Return to Dr. Lederberg

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DEPARTMENT OF BIOPHYSICS

January 20, 1959

Dr. Joshua Lederberg
Department of Genetics
Stanford University
Palo Alto, California

Dear Dr. Lederberg:

Thank you very much for your prompt reply to my letter of December 15 and for your post-card informing me of the derivation by Armitage of the equations for the distribution of numbers of mutants whose growth rate is different from the parent.

A typed copy of the appendices is enclosed which replaces the handwritten copy I sent you. My equations for the average number of mutants check exactly with those of Armitage. There is a mistake in the handwritten copy of Appendix I: the mutation rate per unit time should equal $\alpha \frac{\ln 2}{t_{p+}}$ instead of $\frac{\alpha}{t_{p+}}$ in order to make the definition of α in Appendix I the same as the definition of α in Appendix II. This correction has been made in the typewritten copy.

This changes the value of α from 3.0×10^{-4} to 4.3×10^{-4} in Fig. 4 (a corrected figure is enclosed) and a corresponding change should be made in the last line on page 5. Since Fig. 4 is a log plot this change makes little difference.

As the equation stands now it is not only identical to that of Armitage but also reduces to the Luria-Delbrück equation when the growth rates are equal, as it must. A bonus of the Armitage paper is that the variance as well as the mean has been calculated for the case of unequal growth rates. Theory predicts a greater variance for a greater difference in growth rate, and this effect is found experimentally.

As far as my coming to your laboratory is concerned, I appreciate your consideration of this possibility. I feel that collaboration between us on the genetics of pili would be very productive and I am hoping that this will eventually be possible.

You encouraged me to investigate other opportunities at Stanford and elsewhere because of limitations of space and the size of your group. Dr. Hubert Bloch, chairman of the Microbiology Department of the University of Pittsburgh Medical School has offered me an appointment in his department, which I will probably accept if things at Stanford don't work out for 1959-60. Although this appointment is quite attractive I would prefer to come to Stanford and work in your group. I have told him that I am waiting to hear from you and that it will be at least several weeks before I can expect any answer.

As to an opening on a collaborative basis with Biophysics, Biochemistry, or Medical Microbiology, Biophysics is a distinct possibility. My degree is in Biophysics and I have had further experience in Radiobiology, Electron Microscopy, and Electrophoresis. However, I do not know to whom I should write at Stanford; perhaps you could refer me to the person who is organizing this division or maybe you would rather check on this possibility yourself. I am enclosing the page proof of a chapter on electrophoresis I recently wrote with Dr. Lauffer which might be of interest to the Biophysics group.

Let me say again that I appreciate your interest in my work and your prompt response to my application in spite of the many distractions of organizing a new department.

Sincerely yours,

Charles C. Brinton, Jr.

CCB:lf

Appendix 1

Derivation of the equations for the average numbers of p^+ and p^- cells in a culture when the growth rates of the two forms are different.

Assumptions and Definitions

$p^+ \longrightarrow p^-$ mutation rate = a per bacterium per division

$p^- \longrightarrow p^+$ mutation rate = 0

t_{p^+} = generation time of p^+ cells

t_{p^-} = generation time of p^- cells

N_{p^+} = number of p^+ cells at time t

N_{p^-} = number of p^- cells at time t $\quad t$ = chronological time

At $t = 0$, $N_{p^+} = 1$ and $N_{p^-} = 0$.

$$\left(\frac{dN_{p^-}}{dt}\right)_{total} = \left(\frac{dN_{p^-}}{dt}\right)_{mutation} + \left(\frac{dN_{p^-}}{dt}\right)_{division}$$

In the absence of mutation:

$$N_{p^-} = N_{o\,p^-}\, 2^{(t/t_{p^-})}, \quad \ln N_{p^-} = \ln N_{o\,p^-} + (t/t_{p^-}) \ln 2,$$

$$\frac{dN_{p^-}}{N_{p^-}} = \frac{\ln 2}{t_{p^-}}\, dt, \quad \left(\frac{dN_{p^-}}{dt}\right)_{division} = \frac{\ln 2}{t_{p^-}}\, N_{p^-}$$

In the absence of mutation:

$$N_{p^+} = N_{o\,p^+}\, 2^{(t/t_{p^+})}, \quad N_{p^+} = 2^{(t/t_{p^+})}$$

since $N_{o\ p^+} = 1$ when $t = 0$, or $N_{p^+} = e^{(t/t_{p^+}) \ln 2}$.

If a is small enough so that the fraction of p^+ cells lost by mutation is always small compared to the total number of p^+ cells, this formula may be used to compute the number of p^+ cells at any time. This assumption is valid at 37° up to several hundred generations since $a \approx 2 \times 10^{-4}$.

Since a is defined as the number of mutants occurring per bacterium per division, the rate of mutant production per division equals the number of parent cells times a. The rate of mutant production per unit time is proportional to the number of parent cells times the mutation rate per unit time. The mutation rate per unit time equals a divided by the generation time of the parent cell, times $\ln 2$.

* $$\left(\frac{dN_{p^-}}{dt}\right)_{\text{mutation}} = N_{p^+} \cdot (a/t_{p^+}) \ln 2$$

or

$$\left(\frac{dN_{p^-}}{dt}\right)_{\text{mutation}} = \frac{e^{(t/t_{p^+}) \ln 2}\, a \ln 2}{t_{p^+}}$$

Therefore:

$$\frac{dN_{p^-}}{dt} = \frac{a \ln 2}{t_{p^+}} e^{(\ln 2/t_{p^+})\, t} + N_{p^-} \frac{\ln 2}{t_{p^-}}$$

This is a readily soluble differential equation of the form

$$\frac{dy}{dx} + f(X)y = g(X) \quad \text{where}$$

$$y = N_{p^-}, \quad X = t, \quad f(t) = -\frac{\ln 2}{t_{p^-}}, \quad g(t) = \frac{a \ln 2}{t_{p^+}} e^{(\ln 2/t_{p^+})\, t}$$

* If time is measured in units of division cycles of bacteria rather than chronologically, the unit of time becomes t_{p^+} divided by $\ln 2$ and this equation reduces to Eq. (3) of Luria and Delbrück (11).

The solution of the general equation is:

$$y = e^{-F}\left[\int e^{F} g \, dx + c\right]$$

(Margenau and Murphy, p. 41) where $F = F(x) = \int^{x} f(\zeta)\, d\zeta$.

In our case, $F = F(t) = \int^{t} -\frac{\ln 2}{t_{p-}} = -\frac{\ln 2}{t_{p-}} t$,

so that,

$$N_{p-} = e^{(t/t_{p-})\ln 2}\left[\int e^{(-t/t_{p-})\ln 2} \frac{a \ln 2}{t_{p+}} e^{(t/t_{p+})\ln 2}\, dt + C\right]$$

The integral inside the brackets may be transformed into

$$\int e^{u}\, du = e^{u} \quad .$$

$$\text{Integral} = \frac{\ln 2 \; a \; e^{\ln 2\,([1/t_{p+}] + [1/t_{p-}])\, t}}{t_{p+} \ln 2\,([1/t_{p+}] - [1/t_{p-}])}$$

Also, since $N_{p-} = 0$ when $t = 0$,

$$C = -\frac{a \ln 2}{t_{p+} \ln 2\,([1/t_{p+}] - [1/t_{p-}])}$$

Therefore:

$$N_{p-} = \frac{a\, e^{(t/t_{p-})\ln 2}\left(e^{\ln 2\,[1/t_{p+}] - [1/t_{p-}]\, t} - 1\right)}{t_{p+} \ln 2\,([1/t_{p+}] - [1/t_{p-}])}$$

This equation reduces to the Luria-Delbrück (11) equation (6) when the generation times of the two forms are equal, where $N_{p-} = m$, $\frac{t_{p+}}{\ln 2} = t$, $N_t = e^{\frac{\ln 2}{t_{p+}} t}$, $\alpha = a$.

Equation (6) is: $\frac{m}{N_t} = at$.

The final equation may be written more simply as

$$N_{p^-} = \frac{\alpha \left(e^{\ln 2 \frac{t}{t_{p^+}}} - e^{\ln 2 \frac{t}{t_{p^-}}} \right)}{1 - \frac{t_{p^+}}{t_{p^-}}}$$

or EQ. (71)

This equation is exactly equal to EQ. (10)^ of Armitage (18) where:

$a = \frac{\ln 2}{t_{p^+}}$, $b = \frac{\ln 2}{t_{p^-}}$, $g = \frac{\alpha \ln 2}{t_{p^+}}$, $h = 0$, $x_0 = 1$, $y_0 = 0$.

Appendix 2

Derivation of the equation for mutation rate in terms of the fraction of parallel clones having zero mutants (the "zero-point" method).

The probability of a mutation per bacterium per division = a.

The probability of <u>not</u> having a mutation per bacterium per division = $1 - a$.

The number of divisions that have occurred to produce a clone = $N - 1$ where N is the total number of cells in the clone. (This number is correct whether or not the clone has divided synchronously.)

In order to have <u>no</u> mutants in a clone, there must not have been a mutation during <u>any</u> of the divisions. Since the probability of an event which depends on the simultaneous fulfillment of several separate events is the <u>product</u> of the probabilities of the separate events, the probability of having no mutants is

$$p = (1 - a)^{N-1}$$

When N is large, $p = (1 - a)^N$

$\ln p = N \ln (1 - a)$

When a is $< 10^{-2}$, $\ln p = -Na$ and $a = -(\ln p/N)$.

This equation is exactly equal to the combined equations (4) and (5) of Luria and Delbrück (11), for the case where N_0 is small compared to N_t.

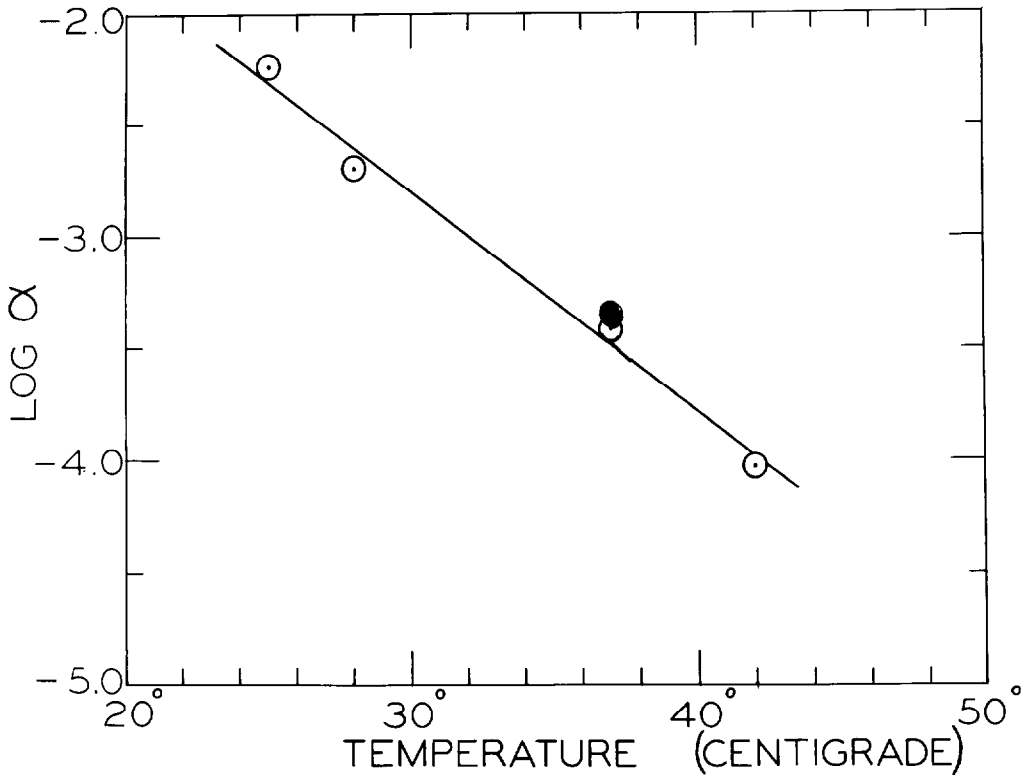

Fig. 4 - The $P^+ \longrightarrow P^-$ mutation rate, α, as a function of incubation temperature. Entire clones arising from presumptively single P^+ cells are plated and the fraction, P, of clones containing zero mutants is determined. α is then estimated from the formula:

$$\alpha = \frac{-\ln P}{N}$$

where N is the average total number of cells in the clones (Appendix 2). This method of determining α is entirely independent of any growth rate difference between parent and mutant. The experimental points are the open circles. The solid circle is the value of α used for plotting the theoretical curve of Fig. 3.