Not indexed in Biblio

JWS Preprints of

Bio 4 (17)

Bio 2 (18)

Bio 5 (19)

x Bio 4

(17) DESCRIPTION OF THE CHEMOSTAT

By Aaron Novick and Leo Szilard

Institute of Radiobiology and Biophysics
The University of Chicago

We have developed a device for keeping a bacterial population in the growth phase, growing at a reduced rate over an indefinite period of time, and we shall refer to this device as the Chemostat.

In the Chemostat, we have a vessel (which we shall call the growth tube) containing V cc. of a suspension of bacteria. A steady stream of the nutrient flows from a storage tank at the rate of $w$ cc/sec into this growth tube. The content of the growth tube is stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the growth tube at all times. An overflow sets the level of the liquid in the growth tube and, through that overflow, the bacterial suspension leaves the growth tube at the same rate at which fresh nutrient enters the growth tube from the storage tank.

The chemical composition of the nutrient in the storage tank is so chosen that it shall contain a high concentration of all growth factors required by the bacterium with the exception of one growth factor, the controlling growth factor, the concentration of which is kept relatively low. The concentration $a$ of the controlling growth factor in the storage tank will then determine the density $n$ of the bacterial population in the growth tube in the stationary state, and it can be shown that, except for very low values of $n$, we have $n = \frac{a}{Q}$, where $Q$ is the amount of the controlling growth factor needed for the production of one bacterium.

The growth rate $\alpha = \frac{1}{n}\frac{dn}{dt}$ of a strain of bacteria is a function of the concentration $c$ of the controlling growth factor in the medium and in general we may expect the growth rate, at low concentrations $c$, first to increase fast with increasing concentration and then, slowly to approach its highest attainable value, $\alpha$ max.

The Chemostat must be so operated that the washing-out time $\frac{w}{V}$ should be lower than the growth rate $\alpha_{max}$ for high concentrations of the controlling

growth factor. It can be shown that in that case a stationary state will become established in which the growth rate $\mathcal{L}$ will be just equal to the washing-out rate $\frac{w}{V}$.

What happens is, that the density n of the bacterial population in the growth tube will increase until it becomes so large that the bacteria will take up the controlling growth factor from the growth tube just as fast as it is necessary in order to reduce the concentration c of the controlling growth factor to the point where the growth rate $\mathcal{L}(c)$ becomes equal to the washing-out rate $\frac{w}{V}$.

Using a tryptophane-requiring strain of coil and a simple lactate medium with tryptophane added, we have used both lactate and tryptophane as the controlling growth factor. Using tryptophane as the controlling growth factor, we have kept bacterial populations over long periods of time at rates up to ten times lower than normal. Thus, we are able to force protein synthesis to proceed very slowly while certain other biochemical processes may continue at an undiminished rate.

A study of this slow-growth-phase, by means of the Chemostat, promises to yield information of value on metabolism, regulatory processes, adaptations and mutations of micro-organisms. A study on the spontaneous mutations of bacteria growing in the Chemostat has been made and is being published elsewhere.

Because, for most investigations, a number of such Chemostats will be needed we attempted to perfect a simple yet adequate design. Of various possible designs, we eliminated those in which changes in the barometric pressure affect the rate of flow of the nutrient from the storage tank into the growth tube. We also discarded designs which permit growth of the bacteria on the inner walls of the growth tube, or permit growth of bacteria in the Chemostat anywhere except homogeneously dispersed in the liquid nutrient in the growth tube. After trying out several designs, we found the one shown in the figure satisfactory.

A tube leading to the bottom of the storage tank (see Figure) is connected to a small air compressor (for instance a little air pump such as used for aerating aquaria). When the compressor is first started, the air rises rapidly in bubbles through the nutrient liquid in the storage tank and accumulates in the space above the liquid level until the pressure in the nutrient liquid at the bottom of the storage tank becomes equal to the air pressure in the tube. The air space in the storage tank above the liquid level communicates through a narrow capillary with the outside air and therefore the air will continue indefinitely to bubble through the nutrient liquid in the storage

tank but at a very slow rate (of perhaps one bubble per minute).

The pressure of the air entering the tube which leads to the bottom of the storage tank is regulated by a simple "pressure regulator" consisting of an air outlet located at the bottom of a glass cylinder which is filled with

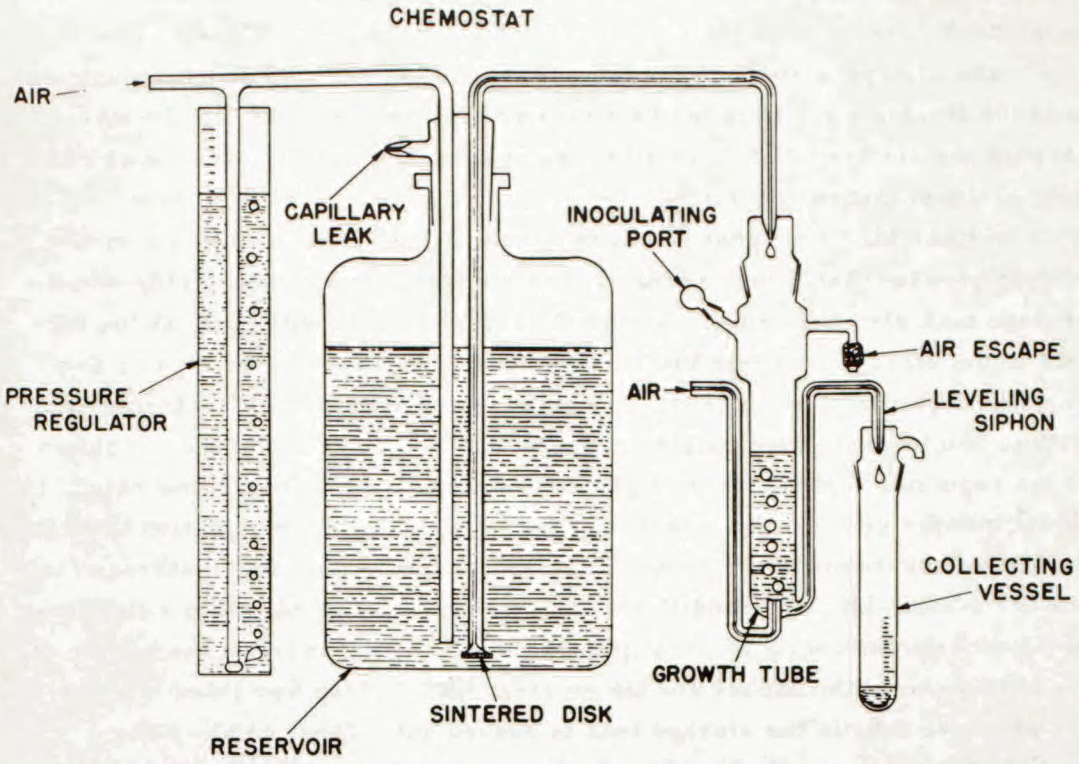


FIGURE 1

water up to a certain level. Above this level, the air communicates freely with the outside air. By changing the water level in the "pressure regulator," the air pressure can be adjusted to any value required for the operation of the Chemostat.

In this arrangement, the pressure at the bottom of the storage tank will always be greater than the pressure of the outside air by the height of the water column in the pressure regulator, and thus the pressure at the bottom of the storage tank will be independent of the height of the level of the nutrient liquid in the storage tank. This is important because the level of the nutrient liquid will gradually fall during the operation of the Chemostat.

From the storage tank, the nutrient liquid is forced through a sintered glass filter into the growth tube, and is mixed drop by drop with the bacterial suspension contained in the growth tube. The growth tube is aerated, and its

content is thus continuously stirred.

The level of the liquid in the growth tube is set by a syphon and the volume of the bacterial suspension in the growth tube is thus maintained constant. The nutrient liquid and the bacteria suspended in it leave the growth tube through the syphon at the same rate at which fresh nutrient enters the growth tube.

The air space above the nutrient liquid in the growth tube communicates with the outside air. Thus the pressure which forces the nutrient liquid through the sintered disk is at all times equal to the height of the water column in the pressure regulator.

If after the Chemostat has been in operation for some time the barometric pressure falls very suddenly, the pressure of the air entering into the storage tank also falls suddenly, and the nutrient liquid will enter at the bottom of the storage tank into the air pressure tube and will rise in it up to a certain height. If this happens, the pressure at the bottom of the storage tank will no longer exceed the outside pressure by the height of the water column in the regulator, but rather by a greater amount, and the flow of the nutrient liquid into the growth tube increases. Because of the communication through a capillary between the air space above the nutrient liquid in the storage tank and the outside air, this condition will be quickly corrected. As air flows out of the storage tank through the capillary outlet, the pressure at the bottom of the storage tank diminishes and the nutrient liquid which had risen into the air pressure tube in the storage tank is pushed out. Thus, within a short period of time, the pressure at the bottom of the storage tank is restored to its former value.

In this manner the Chemostat permits to keep the flow rate of the nutrient liquid into the growth tube constant, independently of changes in barometric pressure and changes in the liquid level in the storage tank. The flow rate can be changed as desired by changing the water level in the pressure regulator.

---

This paper will appear in Science.

XI

# VIRUS STRAINS WHICH ARE OF IDENTICAL PHENOTYPE BUT DIFFERENT GENOTYPE

By A. Novick and Leo Szilard

Institute of Radiobiology and Biophysics
The University of Chicago

Delbruck and Bailey[1] noticed an anomaly in the lysate of bacteria which was obtained by mixedly infecting the B strain of coli with the bacterial viruses T2 and T4. Subsequently, Luria[2] found this anomaly to be even more pronounced when he repeated Delbruck's experiment, using, however, virus T2 which has been exposed to ultraviolet irradiation.

When we undertook experiments in an attempt to understand this anomaly, we were led to the following result: If we infect a culture of the B strain of coli mixedly with the bacterial viruses T2 and T4 and incubate to permit lysis of the bacteria, there are present in the lysate three easily distinguishable types of bacterial viruses. Two of these, as expected, behave like the original parent strains T2 and T4, i.e., one of them behaves like T2 inasmuch as it is unable to attack the mutant strain B/2 (which is resistant to T2) but is able to grow in the mutant strain B/4 (which is sensitive to T2); and the other one behaves like T4, being unable to attack B/4 (which is resistant to T4) but able to grow in B/2 (which is sensitive to T4). The third type of virus present is phenotypically like T4 inasmuch as it is capable of multiplying in the strain B/2 (which is sensitive to T4) but it is genotypically like T2 inasmuch as, after one passage in the strain B/2, it is no longer capable of growing in it but is capable of growing in the strain B/4 (which is sensitive to T2).

The presence of this third type of virus, which may be called "latent T2", can be demonstrated in the following manner: We add to a culture of the B strain of coli viruses T2 and T4 in ratios corresponding to 10 T2 and 10 T4 virus particles per bacterium, incubate to permit lysis of the bacteria and then filter the lysate.

If we plate a sample of this lysate on agar that in inoculated with the strain B/4 (which is sensitive to T2 but resistant to T4), those virus

particles contained in the lysate which have the phenotype T2 will show up as plaques on these plates. T4 virus particles will not give plaques on this plate because B/4 is resistant to T4. The number of plaques is thus a measure of the number of T2 particles in the lysate.

Using a sample of the lysate, we determine in this manner the number of plaques obtained on an agar plate inoculated with the strain B/4. When we repeat this experiment--with the difference that before plating on the B/4 plate we add to the sample of our lysate a certain quantity of the strain B/2, allow five minutes for adsorption, dilute with broth and incubate for an hour to permit lysis of the bacteria--then we obtain a ten to twenty-five times larger number of plaques on the B/4 plate.

This phenomenon appears to show that there is present in our lysate a virus (the "latent T2") which is capable of multiplying in B/2 and subsequently to form plaques on B/4. In order to account for our observation, the concentration of the "latent T2" in the lysate would have to be about ten per cent of the concentration of T2. We were not able to obtain, after one passage in B/2, any appreciable further growth in B/2 of our hypothetical "latent T2". Before drawing the conclusion that the presence of a "latent T2" is in fact responsible for our phenomenon, it is necessary to exclude alternate explanations.

As an alternate explanation of our observation, it appeared a priori conceivable that our lysate contains aggregates of virus particles formed by a T2 and a T4 particle which stick together. Such aggregates might then perhaps be capable of entering into a bacterium of the strain B/2 (by virtue of their T4 component) and once inside, both virus particles T2 and T4 might then be able to multiply, and thus to produce the phenomenon we observed. We were able to rule out this possibility, however, by performing the following experiment:

We add to a sample of our lysate a certain quantity of B/2, using an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We then allow five minutes for adsorption and plate on an agar plate which has been inoculated with both B/2 and B/4. If there are present any B/2 bacteria into which has entered an aggregate of virus particles composed of T2 and T4 and in which both viruses will grow, then a certain number of clear plaques centering around such bacteria (which yield both T2 and T4) should develop on the agar plate. We were not able to find any such clear plaques, however, and found only turbid plaques (in which either the B/2 is lysed by T4 or the B/4 is lysed by T2). This rules out the alternate explanation of our phenomenon.

We ascertained that our phenomenon is produced under conditions in which we use an excess of B/2, so that independent infection of one bacterium by more than one virus particle can be neglected. We also ascertained that our phenomenon is not produced if, in place of our lysate, we use a mixture of T2 and T4.

Thus, we are led to conclude that the phenomenon described is due to virus particles which have the phenotype of T4, but the genotype of T2. The properties of this "latent T2" virus would seem to merit investigation.

---

This paper will appear in Science.

References

1. M. Delbruck and W.T. Bailey, Jr., Cold Spring Harbor Symposia, 11, 33 (1946).
2. S. Luria, Private communication, 1947.

XII

# EXPERIMENTS WITH THE CHEMOSTAT ON SPONTANEOUS MUTATIONS OF BACTERIA

By Aaron Novick and Leo Szilard
Institute of Radiobiology and Biophysics
University of Chicago

## Introduction

All bacteria require for growth the presence of certain inorganic chemical components in the nutrient, such as potassium, phosphorus, sulphur, etc., and with a few exceptions all bacteria require an energy yielding carbon source, such as, for instance, glucose or lactate, etc. In addition to these elements or simple compounds, certain bacteria require more complex compounds, for instance an amino acid, which they are not capable of synthesizing. For the purposes of this presentation, any of the chemical compounds which a given strain of bacteria requires for its growth will be called a growth factor.

In general, the growth rate of a bacterial strain may be within very wide limits independent of the concentration of a given growth factor; but since at zero concentration the growth rate is zero, there must of necessity exist, at sufficiently low concentrations of the growth factor, a region in which the growth rate falls with falling concentration of the growth factor. It therefore should be possible to maintain a bacterial population over an indefinite period of time growing at a rate considerably lower than normal simply by maintaining the concentration of one growth factor--the controlling growth factor--at a sufficiently low value, while the concentrations of all other growth factors may at the same time be maintained at high values.

We shall describe further below a device for maintaining in this manner, over a long period of time, a bacterial population in the growth phase at a reduced growth rate and shall refer to it as the Chemostat.

If the growth rate of a bacterial population is reduced, it is not a priori clear whether the growth rate of the individual cells which constitute the population is uniformly reduced or whether a fraction of the total cell population has ceased to grow and is in a sort of lag phase, while the rest keeps growing at an undiminished rate. We believe that under the conditions of our

experiments, to be described below, we are dealing with the slowing of the growth rate of the individual cells rather than the cessation of growth of a fraction of the population.

By using an amino acid as the controlling growth factor we were able to force protein synthesis in the bacterial population to proceed at a rate ten times slower than at high concentrations of that amino acid. It appears that we are dealing here with a hitherto unexplored "state" of a bacterial population--a state of reduced growth rate under the control of a suitably chosen growth factor.

The study of this "slow-growth-phase" in the Chemostat promises to yield information of some value on metabolism, regulatory processes, adaptations, and mutations of micro-organisms; the present paper, however, is concerned only with the study of spontaneous mutations in bacteria.

There is a well-known mutant of the B strain of coli, B/1, which is resistant to the bacterial virus $T_1$, sensitive to the bacterial virus $T_5$, and which requires tryptophane as a growth factor. We used this strain and mutants derived from it in all of our experiments here reported. As a nutrient medium we used a simple synthetic lactate medium (Friedlein medium) with tryptophane added. As the controlling growth factor we used either lactate or tryptophane.

Experiments on Growth Rates at Low Tryptophane Concentrations.

In order to determine the growth rate of B/1 as a function of the tryptophane concentration (at high lactate concentrations) we made a series of experiments in which we incubated at 37° at different initial tryptophane concentrations, c, flasks inoculated with about 100 bacteria per cc. and obtained growth curves by determining (by means of colony counts) the number of viable bacteria as a function of time. Because the bacteria take up tryptophane, the tryptophane concentration c decreases during the growth of the culture and the growth rate for the initial tryptophane concentration c must therefore be taken from the early part of the growth curve.

The growth rate $\alpha$ is defined by

$$\alpha = \frac{1}{n}\frac{dn}{dt}$$

where n is the number of bacteria per cc. The reciprocal value $\tau = \frac{1}{\alpha}$ we shall designate as the generation time. From the generation time thus defined, we obtain the time between two successive cell divisions by multiplying by ln 2.

In Figure 1 the curve marked SLOW shows the growth rate $\alpha$ as a function of the tryptophane concentration $c$ for 37°. At low tryptophane concentrations $c$, the growth rate at first rises proportionately with the concentration; with increasing concentrations, however, the growth rate approaches a limit, and for concentrations above 10 $\gamma/l$ (micrograms per liter) the

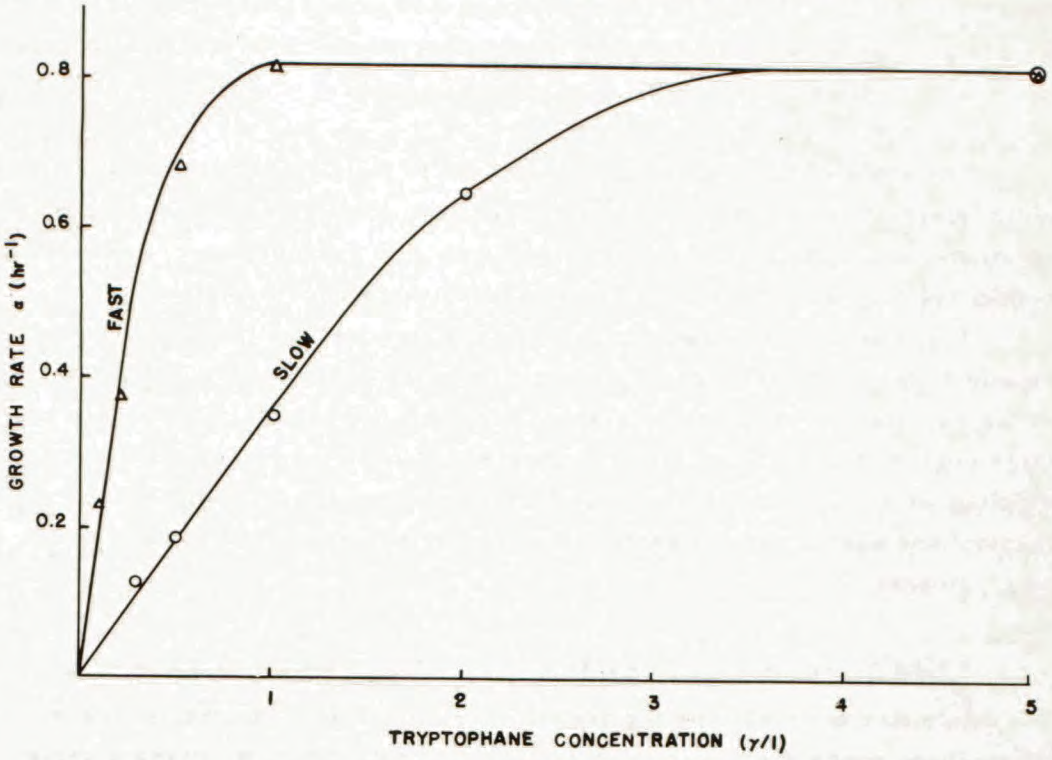


Figure 1. Experiment of September 18, 1950, at 37° C. The curve marked SLOW relates to strain B/1 and the curve marked FAST relates to B/1/f.

growth rate is no longer appreciably different from its highest attainable value. This highest value corresponds to a generation time of $\tau$ = 70 min. One half of the highest value is reached at a tryptophane concentration of about $c = 1\gamma/l$. This concentration corresponds to about three molecules of tryptophane per $10^{-12}$ cc. (The volume of one bacterium is about $10^{-12}$ cc.)

The proportionality of the growth rate with the concentration of tryptophane at low concentrations becomes understandable if we assume that the up-take and utilization of tryptophane by the bacterium requires that a tryptophane molecule interact with a molecule of a certain enzyme contained in the bacterium and that the up-take of tryptophane by these enzyme molecules in the bacterium becomes the rate-limiting factor for the growth of the

bacterium. On the basis of this argument we believe that, down to as low concentrations of tryptophane as the proportionality of growth rate to concentration can be experimentally demonstrated, the observed growth rate of the bacterial culture represents the growth rate of the individual bacterium and that no appreciable fraction of the population goes into lag.

## The Theory of the Chemostat.

In the Chemostat we have a vessel (which we shall call the growth tube) containing V cc. of a suspension of bacteria. A steady stream of the nutrient liquid flows from a storage tank at the rate of w cc/sec. into this growth tube. The content of the growth tube is stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the growth tube at all times. An overflow sets the level of the liquid in the growth tube, and through that overflow the bacterial suspension will leave the growth tube at the same rate at which fresh nutrient enters the growth tube from the storage tank.

After a certain time of such operation at a fixed temperature, a stationary state is reached in the growth tube. We are interested in this stationary state in the particular case in which the growth rate of the bacteria is determined by the concentration in the growth tube of a single growth factor (in our specific case tryptophane). By this we mean that the concentration of a single growth factor (tryptophane) in the growth tube is so low that a small change in it appreciably affects the growth rate of the bacteria, and at the same time the the concentration of all other growth factors in the growth tube is so high that a small change in them has no appreciable effect on the growth rate of the bacteria. As we shall show, under these conditions the concentration c of the growth factor in the growth tube in the stationary state, for a fixed flow rate w, will be independent of the concentration a of this growth factor in the nutrient liquid in the storage tank.

In order to see this we have to consider the following:

1) For zero flow rate of the nutrient (w = 0), the bacterial concentration n would rise in the growth tube according to $\frac{1}{n}\frac{dn}{dt} = \mathcal{L}(c)$, where $\mathcal{L}$ is the growth rate which, according to our premise, is a function of the concentration c of the growth factor.

2) In the absence of growth, the bacterial concentration in the growth tube would decrease for a given flow rate w according to the formula

$$\frac{1}{n}\frac{dn}{dt} = -\frac{w}{V}$$

where $\frac{w}{V} = \mathcal{B}$ may be called the "washing-out rate" of the growth tube, and

$1/\beta$ the "washing-out time."

After a while, for any given flow rate $w$, a stationary state will be reached in the Chemostat at which the growth rate $\alpha$ will be equal to the washing-out rate $\beta$ (and the generation time $\tau$ equal to the washing-out time $1/\beta$), i.e.,

$$\alpha(c) = \beta = \frac{w}{V}; \quad \tau = \frac{1}{\beta} = \frac{V}{w} \tag{1}$$

Thus, in the stationary state for any fixed flow rate $w$, the growth rate $\alpha$ is fixed; since $\alpha$ is a function of the concentration $c$ in the growth tube, it follows that $c$ is also fixed and independent of the concentration $a$ of the growth factor in the storage tank.

It may be asked what is the mechanism by which, for different values of $a$ but the same flow rate $w$, the same concentration $c$ establishes itself in the growth tube in the stationary state. Clearly what happens is this: Suppose that, for a certain concentration $a_1$ of the growth factor in the storage tank, a stationary state with the concentration $c$ in the growth tube has established itself and subsequently the concentration of the growth factor in the storage tank is suddenly raised to a higher value $a_2$. When this change is made, the concentration $c$ in the growth tube will at first rise and along with it will rise $\alpha$, the growth rate of the bacteria, which is a function of $c$. The concentration of the bacteria in the growth tube will thus start to increase, and therefore the bacteria will take up the growth factor in the growth tube at an increased rate. As the increase of the bacterial concentration continues, the growth rate of the bacteria will, after a while, begin to fall and will continue to fall until a new stationary state is reached at which the bacteria again grow at the same rate at which they are washed out, i.e., for which again we have $\alpha = \frac{w}{V}$. When this state is reached, the concentration of the growth factor in the growth tube is again down to the same value $c$ which it had before the concentration of the growth factor in the storage tank was raised from $a_1$ to $a_2$, while the bacterial density is now higher.

In the stationary state the tryptophane balance requires that the following equation hold:

$$a = c + n\frac{V}{w}F(c) \tag{2}$$

or

$$a = c + n\frac{F(c)}{\alpha(c)} \tag{3}$$

where F(c) gives in grams per second the amount of the growth factor which one bacterium takes up per second.

As can be easily seen, the amount $Q$ of the growth factor that is taken up per bacterium produced is given by

$$Q = \frac{F(c)}{\mathcal{L}(c)}$$

so that, for the stationary state, we may also write

$$a = c + n Q \qquad \text{or} \qquad \frac{n = a - c}{Q} \tag{4}$$

and for the $c \ll a$ we may write

$$n = \frac{a}{Q} \tag{5}$$

The Use of Tryptophane as the Controlling Growth Factor.

Since in the stationary state the tryptophane concentration in the growth tube of the Chemostat is always below 10 $\gamma/\ell$ whenever the generation time is appreciably above 70 min., we may use the approximation given in equation (5) whenever the tryptophane concentration a in the storage tank is above 100 $\gamma/\ell$.

In order to determine the amount of tryptophane, $Q$, taken up per bacterium produced, we grew bacterial cultures in lactate medium with varied amounts of tryptophane added. We found that if the initial tryptophane concentration is kept below 10 $\gamma/\ell$, then the amount of tryptophane taken up per bacterium produced is not dependent on the tryptophane concentration and has a value of $Q = 2 \times 10^{-15}$ gm. At higher tryptophane concentrations, however, more tryptophane is used up per bacterium produced.

From equation (5), using the value of $Q = 2 \times 10^{-15}$ gm., we obtain $n = 5 \times 10^{7}$/cc for $a = 100\ \gamma/\ell$ and we obtain $n = 5 \times 10^{8}$/cc for $a = 1000\ \gamma/\ell$.

From this it may be seen that, by choosing suitable values for a and w, we may vary over a wide range, independently of each other, the bacterial concentration n and the tryptophane concentration c.

When we grew B/1 in a Chemostat (V= 20 cc.) for ten days at 37° at a generation time of $\tau$ = 2 hours and at a bacterial density of $5 \times 10^{8}$/cc., we found that a change from the original bacterial strain, B/1, had taken place. The new strain, which we shall designate as B/1/f, differs from the original strain only inasmuch as it grows at very low tryptophane concentrations about

five times as fast as the original strain. The growth rate at higher tryptophane concentrations is not perceptibly different, nor could we detect any other difference between the two strains. The curve marked FAST in Figure 1 gives the growth rate of the B/1/f strain as a function of the tryptophane concentration at $37^{o}$.

The ability of the B/1/f strain to grow faster at very low tryptophane concentrations gives it an advantage over the B/1 strain under the conditions prevailing in the growth tube of the Chemostat, and a mutant of this sort must, in time, displace the original strain of B/1.

Because in our experiments we would want to avoid--as much as possible-- population changes of this type in the Chemostat, we used this new strain, B/1/f, in all of our experiments reported below.

## Spontaneous Mutations in the Chemostat.

If we keep a strain of bacteria growing in the Chemostat and through spontaneous mutations another bacterial strain is generated from it, then the bacterial density $n^*$ of the mutant strain should (for $n^* \ll n$) increase linearly with time, provided that, under the conditions prevailing in the Chemostat, the new strain has the same growth rate as the original strain, so that there is no selection either for or against the mutant. In the absence of selection we have

$$\frac{dn^*}{dt} = \frac{\lambda}{\tau} n \tag{6}$$

where $n^*$ is the density of the mutant population, $n$ is the density of the population of the parent strain, and $\lambda$ is the number of mutations produced per generation per bacterium. Equation (6) holds under the assumption that back mutations can be neglected. From (6), we obtain for $n^* \ll n$

$$\frac{n^*}{n} = \frac{\lambda}{\tau} t + \text{Const.} \tag{7}$$

From this it may be seen that--as stated above--the relative abundance of the mutants must increase linearly with time if there is no selection for or against the mutant.

If the growth rate of the mutant strain is smaller than the growth rate of the parent strain $\alpha^* < \alpha$ so that there is selection against the mutant in the growth tube of the Chemostat, then the density $n^*$ of the mutant population should--after an initial rise--remain constant at the level given by

$$\frac{n^*}{n} = \frac{\alpha}{\alpha - \alpha^*}\ \lambda \qquad (8)$$

Experiments on Spontaneous Mutations in the Chemostat.

Of the various mutations occurring in a growing bacterial population, mutants resistant to a bacterial virus are perhaps the most easily scored with considerable accuracy. In our experiments we mostly worked with mutants of our coli strain which were resistant to the bacterial viruses T5 or T6.

When we grow the strain B/1/f in the Chemostat with a high concentration of tryptophane but a low concentration of lactate in the nutrient in the storage tank, so that lactate rather than tryptophane is the controlling growth factor, we find--after a short initial period--that the bacterial densities of the mutants resistant to T5 or T6 each remain at a constant level. These levels appear to correspond to a selection factor $\frac{\alpha - \alpha^*}{\alpha}$ of a few per cent.

We are inclined tentatively to assume that the behavior of these two mutants exemplifies the general rule that the vast majority of all the different mutational steps leading away from the wild type yield mutants which--under conditions of starvation for the carbon source--grow slower than the parent type.

On the other hand, if we grow our tryptophane-requiring strain in the Chemostat with a high concentration of lactate but a low concentration of tryptophane in the nutrient in the storage tank (so that tryptophane rather than lactate is the controlling growth factor) and if we run the Chemostat at a generation time well above 70 min. (the generation time at high tryptophane concentrations), then there is no reason to expect mutants in general to grow appreciably slower than the parent strain, particularly if the growth of the parent strain is kept very slow by keeping the tryptophane concentration in the growth tube very low. In this case one would rather expect a mutation to affect the growth rate only if it affects the up-take or utilization of tryptophane by the bacterium or if the mutant is a very slow grower. Accordingly, we should in general expect the mutant population to increase linearly with time in the Chemostat when tryptophane is used as the controlling growth factor.

Figure 2 gives for 37° the experimental values for the bacterial density for the mutant population resistant to $T_5$ in the growth tube of the Chemostat as a function of the number of generations through which the parent strain has passed in the Chemostat. (Number of generations $g = \frac{t}{\tau}$ .) The three

curves in the figure correspond to generation times of 2 hours, 6 hours, and 12 hours. The slope of the straight lines gives $\lambda$, the mutation rate per generation, as $2.5 \times 10^{-8}$, $7.5 \times 10^{-8}$, and $15 \times 10^{-8}$ per bacterium. We see

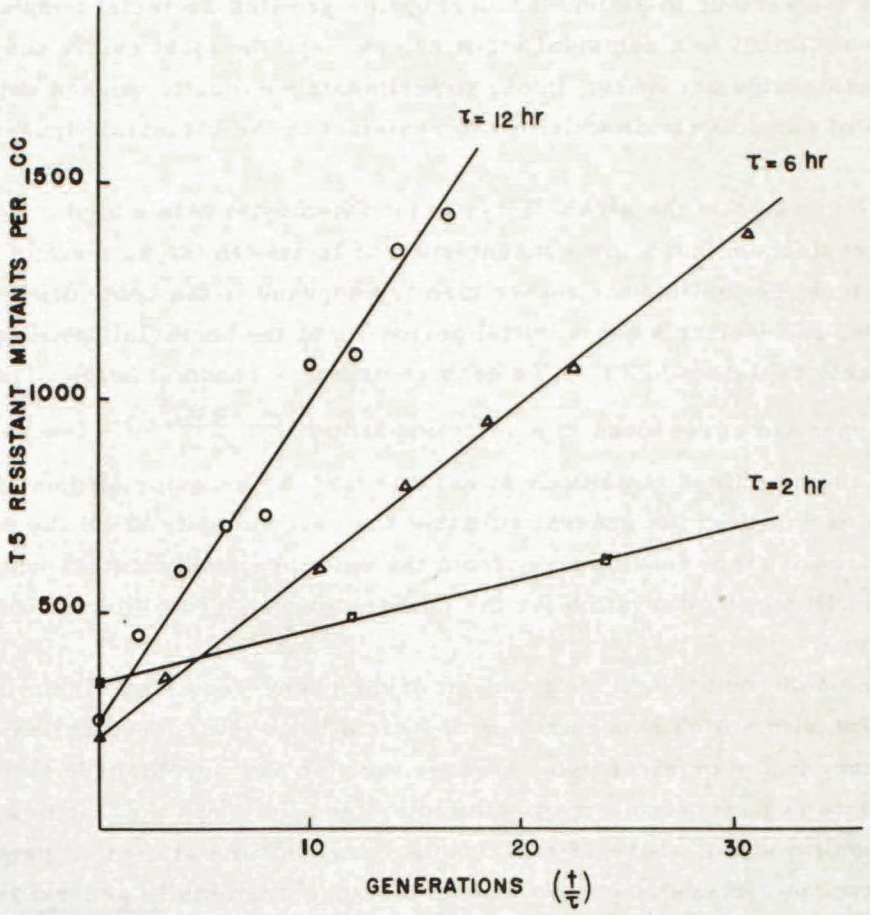


Figure 2. Experiments of May 3, 8, and 28, 1950, at 37° C. giving for strain B/1/f for three different values of the generation time $\tau$ the concentration of the mutants resistant to T5, for a population density of $5 \times 10^8$ bacteria per cc.

that the mutation rate per generation for $\tau$ = 6 hours is three times as high and for $\tau$ = 12 hours is six times as high as it is for $\tau$ = 2 hours. Thus the mutation rate per generation is, in our experiment, not constant but increases proportionately with $\tau$, and what remains constant is the number of mutations produced per unit time per bacterium. According to the above figures, we have $\frac{\lambda}{\tau} = 1.25 \times 10^{-8}$ per hour per bacterium.

This result is not one that could have been foreseen. If mutants arose, for instance, as the result of some error in the process of gene duplication, then one would hardly expect the probability of a mutation occurring per cell division to be inversely proportionate to the rate of growth.

If the processes of mutation could be considered as a monomolecular reaction--as had been suggested at one time by Delbruck and Timofeeff-Ressovsky--then, of course, the rate of mutation per unit time should be constant. The rate k of a monomolecular reaction is given by

$$k = Ae^{-W/RT} \tag{9}$$

the value of the constant A can be calculated from the observed reaction rate k and the heat of activation W (which can be obtained by determining the temperature coefficient of the reaction).

Using the Chemostat, we have determined the rate of mutation to resistance to $T_5$ at 25° (for $\tau$ = 6 hrs. and $\tau$ = 12 hrs.) and found it to be about one half of the mutation rate at 37°. From this value and the mutation rate of $\frac{\lambda}{\tau} = 1.25 \times 10^{-8}$ per hour per bacterium at 37°, we compute $A \approx 10^{-3}$ per sec.

In a condensed system, such as an aqueous solution, A has been found to lie between $10^5$ and $10^{14}$ per sec. for known monomolecular reactions. Therefore, if the mutation studied by us were due to a monomolecular reaction, it would have an A value $10^8$ times lower than the lowest value so far found.

The density of the mutants resistant to the bacterial virus $T_6$ in the Chemostat, with tryptophane as the controlling growth factor, also appears to rise linearly with time for $\tau$ = 2 hours, $\tau$ = 6 hours, and $\tau$ = 12 hours, but our results so far are not sufficiently accurate to say whether this mutation also occurs at a constant rate per unit time for different generation times $\tau$. The temperature coefficient of the mutation rate appears to be very low, but again this conclusion must await more accurate experiments.

The result obtained for mutation to resistance to the virus $T_5$, showing that this mutation occurs at a constant rate per unit time up to a generation time of $\tau$ = 12 hours, raises the question whether this is generally true of spontaneous bacterial mutations or whether we are dealing in our case with certain exceptional circumstances. Clearly, a number of different mutations will have to be examined, different amino acids will have to be used as the controlling growth factor, and other conditions will have to be varied before

one would draw the far-reaching conclusion that our observation on mutation to resistance to the virus $T_5$ exemplifies a general rule.

## Mutants Resistant to $T_4$.

We find that mutants resistant to $T_4$ are selected against in the Chemostat when grown either with lactate or with tryptophane as the controlling growth factor, i.e., the number of mutants remains--after an initial rise--at a fixed level.

It is known that, of the different mutants of the B strain of coli which are resistant to the virus $T_4$, the most frequent one is also resistant to the viruses $T_3$ and $T_7$ and that this mutant is a very slow grower under ordinary conditions of culture. It is conceivable that this might explain why the mutants resistant to $T_4$ are selected against in the Chemostat even when the bacterial population grows under tryptophane control and at a much reduced rate.

## Manifestation of "Evolution" in the Chemostat.

If a bacterial strain is grown over a long period of time in the Chemostat, from time to time a mutant might arise which grows faster, under the conditions prevailing in the Chemostat, than the parent strain. If this happens, practically the entire bacterial population in the Chemostat will change over from the parent strain to the new strain. We have discussed one change-over of this sort, i.e., the change-over from the strain B/1 to the strain B/1/f. There is no reason to believe, however, that no further change-over may take place when we start out with B/1/f as the parent strain and continue to grow it in the Chemostat over a long period of time.

We have seen that the mutants resistant to $T_5$ accumulate in the Chemostat and that their number rises linearly with the number of generations, giving a straight line, the slope of which is given by $\lambda$. If now at a certain time the population changes over in the Chemostat from the parent strain to a faster-growing strain, the accumulated mutants resistant to the bacterial virus $T_5$ which were derived from the parent strain should disappear from the Chemostat along with the parent strain. This should lead to a fall in the number of mutants resistant to the bacterial virus $T_5$ during a change-over from the parent strain to the faster-growing strain. After the change-over to the new strain the concentration of the mutants resistant to $T_5$ may be expected again to increase linearly with the number of generations, giving a straight line which has the same slope as before the change-over, because the new strain

which displaces the parent strain may be expected to mutate to resistance to $T_5$ at an unchanged rate $\lambda$.

Thus, we may in general expect when a change-over in the population takes place the concentration of the mutants resistant to $T_5$ to shift from one straight line to another which lies lower. The magnitude of this shift may be somewhat different from experiment to experiment, depending on when mutants resistant to $T_5$ happen to make their first appearance in the population of the new strain.

At the outset the bacteria belonging to the new strain will be few in number, but their number will increase exponentially with the number of generations until--at the time of the change-over--the bacteria belonging to the new strain become an appreciable fraction of the total population. If the mutation rate to resistance to $T_5$ is of the order of magnitude of $10^{-8}$, then it is unlikely that such a mutant would appear in the population of the new strain until its population has reached perhaps $10^7$. However, because an element of chance is involved, occasionally a mutant resistant to $T_5$ may appear earlier, and if that happens the shift associated with the change-over will be smaller and in principle it might even be negative.

If a bacterial population remains growing in the Chemostat for a sufficiently long time, a number of such change-overs might take place. Each such step in the evolution of the bacterial strain in the Chemostat may be expected to manifest itself in a shift in the ascending straight line curve of the $T_5$ resistant mutants.

As we have seen, the mutants resistant to $T_4$ remain--apart from an initial rise--at a constant level in the Chemostat. However, when the bacterial population in the Chemostat changes over from a parent strain to a new strain, the $T_4$ resistant mutants might change over from one level to another because the selection against the two strains might be different.

Figure 3 shows, for mutants resistant to $T_5$ and for mutants resistant to $T_4$, the number of mutants as a function of the number of generations $\frac{t}{\tau}$ in a Chemostat which was run for 300 hours at $\tau$ = 4 hours with tryptophane as the controlling growth factor.

It may be seen that these two curves show a population change-over of the type just described. The curve for the $T_5$ resistant mutants shows a shift $\underline{P}$ of P = 32 generations.

A number of shifts of this type were observed in different experiments. We verified that these shifts represent population change-overs by showing in one case that (under the conditions prevailing in the Chemostat) bacteria taken from the Chemostat before the change-over in fact grow slower than bacteria taken from the Chemostat after the change-over.

In order to show this, we took from the Chemostat before the change-over a bacterium resistant to $T_5$ and after the change-over a bacterium sensitive to $T_5$ and inoculated a second Chemostat (operated under identical

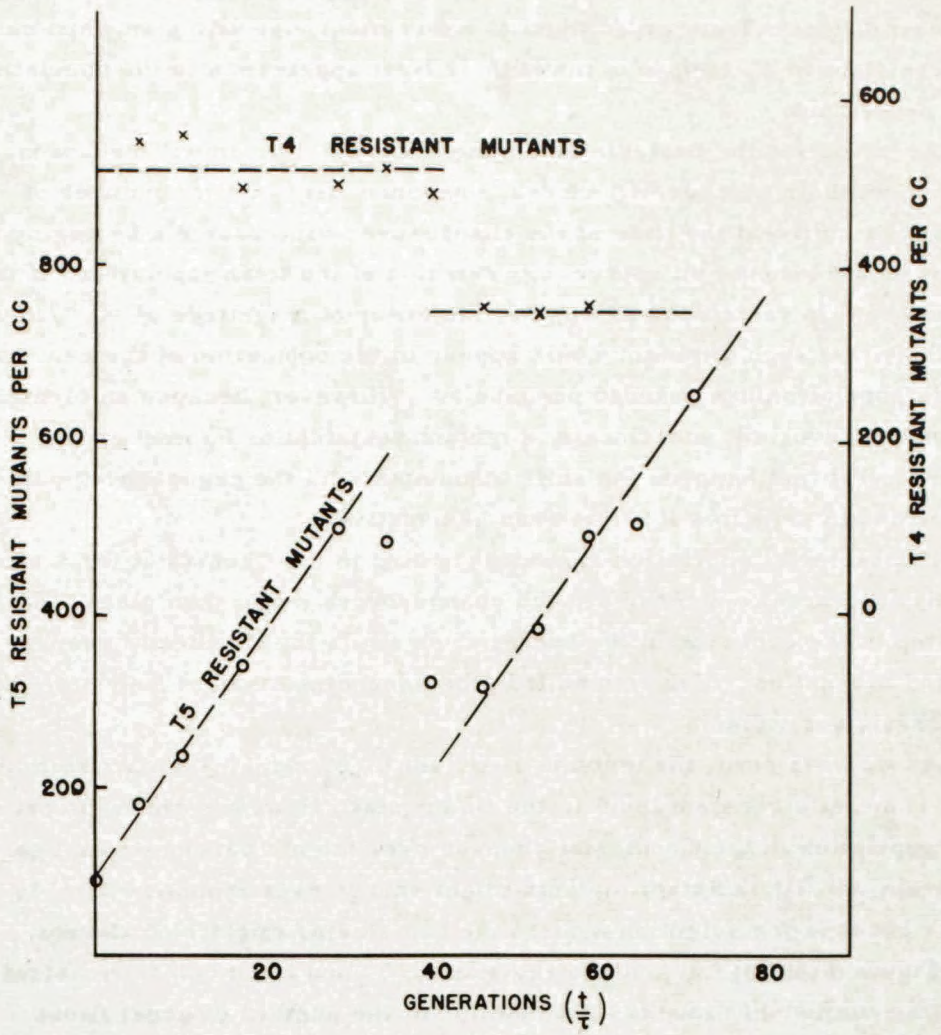


Figure 3. Experiment of July 19, 1950, at 37° C. giving for strain B/1/f the concentration of mutants resistant to T5 (left hand scale) and mutants resistant to T4 (right hand scale) for a population density of $2.5 \times 10^8$ bacteria per cc. In this experiment oxygen containing 0.25% $CO_2$ was used for aeration.

conditions) with a 50-50 mixture of these two strains. We then found that the relative abundance of the resistant strain rapidly diminished. In the

corresponding control experiment we took a sensitive bacterium from the Chemostat before the population change-over and a resistant one after the population change-over and again found that the strain prevalent before the change-over (this time the sensitive one) was the slower grower.

In the later stages of the change-over the concentration $x$ of the original strain falls off exponentially with the number of generations, $g = \frac{t}{\tau}$, so that we may write $x = Ce^{-g/\Delta}$. In our experiment we obtained for $\Delta$ a value of $\Delta = 3.25$.

It should be noted that the value of $\Delta$ can also be read directly (though not accurately) from the curve, which gives the concentration $n^*$ of the resistant mutants as a function of $g$, the number of generations. During the change-over the concentration $c$ of the tryptophane in the growth tube goes over from an initial value $c_1$ to a final lower value $c_2$, and it can be shown that for the midpoint of the change-over at which $c = \frac{c_1 \div c_2}{2}$ we have

$$\Delta = \frac{P/4}{1 - \frac{1}{\lambda n} \frac{d n^*}{d g}} + \frac{1}{2} \qquad (10)$$

where $P$ is the magnitude of the shift expressed in the number of generations by which the ascending straight line of the resistant mutants is shifted in the change-over. This formula holds only if $\tau$ is large so that the rate of growth of the bacteria in the Chemostat is proportionate to the tryptophane concentration $c$. Because the exact position on the curve of the midpoint of the change-over on the curve $n^*$ is not known, this formula can give only a rough indication for the value of $\Delta$.

In our case, the estimate based on it gave for $\Delta$ a value of $\Delta = 2.4$ in place of the directly observed value of $\Delta = 3.25$. Within the limits of the accuracy of our curve for $n^*$, these two values are consistent with each other.

Population change-overs manifesting themselves in a shift in the ascending straight line of the $T_5$ resistant mutants occurred in every experiment carried, at $\tau = 4$ hrs., beyond the 50th generation. In an experiment carried to the 450th generation at a bacterial density of $2.5 \times 10^8$/cc., a number of such shifts occurred, the last one at about the 350th generation. (In the course of this experiment the mutants resistant to $T_4$ rose twice from a low level to high peak, the first of which reached $4.6 \times 10^4$ and the second

$4.5 \times 10^6$ mutants per cc. This phenomenon is now being investigated.)

It may be said that our strain, if grown in the Chemostat at low tryptophane concentrations for a long period of time, undergoes a number of mutational steps, each one leading to a strain more "fit" than the previous one, and that each step in this process of evolution becomes manifest through the shifts appearing in the curve of the mutants resistant to $T_5$.

---

This paper will appear in the December, 1950, issue of the Proceedings of the National Academy of Sciences.

Reprinted from SCIENCE, December 15, 1950, Vol. 112, No. 2920, pages 715–716.

# Description of the Chemostat

Aaron Novick and Leo Szilard
*Institute of Radiobiology and Biophysics, University of Chicago*

Reprinted from SCIENCE, December 15, 1950, Vol. 112, No. 2920, pages 715–716.

# Description of the Chemostat

**Aaron Novick and Leo Szilard**

*Institute of Radiobiology and Biophysics, University of Chicago*

We have developed a device for keeping a bacterial population growing at a reduced rate over an indefinite period of time. In this device, which we shall refer to as the Chemostat, we have a vessel (which we shall call the growth tube) containing $V$ ml of a suspension of bacteria. A steady stream of nutrient flows from a storage tank at the rate of $w$ ml/sec into the tube. The contents of the tube are stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the tube at all times. An overflow sets the level of the liquid in the growth tube, and through that overflow the bacterial suspension leaves the tube at the same rate at which fresh nutrient enters it.

The chemical composition of the nutrient is such that it contains a high concentration of all growth factors required by the bacterium, with the exception of one, the controlling growth factor, the concentration of which is kept relatively low. The concentration of the controlling growth factor, $a$, in the storage tank will then determine the density, $n$, of the bacterial population in the growth tube in the stationary state, and it can be shown that, except for very low values of $n$, we have $n=\frac{a}{A}$, where $A$ is the amount of the controlling growth factor needed for the production of one bacterium.

The growth rate $\alpha=\frac{1}{n}\frac{dn}{dt}$ of a strain of bacteria is a function of the concentration, $c$, of the controlling growth factor in the medium, and in general we may expect the growth rate, at low concentrations $c$, first to increase rapidly with increasing concentration and then slowly to approach its highest attainable value, $\alpha_{max}$.

The Chemostat must be so operated that the washing-out time, $\frac{w}{V}$, should be lower than the growth rate $\alpha_{max}$ for high concentrations of the controlling growth factor. It can be shown that in that case a stationary state will become established in which the growth rate, $\alpha$, will be just equal to the washing-out rate, $\frac{w}{V}$.

What happens is that $n$ will increase until it becomes so large that the bacteria will take up the controlling growth factor from the tube just as fast as it is necessary in order to reduce $c$ to the point where the growth rate $\alpha(c)$ becomes equal to the washing-out rate, $\frac{w}{V}$.

Using a tryptophane-requiring strain of coli and a simple lactate medium with tryptophane added, we have used both lactate and tryptophane as the controlling growth factor. Using tryptophane, we have kept bacterial populations growing over long periods of time at rates up to ten times lower than normal. We are thus able to force protein synthesis to proceed very slowly while certain other biochemical processes may continue at an undiminished rate.

A study of this slow-growth phase by means of the Chemostat promises to yield information of some value on metabolism, regulatory processes, adaptations, and mutations of microorganisms. A study of the spontaneous mutations of bacteria growing in the Chemostat has been made and is being published elsewhere.

Because for most investigations a number of such Chemostats will be needed, we attempted to perfect a simple yet adequate design. Of various possible designs, we eliminated those in which changes in the barometric pressure affect the rate of flow of the nutrient from the storage tank into the growth tube. We also discarded designs that permit growth of the bacteria on the inner walls of the growth tube, or permit growth of bacteria in the Chemostat anywhere except homogeneously dispersed in the liquid nutrient in the tube. After trying out several designs, we found the one shown in Fig. 1 satisfactory.

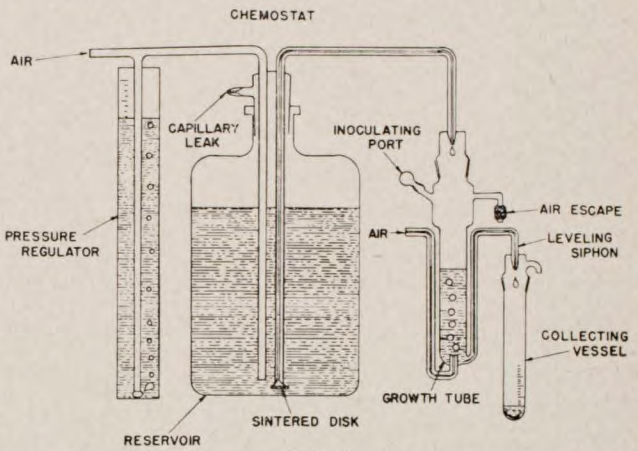


FIG. 1

A tube leading to the bottom of the storage tank is connected to a small air compressor (for example, an air pump such as is used for aerating aquaria). When the compressor is first started, the air rises rapidly in bubbles through the nutrient liquid in the storage tank and accumulates in the space above the liquid level until the pressure in the nutrient at the bottom of the tank becomes equal to the air pressure in the tube. The air space in the storage tank above the liquid level communicates through a narrow capillary with the outside air, and therefore the air will continue indefinitely to bubble through the nutrient liquid in the storage tank, but at a very slow rate (of perhaps one bubble per minute).

The pressure of the air entering the tube is regulated by a simple pressure regulator consisting of an air outlet located at the bottom of a glass cylinder filled with water

up to a certain level. Above this level, the air communicates freely with the outside air. By changing the water level in the pressure regulator, the air pressure can be adjusted to any value required for the operation of the Chemostat.

In this arrangement, the pressure at the bottom of the storage tank will always be greater than the pressure of the outside air by the height of the water column in the pressure regulator, and hence will be independent of the height of the level of the nutrient liquid. This is important because the level of the nutrient will gradually fall during the operation of the Chemostat.

From the storage tank the nutrient liquid is forced through a sintered glass filter into the growth tube, where it is mixed drop by drop with the bacterial suspension. The content of the growth tube is continuously stirred by aeration.

The level of the liquid in the tube is set by a siphon, and the volume of the bacterial suspension is thus maintained constant. The nutrient liquid and the bacteria suspended in it leave the tube through the syphon at the same rate at which fresh nutrient enters. The air space above the nutrient liquid in the growth tube communicates with the outside air, hence the pressure which forces the nutrient liquid through the sintered disk is at all times equal to the height of the water column in the pressure regulator.

If, after the Chemostat has been in operation for some time, the barometric pressure falls very suddenly, the pressure of the air entering into the storage tank also falls suddenly, and the nutrient liquid will rise in the air pressure tube to a certain height. If this happens, the pressure at the bottom of the storage tank will no longer exceed the outside pressure by the height of the water column in the regulator, but rather by a greater amount, and the flow of the nutrient liquid into the growth tube increases. Because of the capillary communication between the air space above the nutrient liquid and the outside air, this condition will be quickly corrected. As air flows out of the storage tank through the capillary outlet, the pressure diminishes, and the liquid which had risen into the air pressure tube in the tank is pushed out. Thus, within a short period of time, the pressure at the bottom of the storage tank is restored to its former value.

In this manner the Chemostat keeps the rate of flow of the nutrient liquid into the growth tube constant, independent of changes in barometric pressure and in the liquid level in the tank. The flow rate can be changed as desired by changing the water level in the pressure regulator.

Rough draft, for possible publication in SCIENCE - not for release

August 5, 1950

## Description of the Chemostat

By A. Novick and Leo Szilard

We have developed an apparatus for keeping a bacterial population in the growth phase, growing at a reduced rate, over an indefinite period of time, and we shall refer to it hereafter as the Chemostat.

In the Chemostat, we have a vessel (which we shall call hereafter the growth tube) containing $V$ cc. of a suspension of bacteria. A steady stream of the nutrient flows from a storage tank at the rate of $w$ cc/sec into this growth tube. The content of the growth tube is stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the growth tube at all times. An overflow sets the level of the liquid in the growth tube and through that overflow, the bacterial suspensions will leave the growth tube at the same rate at which fresh nutrient enters the growth tube from the storage tank.

The chemical composition of the nutrient in the storage tank is so chosen that it shall contain a high concentration of all growth factors required by the bacterium with the exception of one growth factor, the controlling growth factor, the concentration of which is kept relatively low. The concentration $a$ of the controlling growth factor in the storage tank will then determine the density $n$ of the bacterial population in the growth tube and it can be shown that, in the stationary state, we have $n = \frac{a-c}{A}$, where $c$ is a concentration of the controlling growth factor in the growth tube, and $A$ is the amount of the controlling growth factor needed for the production of one bacterium.

The growth rate $\mathcal{L} = \frac{1}{n}\frac{dn}{dt}$ of a strain of bacteria is a function of the concentration, $c$, of the controlling growth factor in the medium and in general we may expect the growth rate, at low concentrations $c$ first to increase fast with

increasing concentration and then, slowly to approach its highest attainable value $\mathcal{L}_{max}$.

The Chemostat must be so operated that the washing-out time $\frac{1}{w}$ should be lower than the growth rate $\mathcal{L}_{max}$ of the bacteria holding for high concentrations of the controlling growth factor. It can be shown that in that case there establishes itself a stationary state in which the growth rate $\mathcal{L}$ will be just equal to the washing-out rate $w$.

What happens is, that the density $n$ of the bacterial population in the growth tube will, quite automatically become so large that the bacteria will take up the controlling growth factor from the growth tube as fast as it is necessary in order to make the concentration $c$ of the controlling growth factor just as low as is needed to make the growth rate $\mathcal{L}(c)$ equal to the washing-out rate $w$.

Using a tryptophane-requiring strain of coli and a simple lactate medium with tryptophane added, we have used both lactate and tryptophane as the controlling growth factor. Using tryptophane as the controlling growth factor, we have kept bacterial populations growing over long periods of time at rates ten to twenty times lower than normal. Thus, we are able to force protein synthesis to proceed very slowly while other biochemical processes are not necessarily interfered with.

A study of this slow-growth-phase, by means of the Chemostat, promises to yield information of some value on metabolism, regulatory processes, adaptations and mutations of micro-organisms. The result of a study on the spontaneous mutations of bacteria growing in the Chemostat is being published elsewhere.

Because, for most investigations, a number of such Chemostats will be needed we attempted to perfect a simple design that will meet the requirements. Of various possible simple designs, we eliminated those in which changes in the barometric pressure affect the rate of flow of the nutrient from the storage tank into the growth tube. Growth of the bacteria on the inner walls of the growth tube or

bacteria growing in the Chemostat anywhere except homogeneously dispersed in the liquid nutrient in the growth tube must be avoided and after trying out and rejecting several designs, we found the design shown in the figure the most satisfactory.

A tube leading to the bottom of the storage tank (see Figure) is connected to a small air compressor (for instance a little air pump such as used for aereating aquaria*. When the compressor is first started, the air rises rapidly in bubbles through the nutrient liquid and accumulates in the space above the liquid level until the pressure in the nutrient liquid at the bottom of the storage tank becomes equal to the air pressure in the tube. The air space in the storage tank above the liquid level communicates through a narrow capillary with the outside air and therefore the air will continue to bubble through the nutrient liquid in the storage tank even though at a very slow rate (of perhaps one bubble per minute).

The pressure of the air entering the tube which leads to the bottom of the storage tank is regulated by a simple "pressure regulator" consisting of an air outlet located at the bottom of a glass cylinder which is filled with water up to a certain level. Above this level, the air communicates freely with the outside air. By changing the water level in the "pressure regulator," the air pressure can be adjusted to any value required for the operation of the Chemostat.

In this arrangement, the pressure at the bottom of the storage tank will always be greater than the pressure of the outside air by the height of the water column in the pressure regulator, and thus the pressure at the bottom of the storage tank will be independent of the height of the level of the nutrient liquid in the storage tank. This is important because the level of the nutrient liquid will gradually diminish during the operation of the Chemostat.

From the storage tank, the nutrient liquid is pressed through a sintered glass filter and a capillary, into the growth tube, and is mixed drop by drop with the liquid nutrient contained in the growth tube. The growth tube is aereated, and its content is thus continuously stirred.

The level of the liquid in the growth tube is set by a syphon and is maintained constant. The nutrient liquid and the bacteria suspended in it leave the growth tube through the syphon at the same rate at which fresh nutrient enters the growth tube.

The air space above the nutrient liquid in the growth tube communicates with the outside air. Thus the pressure which forces the nutrient liquid through the sintered disk and the capillary is at all times equal to the height of the water column in the pressure regulator.

If the Chemostat has been in operation for some time and then the barometric pressure falls very suddenly, the pressure of the air entering into the storage tank also falls suddenly, and the nutrient liquid will enter at the bottom of the storage tank into the air pressure tube and will rise in it up to a certain height. If this happens, then the pressure at the bottom of the storage tank will no longer exceed the outside pressure by the height of the water column in the regulator, but rather by a greater amount, and the flow of the nutrient liquid into the growth tube increases. Because of the communication between the air space above the nutrient liquid in the storage tank through a capillary with the outside air, this condition will be, however, very quickly corrected. As air flows out of the storage tank through this capillary outlet, the pressure at the bottom of the storage tank diminishes andthe level of the nutrient liquid in the air pressure tube in the storage tank falls again. Thus within a short period of time, the pressure at the bottom of the storage tank is restored to its former value.

Thus the Chemostat permits to keep the flow rate of the nutrient liquid into the growth tube constant independently of changes in barometric pressure and of the changes in the liquid level in the storage tank. The flow rate can be changed as desired by changing the water level in the pressure regulator.