POSSIBILITY OF GROWTH OF AIRBORNE MICROBES IN OUTER PLANETARY ATMOSPHERES

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ABSTRACT

In the laboratory, bacterial aerosols can be created with consistent properties, and the physics of particles in test chambers is well understood. Particles fall out on walls of chambers at an exponential rate often described in terms of a half-life. The "biological behavior", or extent of survival in a given environment, is amenable to quantitative assay, but the results often vary. There are several theories that attempt to explain mechanisms of survival. We have now shown that airborne bacteria can maintain metabolic functions in a suitable atmosphere. We theorize that particles in the Jovian atmosphere would have physical half-lives of 10 to 1500 years, depending upon which of two turbulent models is chosen.

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INTRODUCTION

There is a poem I recall somewhat vaguely -- I do not know who wrote it -- but the content is something like this:

I sat upon a chair
And felt it had no back.
It had no feet, no legs, I swear,
But there I sat,
Disregarding such inconsequential
Things as that.

One cannot approach the study of the possibility of propagation of microbes on Jupiter -- especially earth-type microbes, without disregarding a great number of "inconsequential" threads, all needed to support the fabric of truth. The very title of this paper implies a number of elusive concepts so ethereal, in fact, that an attempt to study this problem in its entirety at this time would appear to be futile.

There are, however, planetary quarantine restrictions agreed upon by several nations, so the onus is upon us to attempt to discover what we can about any of the factors involved in the possibility that Jupiter's

atmosphere might become contaminated if entry of a space probe occurs into the biological stratum. It is obvious that the contamination would be via aerosols. Here is a list of some of the other important "ifs" with probabilities smaller, perhaps by orders of magnitude, than the first "if":

(a) If the probe is contaminated;

(b) If the contaminant survives to Jupiter;

(c) If the contaminant is an anaerobe;

(d) If the cell survives a detachment process;

(e) If the cell is in (or on) a sufficiently small particle to remain airborne for some length of time;

(f) If the cell contacts organic material and water at a suitable rate;

(g) If the cell can utilize such organic material anaerobically (It must obtain nutrients and energy and it must have an energy sink -- a proton acceptor) to allow an increase in mass;

(h) If the cell can then form two or more cells (propagate);

(i) If the last three processes can occur in the airborne state.

Our laboratory is studying the possibility of propagation of airborne cells occurring in Earth's atmosphere, the rationale being that it is easier to determine whether Earth's microbes can propagate

in an air environment where they have had some billions of years to acquire the necessary adaptive systems than to compound the difficulty by simulating a Jovian environment -- whatever that environment might be said to be at this time.

MICROBIAL AEROSOL FORMATION

It will be necessary to bring you up-to-date on the state of the art in aerobiological research - - as briefly as practicable - - and to review current knowledge, for without this there can be no real exchange of information about how we study airborne survival and propagation of microbes, and how the data can be interpreted.

Studies in aerobiology have been quite diverse, but for our purposes we will confine our discussion to microbes in or on particles or droplets in the 1 to 20 μm diameter range and we will label collections of such particles with the term aerosol. Particles larger than this do not remain airborne for appreciable lengths of time.

Aerosols arise from many sources, but in the laboratory uniform and consistent aerosols are most frequently created by twin-fluid atomizers. Shear forces from compressed air stretch liquid surfaces exposed to the air flow until the surface tension is overcome. Threads or sheets are formed that break into droplets varying in size from less than 0.1 μm to as large as 100 μm, with the median diameter being about 10 to 12 μm, and the distribution of sizes being almost log-normal. Within a second

after droplets are formed, most of the water evaporates and the equilibrium size of the particles then depends on the amount of dissolved solids in the fluid being atomized. With 2% solids, a usual concentration for cultivating or maintaining bacteria, the median aerosol particle diameter is reduced to about 2 μm by evaporation (Green and Lane, 1964).

When bacteria are suspended within the atomizer fluid, the probability of their being included within a given droplet of their size or larger depends directly on the number of microbes per cm^3. For example, there are 2×10^9, $10\mu m^3$ spaces in a cm^3; if there are 2×10^9 microbes in the fluid, then most droplets will contain at least 1 bacterium, but the distribution of cells per droplet is Poisson. A simple nomograph is helpful in determining the distribution (Figure 1).

STIRRED SETTLING

The usual second step in aerobiology studies is to confine the aerosol so that it can be studied. An easy way is to use a box, in the bottom of which is located an upward-directed fan. This is called a stirred settling chamber (Sinclair, 1950). Besides being simple, this device allows aerosols to be studied in turbulent air. Since air in motion within a closed system is neither entering or leaving the chamber (the air is not expanding or contracting), then the sum of all vectorial direction and velocity values must equal zero. Hence, stirring

the air neither adds nor substracts from the average rate of fall of the contained particles. The average number of particles swept downward equals the number swept upward.

In still air, particles reach a terminal velocity according to Stokes' Law, and the velocity of fall depends on size, density, and shape of particles as well as the gravitational constant of acceleration. Stirring just acts to keep the particle concentration relatively uniform within the contained air (Figure 2). Because the concentration is uniform, a constant percentage of the particles remaining airborne are brought close enough to horizontal surfaces to impact (fall-out) in a given unit of time. We say the "physical" decay, or fallout, of the mass of the contained particles is exponential for a given particle size and density, and we usually refer to the decay in terms of either the half-life or the exponent of e (see appendix).

Obviously, a measured decay rate can be used to estimate particle size in a given chamber. This measurement can be accomplished by counting the remaining particles, either by photometric, electronic, or chemical measurements. In a cubical chamber 1 meter high the half-life (decay) of 1μm particles of unit density is 400 minutes. The relationship between chamber height and half-life is direct, so if the average depth of the turbulent, biological (0-100°) atmospheric stratum of Jupiter - - even assuming adiabatic expansion or contraction of counter-flowing air streams - - is 80 km, then the half-life of a 1 μm particle could be as large as 2×10^7 minutes or about 50 years. When this value is corrected for the

differences in gravitational constants (Jupiter's is about 2.2 times that of Earth) and viscosity differences (a factor of about 0.44), the half-life reduces to approximately 10 years. The half-life of a 10μm particle would be about 0.12 years (terminal velocity is a function of diameter squared) or about 40 days.

Stated another way, a single 1 μm particle in Jupiter's atmosphere, randomly located, would probably have a 50-50 chance every 10 years or so of being wafted down into the interior far enough to exceed the approximate 100° C biological temperature level if the stirred settling model can be said to represent the average behavior of the whole Jovian atmosphere (see appendix).

ROTATING DRUM

If a box or drum is slowly rotated about a horizontal axis, the effect, with respect to the air in the drum, is to rotate the relative direction of the gravitational force acting on a suspended particle (Goldberg, 1971). Particles move in small orbits with respect to the rotating air, and the system can be arranged such that centrifugal force is the principal force causing fallout. The half-life of a 1μm particle in a drum can be 72 hrs or more (Dimmick and Akers, 1969).

In use, the air in these chambers is purged until the exit air is the same as the entering air, which has a pre-set, controlled humidity and constant temperature. Then a fluid suspension is atomized, the aerosol is conducted via tubing into the chamber while air is withdrawn

at the same rate. The filling process takes about 5 minutes for a 1500 L drum. The end of this process is called "zero" aerosol time. Samples are removed at appropriate intervals via a separate set of tubing into impingers*, which are like bubblers except that the sampled air is drawn through the collecting fluid at sonic velocity. Impingers are about 99% efficient for particles 1μm or larger, and the most commonly used impinger samples 12.5 L of air per minute.

BEHAVIOR OF BACTERIA IN AIR

The typical aerobiological study is conducted to establish baseline data (Hatch and Wolochow, 1969). One starts by seeding a selected growth medium with a selected bacterial species. After a short time, known as the lag period -- while they become acquainted with each other -- the cells multiply at an exponential rate by, of all things, dividing. When the number of cells has reached some desired level, they are either atomized from the original growth medium or they are removed by centrifugation and suspended in the atomizer fluid of choice.

The cells are atomized, and samples are removed via the impinger containing solutions calculated to encourage maximal survival. The liquid is then diluted and seeded onto semi-solid growth medium where, it is expected, live cells will propagate to produce spots called colonies that can be counted to ascertain the number of live cells collected from

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* Ace Glass Co., Vineland, N.J., U.S.A.

the air. The numbers of colonies, adjusted for sampling volume and time, are plotted on semi-log paper, the X axis being aerosol time, and a line through the points is an analogue of the biological decay "rate". Note that this rate of decay includes the physical fallout of airborne particles as well as the measure of survival. When physical decay, measured by other means, is subtracted from the observed changes in viable numbers, the remainder is called "biological decay" (Figure 3).

For years it has been usual for the investigator to repeat the previously described experiment with a variety of conditions of humidity, make a plot of survival rate versus humidity, and publish a paper titled THE survival of pogo gregarious (or whatever) as a function of humidity -- or temperature, etc. (Figure 4). These experiments are, indeed, performed with a level of expertise high enough to classify them as being within the state of the art (Brown, 1954). I am responsible for some of them myself (Dimmick, 1960). That is not, however, the whole story.

There are two particularly interesting characteristics of such experiments. One is THE in the title; the trend shown in the typical paper can usually be modified in a variety of ways not having to do with humidity, (e.g., age of culture, growth conditions, additives), although it is true that, in general, the mid-range of humidity is most lethal, regardless of the history of the microbes (Sawyer, et al., 1966). The second characteristic is that not a single instance has been reported where the biological decay was less then the physical decay, a situation

that would have to exist if true propagation had occurred.*

There may be, however, some reasons why propagation has never been observed -- assuming it is possible -- and this brings me to the atypical and to the problem of heterogeneity. Aside from the cells-per-particle problem previously mentioned, individual bacterial cells of the same species, grown in the same culture, differ at any given time during growth, in a variety of ways (Dark and Callow, 1973), one of which is the capacity to survive for a given time in a given environment. When a population just starts to propagate in growth medium ("young cells"), most cells become more sensitive to stress than in any other period of growth. An "older" population, however, still contains some "young" cells, and vice versa, and the distribution of the two age groups is often unpredictable.

Since it requires at least 5 minutes of atomization time to attain a suitable concentration in an aerosol chamber, then a given air sample contains bacteria of different "aerosol ages". This is hardly important after, say, 300 minutes of aerosol time, but it has been responsible for some interesting conclusions made about viability during the first 30 minutes of aerosol time (Ferry et al. 1951).

In an attempt to attain better cellular homogeneity, one may employ synchronous cultures wherein the majority of the cells are induced to divide at the same time during the growth of the culture in vitro (Dimmick and Heckly, 1965). We have done this many times, and the resulting

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* Subsequent to the delivery of this paper, our laboratory has shown that at least 1 generation of new airborne cells can be formed.

survival curves were like something offered by nature as a joke: that is, it was better to laugh about the lack of replicability than to cry about it (Figure 5). But out of these studies has come a theory based on evidence of repeatable trends. The theory says that survival of an individual cell is a function of a large number of metabolic and genetic events within the cell, interlinked in such a way that there are numerous feedback systems, both positive and negative, within each cell. Response times vary from cell to cell and within a cell, and the system may have many stable conditions intended to maximize the chance of survival, but these conditions may alternate with unbalanced interim states that can be lethal for the cell. If environmental changes are slow enough, a new stability is attained; if they occur too fast, then instability is initiated, and the cell dies for lack of control (Heckly, et al., 1967).

There are other theories for the mechanism of death of microbes in the airborne state. Hess (1965) found oxygen to be toxic, but only at low humidities. Cox (1973) has proposed a mathematical model for the oxygen-induced death. Webb (1965) postulated that loss of hydrogen bonding in the DNA structure caused death. Israeli (1973) has shown supression of DNA synthesis in airborne bacteria, and irreversible blockage of the β-galactocidase enzyme system. Hatch et al. (1973) are studying genetic factors; they attribute aerosol resistance to a genetic determinant. Benbough and Hambleton (1973) have shown that changes in the membrane integrity occur such that vital materials can leak from the cell. None of these observations have yielded evidence

that might lead one to believe that airborne microbes could propagate, and you see the further difficulty of applying laboratory data to field studies where the source ("history") of the species is usually unknown.

METABOLISM IN THE AIRBORNE STATE

In our studies of the possibility of propagation of microbes on Jupiter, we decided that the egg really comes before the hen. That is, there was presumptive evidence that airborne microbes might sustain metabolic functions, but no real proof had been found. Obviously, cells cannot grow -- much less propagate -- if they cannot carry out metabolic functions, so an attempt to demonstrate metabolism seemed to be the easiest starting point.

One obvious proof of metabolic function would be to demonstrate uptake of glucose and evolution of CO_2. The approach was to mix two aerosols (relative humidity 90 - 95%), one containing bacteria and the other containing uniformly labelled C^{14} glucose. Some particles would collide, and if cells were metabolically active, the glucose would be taken into the cell, converted to H_2O and CO_2, and $^{14}CO_2$ would accumulate in the aerosol chamber. To measure the uptake of glucose, we collected samples of air on membrane filters, washed the filters thoroughly and tested them for insoluble, labelled content.

Complete details of these experiments have been submitted and accepted for publication (Dimmick et al. 1975), but the findings are summarized here in Figure 6. Some cells did take up glucose -- termed

insoluble particulate label (IPL) -- and the IPL remained within cells for about 5 hours. During that time $^{14}CO_2$ was produced, after which the concentration remained relatively constant. Since some bacteria could settle onto the walls of the aerosol chamber and produce $^{14}CO_2$, suitable controls were performed, and this effect was shown to be insignificant during the first 5 hours of aerosol time.

This seems to be clear proof that metabolism can occur in airborne microbes. The next step is to search for evidence that airborne cells can form new genetic material needed for cell division. If this is found to be true, then we will search for evidence that one cell might form two cells -- true propagation.

Finally, it is reasonable to presume that the turbulent system in the Jovian Biostratum is a blending of the stirred settling and the rotating air-mass models (Libby, 1974). The half-life of 1 μm particles is not likely to be less than about 10 years, as shown above, and could not possibly be more than about 10^3 years, as developed in the appendix. This wide range of half-life values is reflective of the lack of real knowledge of conditions on Jupiter, but at least the values provide limitations within which theories of contamination or "life on Jupiter" must be constrained.

We gratefully acknowledge the assistance of Caro Hopper, Sharon Tritt and Guy Vinson in preparation of this paper.

Benbough, J.E. and Hambleton, P.: 1973, Airborne Transmission and Airborne Infection (Hers, J.F. and Winkler, K.C., eds) Oosthoek Publ. Co., Utrecht, The Netherlands, p. 135.

Brown, A.D.: 1954, Aust. J. Biol. Sci., 7,444-451.

Cox, C.S.: 1973, Airborne Transmission and Airborne Infection (Hers, J.F. and Winkler, K.C., eds.) Oosthoek Publ. Co., Utrecht, The Netherlands, p. 108.

Dark, F.A. and Callow, D.S.: 1973, Airborne Transmission and Airborne Infection (Hers, J.F. and Winkler, K.C., eds.) Oosthoek Publ. Co., Utrecht, The Netherlands, p. 97.

Dimmick, R.L.: 1960, J. Bacteriol. 80,289-296.

Dimmick, R.L. and Heckly, R.J.: 1965, in Proceedings of Atmospheric Biology Conference (Tsuchiya, H.M. and Brown, A.H., eds.) Univ. of Minnesota (Lib. Cong. Cat. #65-22526).

Dimmick, R.L. and Akers, Anne: 1969, Introduction to Experimental Aerobiology, John Wiley and Sons, New York.

Dimmick, R.L., Straat, P.A., Wolochow, H., Levin, G.V., Chatigny, M.A. and Schrot, J.R.: 1975, J. Aerosol Sci. (January, in press).

Ferry, R.M., Farr, L.E., Rose, J., and Blau, M.R.: 1951, J. Infect. Diseases, 88, 256-271.

Goldberg, L.J.: 1971, Appl. Microbiol. 21, 244-252.

Green, H.L. and Lane, W.R.: 1964, Particulate Smokes and Dusts (2nd ed.) E. and F.N. Spon Ltd., London.

Hatch, M.T., Bondurant, M.C. and Ehresmann, A.W.: 1973, Airborne Transmission and Airborne Infection (Hers, J.F. and Winkler, K.C., eds.) Öosthoek Publ. Co., Utrecht, The Netherlands, p. 103.

Heckly, R.J., Dimmick, R.L. and Guard, N.: 1967, Appl. Microbiol., 15, 1235-1239.

Israeli, E.: 1973, Airborne Transmission and Airborne Infection (Hers, J.F. and Winkler, K.C., eds.) Oosthoek Publ. Co., Utrecht, The Netherlands, p. 110.

Libby, W.F.: 1974, Origins of Life, 5, 483-486.

Sawyer, W.D., Jemski, J.V., Hogge, A.L., Eigelsbach, H.T., Wolfe, E.K., Dangerfield, H.G., Gochenour, W.S., and Crozier, D.: 1966, J. Bacteriol. 91, 2180 - 2184.

Sinclair, D.: 1950, Handbook of Aerosols, U.S. Government Printing Office, Wash., D.C.

Webb, S.J.: 1965, Bound Water in Biological Integrity, Charles C. Thomas, Publisher, Springfield, Ill.

APPENDIX 1

Laws of stirred settling in an enclosed chamber are:

(1) For tranquil air and a given particle size and density,

$$v = \frac{\rho\, d^2\, g}{18\, \eta}$$

where

v = Stokes' velocity of fall, cm/sec

ρ = density, gm/cm^3

d = particle diameter, cm

g = gravity constant, cm/sec^2

η = viscosity of air, gm/cm sec

and for average behavior of a collection of particles,

(2)

$$\frac{N_t}{N_o} = e^{-Kt}, \text{ and } K = \frac{v}{H}$$

where

N = number of particles/cm^3

H = effective chamber height, cm, (volume to horizontal area ratio)

t = time, sec

or

(3) half life ($t_{\frac{1}{2}}$) = $.693 \frac{H}{v}$

APPENDIX 2

Laws of particles in a rotating body of air, e.g., a drum, are:

$$(4) \qquad v = \frac{\rho d^2 R \omega^2}{18\eta}$$

where

R = Radius, cm

ω = radians/sec

and by setting K for stirred settling equal to K for rotating air, it can be shown that

$$\frac{g}{H} = 8 \pi^2 \theta^2 , \quad \text{or} \quad H = \frac{g}{8 \pi^2 \theta^2}$$

where

θ = revolutions per second

and

H = effective height if the system were a stirred settling chamber.

If it is assumed that the period of rotation of the bands on Jupiter are 1 month (Figure 7), then

$$H_{(\text{effective})} = \frac{2500}{78.96 \times (3.86 \times 10^{-7})^2}$$

$$= 2.13 \times 10^{14} \text{ cm}$$

and

$$t_{1/2} = .693 \frac{H}{v} = \frac{0.693 \times 2.13 \times 10^4}{1.74 \times 10^{-2}}$$

$$t_{1/2} = 4.9 \times 10^{16} \text{ sec} = 1.5 \times 10^3 \text{ years}$$

where

v is calculated using g = 2500 cm/sec^2

and $\eta = 8 \times 10^{-5}$ for a 1 μm particle, density 1 in hydrogen.

i.e.,

$$v = \frac{10^{-8} \times 2500}{18 \times 8 \times 10^{-5}} = 1.74 \times 10^{-2} \text{ cm/sec}$$

NOTE TO PRINTER:

Symbols:

ρ = rho

π = pi

η = eta

ω = omega

θ = theta

μ = mu

g = gee

v = same letter, lower case

R = same letter, Caps

K = same letter, Caps

t = same letter. l.c.

FIGURE LEGENDS

Figure 1 Relationship between number of particles in fluid to be atomized and expectation of finding at least one particle in airborne droplets of a given size.

Figure 2 Approximate vector diagram of air velocities in a stirred settling chamber.

Figure 3 Illustration of typical behavior of particles and bacteria in a stirred settling chamber or a drum.

Figure 4 Illustrative relationship of survival of airborne bacteria with humidity.

Figure 5 Atypical survivor curves in bacterial aerosols from "synchronous" cultures.

Figure 6 Dynamics of $^{14}CO_2$ production by bacteria in the airborne state.

A = $^{14}CO_2$ production, air + wall

B = $^{14}CO_2$ production from walls alone

C = Uncorrected IPL amounts

D = IPL corrected for particle fallout

E = $^{14}CO_2$ production from used chamber after cleaning for additional tests.

Continued:

Figure Legends: Cont'd.

Figure 7 Suggested concept of possible atmospheric velocity pattern in Jovian Biosphere. Cross-section of one band.

The illustration is intended to show how the central core is like a box with a "floor", "ceiling", and sides that particles can "fallout" onto and be lost. The contained air is both rotating and turbulent. Hence, it is equivalent to a stirred settling chamber.

Fig. 1. Relationship between number of particles in fluid to be atomized and expectation of finding at least one particle in airborne droplets of a given size.

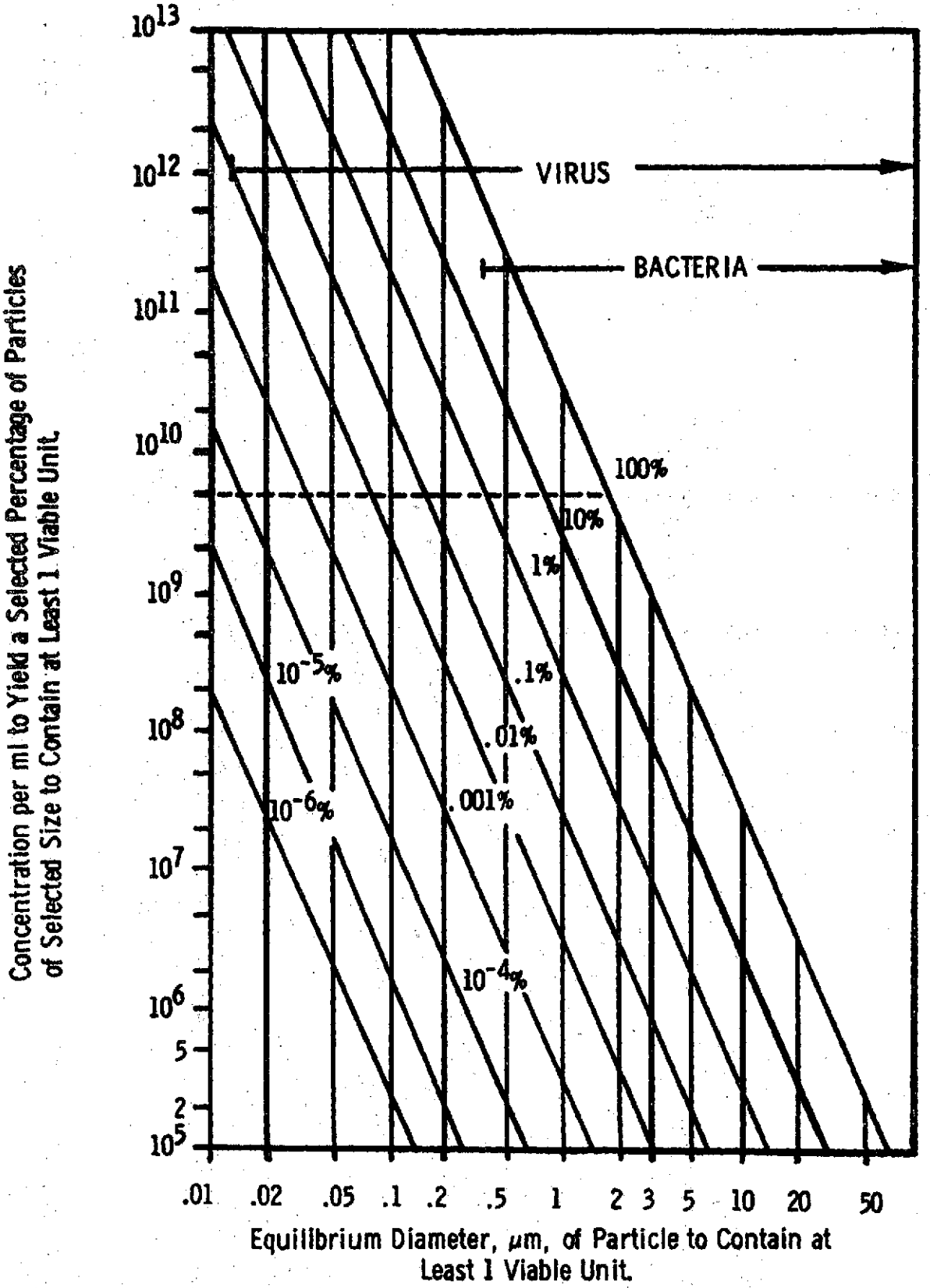
Concentration per ml to Yield a Selected Percentage of Particles of Selected Size to Contain at Least 1 Viable Unit
10^{13}
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
5
2
10^{5}
VIRUS
BACTERIA
100%
10%
1%
.1%
.01%
.001%
10^{-4}%
10^{-5}%
10^{-6}%
.01
.02
.05
.1
.2
.5
1
2
3
5
10
20
50
Equilibrium Diameter, μm, of Particle to Contain at Least 1 Viable Unit.

Fig. 2. Approximate vector diagram of air velocities in a stirred settling chamber.

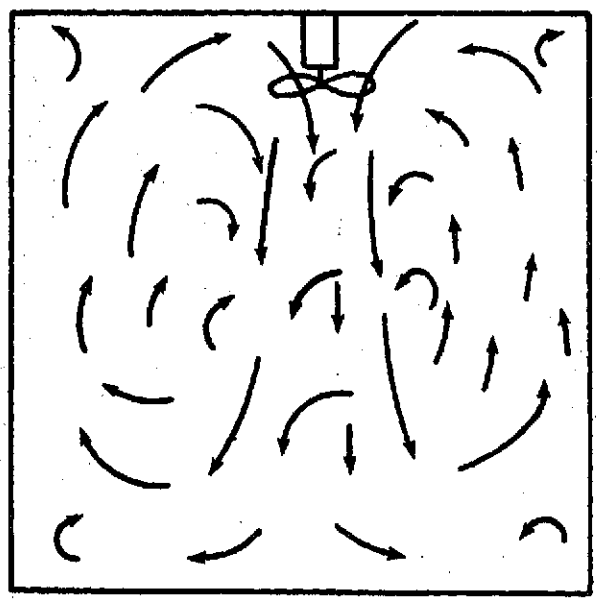

Fig. 3. Illustration of typical behavior of particles and bacteria in a stirred settling chamber or a drum.

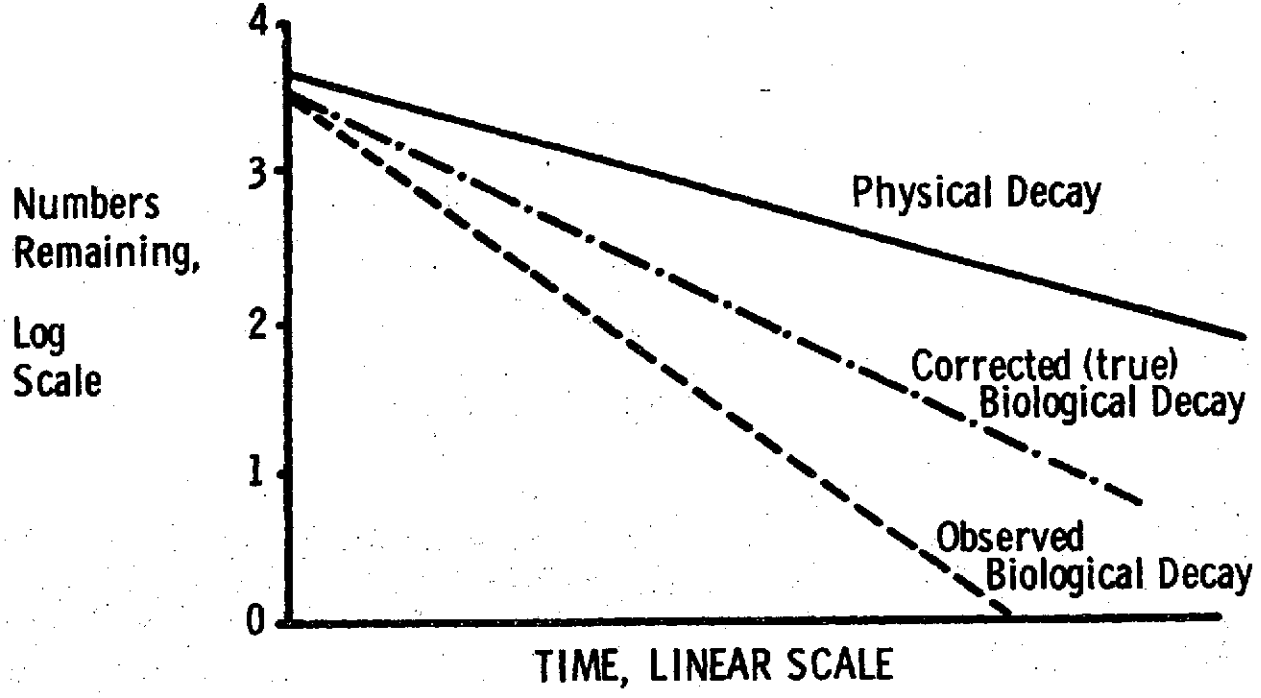
Numbers
Remaining,
Log
Scale
4
3
2
1
0
Physical Decay
Corrected (true)
Biological Decay
Observed
Biological Decay
TIME, LINEAR SCALE

Fig. 4. Illustrative relationship of survival of airborne bacteria with humidity.

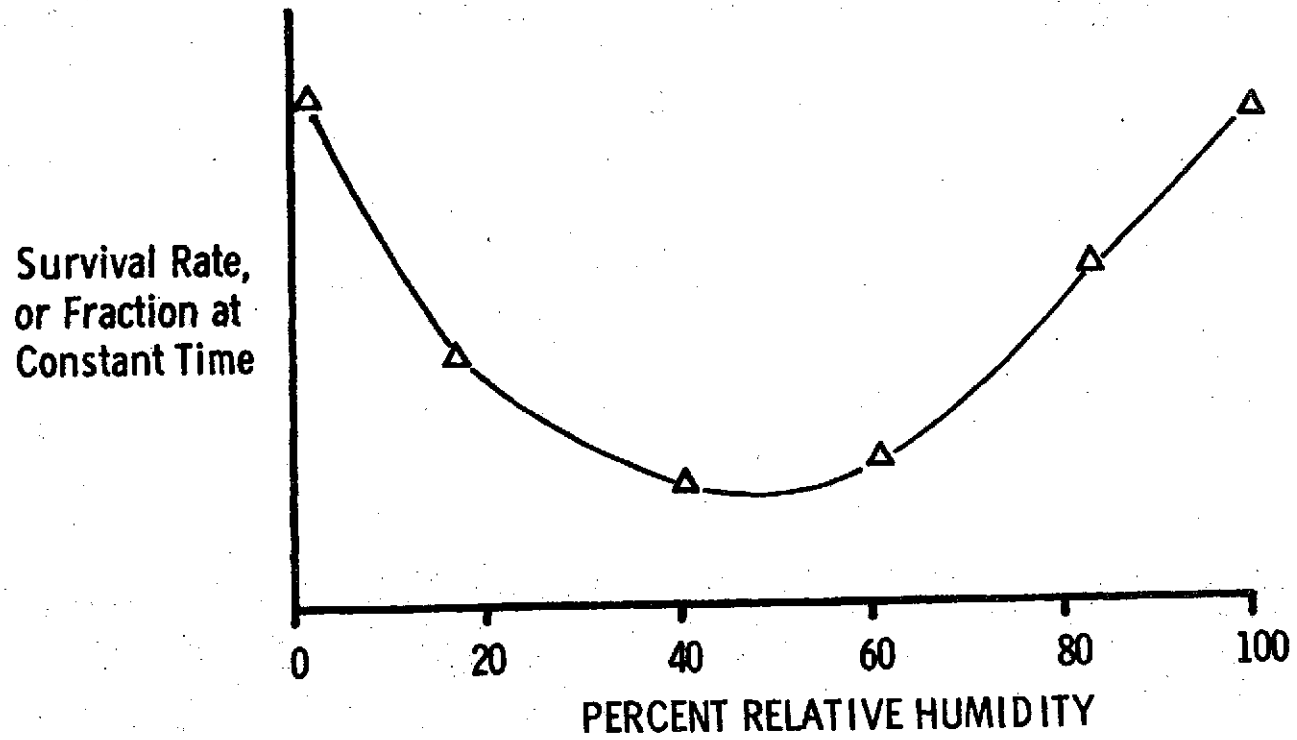
Survival Rate,
or Fraction at
Constant Time
0
20
40
60
80
100
PERCENT RELATIVE HUMIDITY

Fig. 5. Atypical survivor curves in bacterial aerosols from "synchronous" cultures.

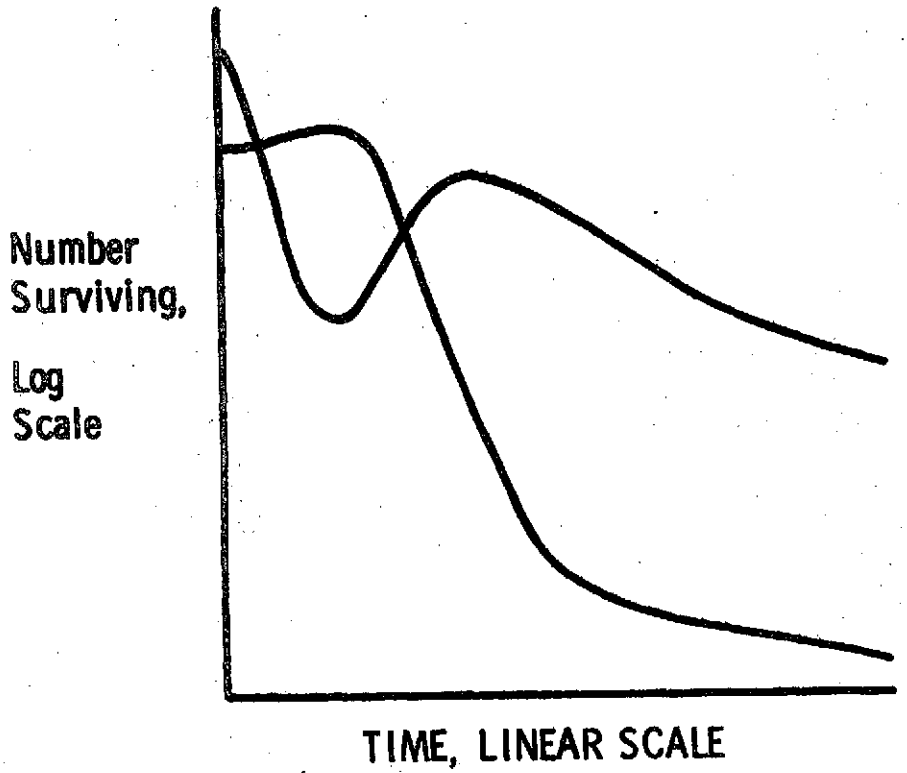
Number
Surviving,
Log
Scale
TIME, LINEAR SCALE

Fig. 6. Dynamics of $^{14}CO_2$ production by bacteria in the airborne state.

A = $^{14}CO_2$ production, air + wall

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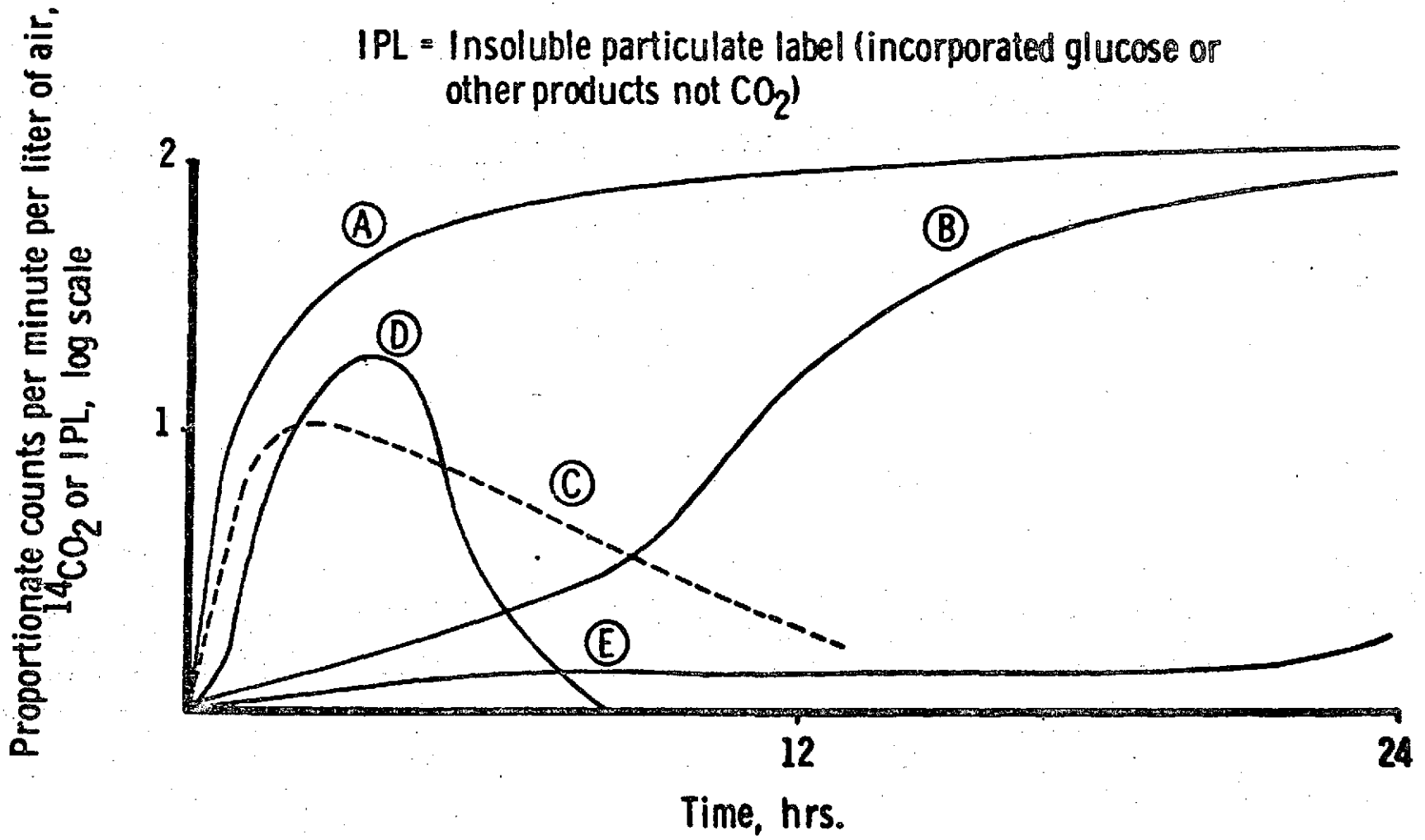
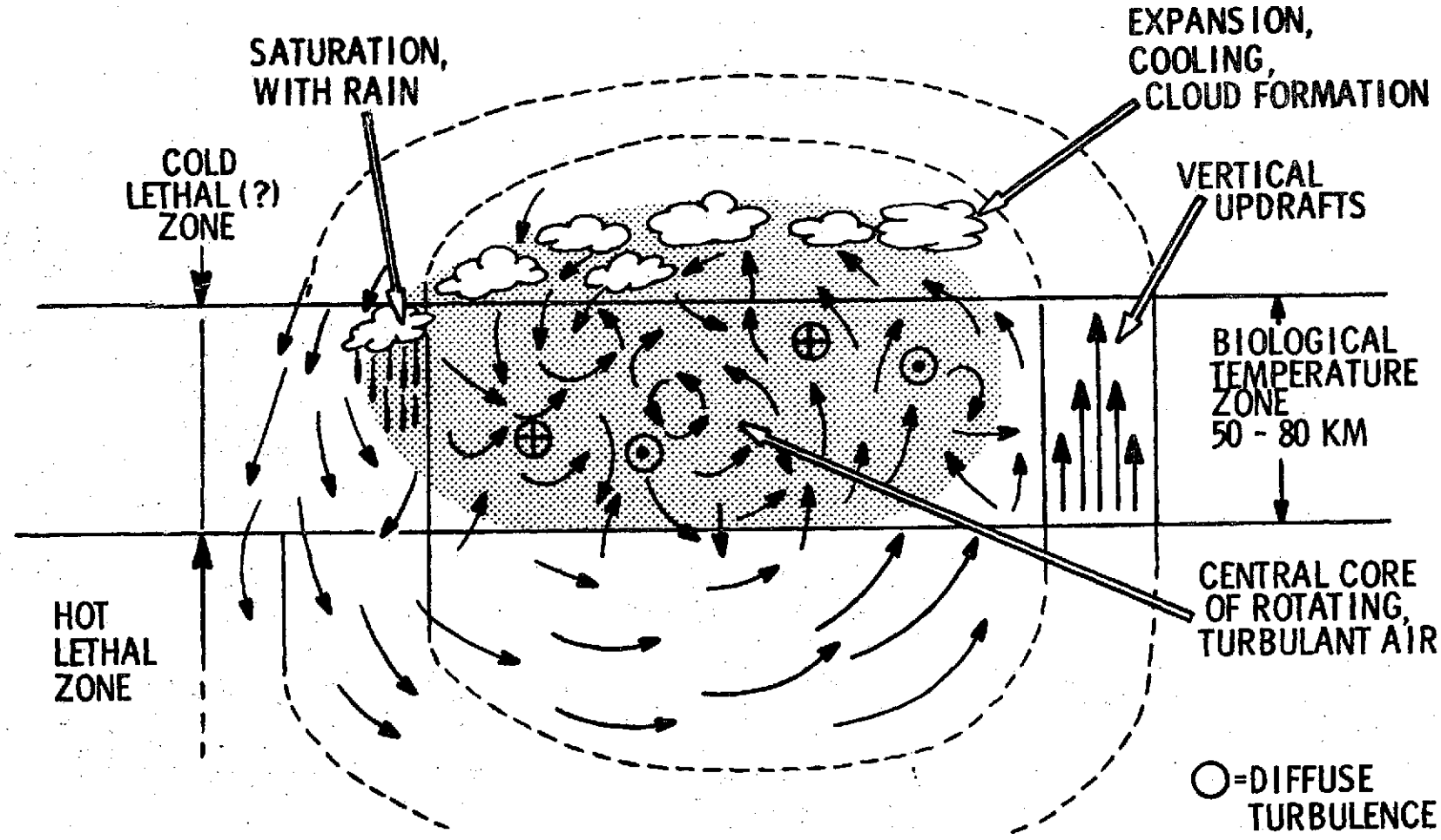

IPL = Insoluble particulate label (incorporated glucose or other products not CO_2)
Proportionate counts per minute per liter of air, $^{14}CO_2$ or IPL, log scale
2
1
A
B
C
D
E
12
24
Time, hrs.

Fig. 7. Suggested concept of possible atmospheric velocity pattern in Jovian Biosphere. Cross-section of one band.

The illustration is intended to show how the central core is like a box with "floor", "ceiling", and sides that particles can "fallout" onto and be lost. The contained air is both rotating and turbulent. Hence, it is equivalent to a stirred settling chamber.


SATURATION,
WITH RAIN
EXPANSION,
COOLING,
CLOUD FORMATION
COLD
LETHAL (?)
ZONE
VERTICAL
UPDRAFTS
BIOLOGICAL
TEMPERATURE
ZONE
50 - 80 KM
HOT
LETHAL
ZONE
CENTRAL CORE
OF ROTATING,
TURBULANT AIR
=DIFFUSE
TURBULENCE