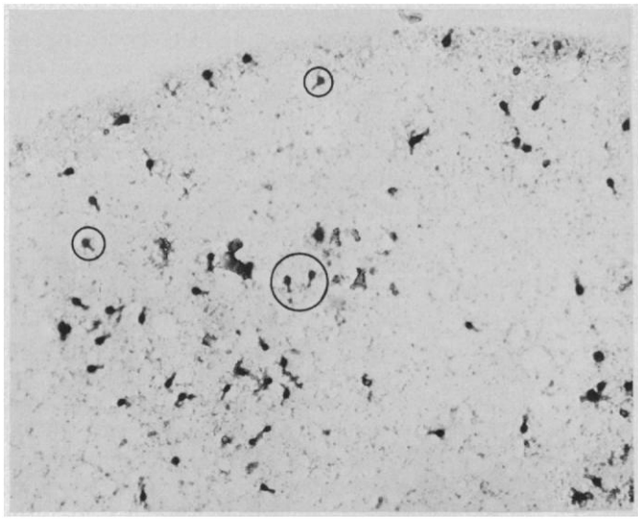

Fig. 1. Electron micrograph of a portion of a dried jet droplet. Coliphage T4 (encircled) with heads and extended tails are randomly distributed and are easily identified within the matrix of detritus demarking the site of the droplet. (Uranyl acetate, × 7500)

umn containing 5.7×10^8 phages per milliliter and 5×10^6 bacteria per milliliter showed an average number of $2.3 \times 10^{10} \pm 1.9 \times 10^{10}$ phage per milliliter (± standard deviation). The average number of bacteria per milliliter was $1.5 \times 10^8 \pm 1.4 \times 10^8$. The high variance in the number of bacteria is to be expected from the small sample size and the relatively rare events.

The variance of phage concentration in the droplets has at least two causes: phage particle losses during staining, and differences in phage concentration between the first and second drops of the jet set. It is clear from the work of Blanchard and Syzdek (*9*) with bacteria that such differences do exist.

Our data show that both phages and bacteria were concentrated in the droplets (by 50 times for the phages and 30 times for the bacteria) relative to the suspension. The concentration factor C of Blanchard and Syzdek (*1*) is a function of drop size and reaches a maximum value for drops that are 60 to 80 μm in diameter. Our drops had a mean diameter of 34 μm with a range of 24 to 44 μm and showed a negative correlation (−0.5) of concentration factor with drop diameter, in reasonable agreement with Blanchard and Syzdek. This correlation is significantly different from zero at the 95 percent confidence level.

The concentration of phages and bacteria in the bulk solution was determined from the number of plaques and colonies formed according to standard biological assays. Attempts to determine phage and bacterial concentrations in the jet droplets by biological counts permitted us to say only that viable phage and bacteria were present. In one experiment we found an average of three bacteria and 390 phages per jet droplet. The difficulties we encountered in doing the assays may have come from the possibility that the droplets diffused into the agar on contact, so we were unable to spread the dish successfully. On the other hand, the drops may have bounced off the agar surface, as has been observed by others (*10*). We do not understand the phenomena surrounding droplet bouncing; however, we have reduced droplet loss on electron microscope grids by adding fresh lysate to the bulk fluid. By contrast, the addition of fresh lysate did nothing to improve the biological assays.

Figure 1 shows that phages and other material were present in the droplet. This droplet was collected from bulk fluid in which the phage was added from lysates. In three or more experiments where both phages and cells were washed by several alternate centrifugations and resuspensions, no indication of droplets was found. Perhaps a surface film on the droplets was needed to make them adhere to the electron microscope grids.

The experiments of Blanchard and Syzdek (*1*) established that viable bacteria were present in jet droplets and that the concentration in the droplets was between 10 and 1000 times that of the bulk fluid. The experiments we report here confirm their results and show further that bacterial viruses also were transferred and concentrated in aerosols. Electron microscopy (Fig. 1) showed that the complex morphology of the T2 and T4 coliphages was not disrupted extensively and that many of the particles appeared normal. From limited data we have also shown that viable particles were present in the aerosol.

The T2 and T4 coliphages were chosen for these experiments because their poor resistance to drying and to ultraviolet light would prevent them from becoming an airborne nuisance contaminant in the laboratory.

Phage counts, bacterial counts, and measurements of diameter made on electron micrographs may be low because of material being washed away or lost during the staining process. On the other hand, the circle of phages, bacteria, and debris that we used to define the original diameter of the hemisphere formed by the impacting jet droplet may be an underestimate of the correct volume. It is impossible to know whether the material we see deposited in a circle on the electron micrographs represents the original area wetted by the hemisphere or whether some of the water evaporated from the hemisphere before the debris was laid down.

The most important aspect of Blanchard's results and of ours is the increase in concentration of bacteria and viruses in the jet drops. The public health implication of this work is its addition of viable viruses to a growing list of microorganisms and other potentially noxious materials that may not remain in the ocean or other waters to which they have been consigned but instead may be lofted into the winds wherever bubbles are generated, such as in whitecaps at sea, in breakers in the surf, or during the passage of ships. The unanticipated aspect of our experiments as well as those of Blanchard's is that both viruses and bacteria are actually concentrated by adsorption and ejection from water.

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