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A DEVICE FOR GROWING BACTERIAL POPULATIONS UNDER STEADY STATE CONDITIONS*

BY MAURICE S. FOX‡ AND LEO SZILARD

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A number of photocell-controlled devices for growing bacterial populations under steady state conditions have been described (1–3). We wish to describe here a device, similar in principle though different in operation, that was used by one of us in a series of mutation rate studies carried out in 1951–52 (4). In this device, the turbidity of an exponentially growing population of bacteria is maintained at some preset value by controlling the rate of influx of fresh nutrient solution into the culture while the total culture volume remains constant. The rate of influx of the nutrient solution into the growth tube is controlled by a photocell which responds to the turbidity of the culture, and the culture volume is held constant by means of an overflow siphon from the growth tube.

For a device of this kind to be useful in the study of bacterial populations, it is necessary that the bacteria grow solely in liquid suspension and do not grow on the walls or other surfaces of the growth tube. This consideration was of primary concern in the design of the device to be described.

The "breeder," as we call this device for the sake of brevity, includes a nutrient reservoir and an air pressure–controlled feeding system from the reservoir into the growth tube similar in design to the chemostat (5) (see Fig. 1). Feeding or addition of fresh nutrient liquid is accomplished in the following way. A pressure h_3 plus h_4 in Fig. 1 is maintained in the air space of the storage tank, this pressure head plus h_2 maintains a level of liquid, h_1, in the U-shaped capillary. At certain fixed time intervals a microswitch,[1] periodically activated by a ¼ R.P.M. synchronous motor,[2] opens a solenoid valve for a brief interval so that a pressure h_4 is allowed to act on the liquid in the capillary and force the liquid volume contained in the U-bend into the growth tube. When the solenoid valve closes, the original liquid level in the capillary is re-established. In the breeder this synchronous motor which controls feeding is switched on when the turbidity of the culture exceeds a certain set value.

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[1] Acro snap switch SPDT, RD5L, ½ HP 125 to 250 volt, Newark Electric Co., Chicago.

[2] ¼ R.P.M., 1600 series, Hayden Manufacturing Co., Torrington, Connecticut.

For maintaining constant turbidity, a differential photocell circuit is used. This circuit is activated by a projection lamp,[3] whose beam passes through a 70 to 30 beam splitter. The 30 per cent fraction of the beam is reflected through a prism onto a reference photocell[4] (see Fig. 2), while the 70 per cent fraction of the beam passes through the growth tube and then onto a second photocell. By opposing the two (see Fig. 3) photocells through a variable resistance the current output across the resistance can be adjusted to zero for any given culture turbidity. An increase in turbidity registers as a positive current on an adjustable microammeter relay.[5] The relay is set to

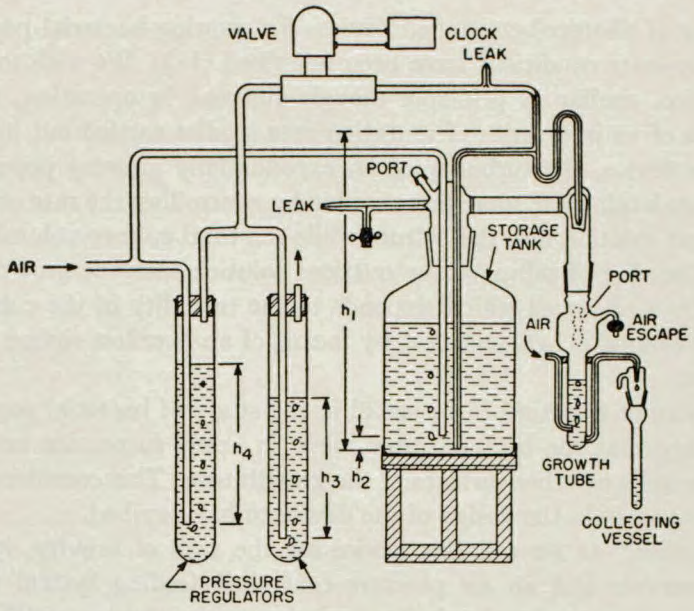

FIG. 1. The chemostat.

lock for readings greater than 7 microamperes which corresponds to a turbidity change of 5 to 10 per cent in a population of bacteria of about 2×10^8/ml. When the relay is in the locked position the synchronous motor[2] controlling the influx of fresh medium is energized.

A magnetic stirrer is used to provide adequate agitation at the bottom of the growth tube for the purpose of avoiding stagnant spaces where growth on the wall may occur.

A shaft driven by an additional synchronous motor,[6] geared down from 1

[3] Spartus 35 mm. slide projection lamp.

[4] Barrier layer selenium photocell No. 735, Photovolt Corporation, New York.

[5] Model 261-c, Assembly Products, Chagrin Falls, Ohio.

[6] 1 R.P.M. motor, model C5, Telechron, Inc., Ashland, Massachusetts.

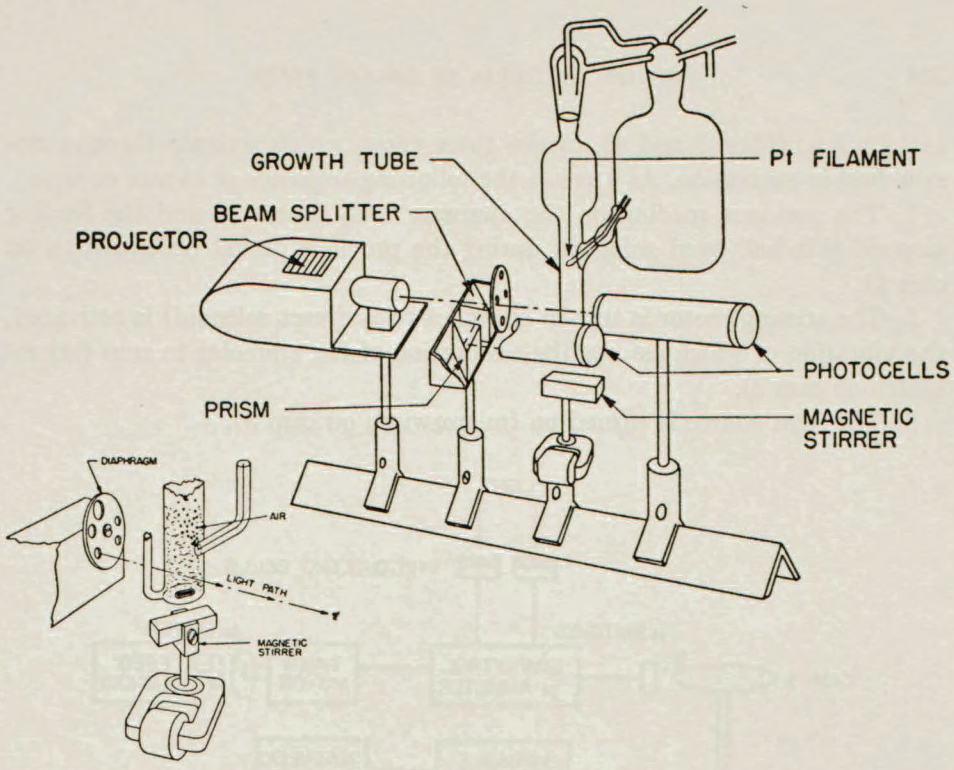

FIG. 2. The breeder.

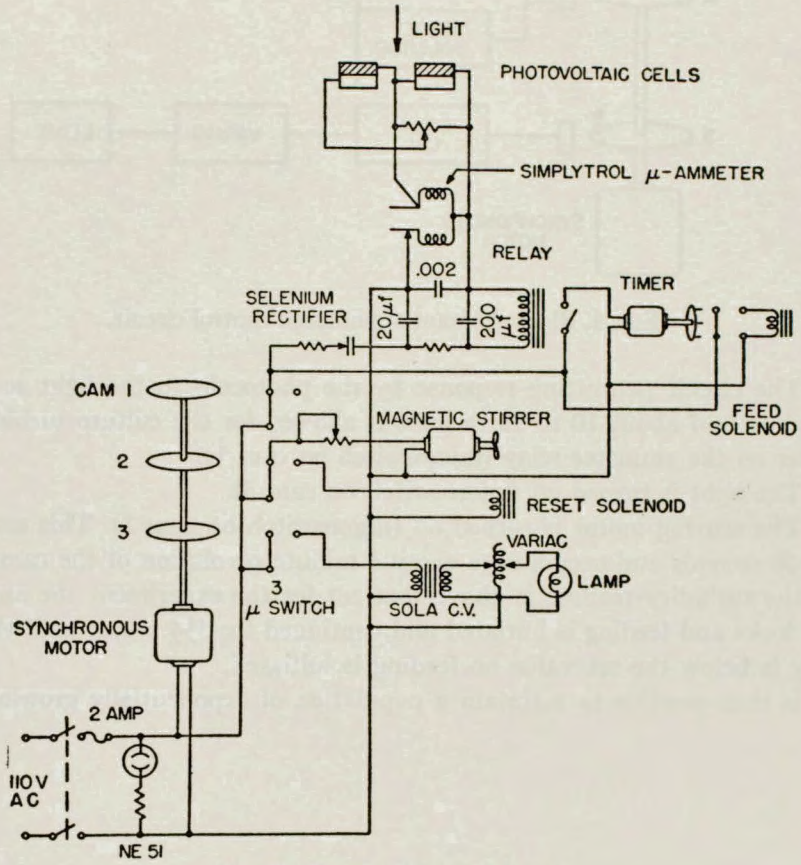

FIG. 3. Photocell control circuit.

to ¼ R.P.M. (Figs. 3 and 4), carries three cams, which activate three microswitches[1] in succession. As a result the following sequence of events occurs:

1. The previous reading on the ammeter relay is erased and the feeding stopped if it had been going on during the previous period (microswitch on cam 1).

2. The stirring motor is turned off and a relay (reset solenoid) is activated, the vibration of which assures the restoration of the ammeter to zero (microswitch on cam 2).

3. The light source is turned on (microswitch on cam 3).

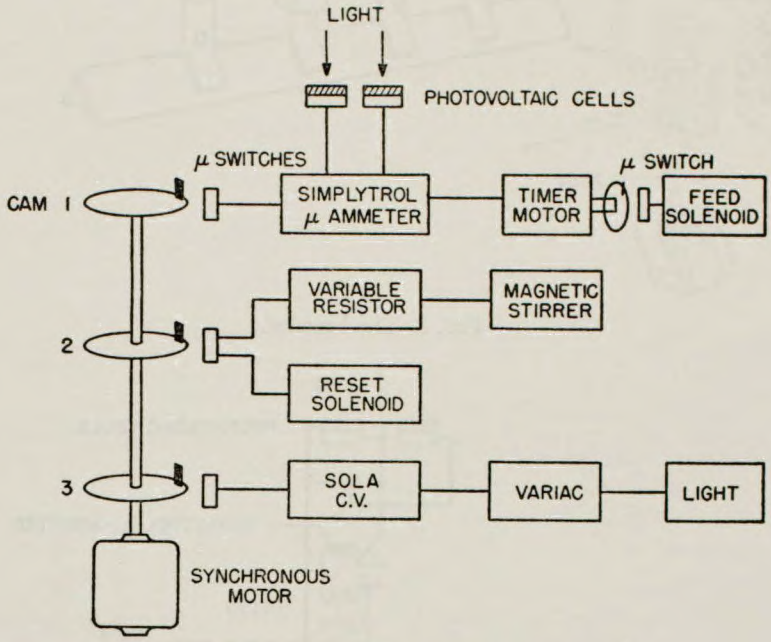

FIG. 4. Block diagram of photocell control circuit.

4. The circuit permitting response by the photocells to the light source is turned on and about 10 to 15 seconds is allowed for the culture turbidity to register on the ammeter relay (microswitch on cam 1).

5. The light is turned off (microswitch on cam 3).

6. The stirring motor is turned on (microswitch on cam 2). This sequence lasts 30 seconds and occurs once every 4 minute revolution of the cam shaft.

If the turbidity reading is above that set for the experiment the ammeter relay locks and feeding is initiated and continued for 3½ minutes. If the turbidity is below the set value no feeding is initiated.

It is thus possible to maintain a population of exponentially growing bac-

teria in a steady state. If n is the number of bacteria per milliliter in a growing culture, then the rate of increase of bacterial density is given by:

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

in which α is the growth rate given as one over the time required for an e-fold increase in bacterial density. If the bacterial suspension is removed and replaced by sterile nutrient in an approximately continuous manner at some given rate w/V, in which w is the rate at which fresh nutrient enters the growth tube in milliliters per hour, and V is the total volume of the culture in the growth tube in milliliters, then the rate of change of bacterial density will be given by:

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

When the breeder maintains the culture at constant turbidity, $dn/dt = 0$, and $\alpha = w/V$. The growth rate, α, is thus given by the volume overflow from the growth tube per hour, divided by the liquid volume contained in the growth tube.

It is convenient to operate the breeder at a bacterial density of about $\frac{1}{10}$ of the saturation density for a given medium in order to avoid reduction of the growth rate due to metabolic limitations.

The principal difficulty encountered in the operation of the device here described is due to the tendency of the organism used (strain B of *Escherichia coli* and B/1t, a tryptophan-requiring bacteriophage T_1-resistant mutant of the same strain) to adhere to the walls of the growth tube. The first manifestation of such "wall growth" is a marked increase in the rate of overflow from the growth tube which simulates an increase of the bacterial growth rate. This can be readily understood on the basis of a dual source of bacteria in the liquid suspensions, those originating by growth in the liquid phase, and those being thrown off by the population growing on the walls of the growth tube. Soon after the first observation of such an increased growth rate, areas of bacterial growth on the walls of the growth tube become visible.

This difficulty was overcome by coating the walls of the growth tube with General Electric drifilm No. 9987, supplementing the medium with 0.05 per cent tween 80, and agitating the culture vigorously with a magnetic stirrer. The stirring element was encased in glass and coated with drifilm. The introduction of tween 80 resulted in excessive foaming of the culture under aeration. A fine platinum filament (0.003 inch) maintained just below red heat set about 1 cm. above the surface of the growing culture was found adequate to destroy the foam and to prevent its rising beyond the filament in the growth

tube (see Fig. 2). Control experiments demonstrated that neither the light beam used to measure turbidity, nor any of these expedients used to prevent wall growth, had any significant effect on either the growth rate of the bacteria or the mutation rates observed in the bacterial population (4).

Using the breeder, mutation studies have been carried out by one of us on the B/1t mutant of *E. coli* B in various complex and synthetic media under aerobic and anaerobic conditions. The results of these experiments are described elsewhere (4).

SUMMARY

A device for maintaining an exponentially growing population of bacteria in the steady state at maximal growth rates is described.

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MUTATION RATES OF BACTERIA IN STEADY STATE POPULATIONS

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(Received for publication, May 31, 1955)

A number of observations are presented here concerning the occurrence of mutations in steady state populations of bacteria as a function of the growth rate of the bacteria and the nutritional conditions of the culture. These observations are made possible by the use of two different steady state devices operated under a variety of defined nutritional conditions. These devices maintain exponentially growing populations of bacteria at constant cell density (steady state) by different control mechanisms. One, the breeder, which is described elsewhere (1), permits observation of mutation rates at a maximum growth rate, and the other, the chemostat (2), permits observation of mutation rates at various controlled growth rates.

In the breeder, a photocell-activated circuit, responding to bacterial turbidity in the growth tube, controls the rate of addition of fresh nutrient solution to the culture in the growth tube. Increases in turbidity result in the addition of fresh nutrient and simultaneous removal of culture *via* an overflow siphon, the culture volume remaining constant. Operating at turbidities considerably below (about $\frac{1}{10}$) saturation turbidity for a given set of culture conditions, the bacteria grow at a maximal rate.

In the chemostat the cell density in the growth tube is determined by a limiting concentration of a required growth factor in the incoming nutrient solution. The generation time of the bacteria is determined by the steady state concentration of this growth factor established in the growth tube. This concentration is controlled by externally regulating the rate of influx of nutrient solution into the growth tube. In this way, using tryptophan as the limiting growth factor, it was possible to maintain the bacteria studied at various generation times from a minimum of about 35 minutes to about 4 hours. In all the experiments to be described the generation time is defined as the time required for an *e*-fold increase in numbers.

In the chemostat with minimal (F) medium when tryptophan was used as the controlling growth factor, Novick and Szilard, and Lee (3, 4) found, for the two mutations investigated, that the mutation rates were independent of

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generation time and dependent only on absolute time for generation times from 2 hours to 12 hours. In addition they found mutagenic effects with some purines (3, 4) and an antimutagenic effect with some purine ribosides (5).

In the following experiments, the mutation rates for these same two mutations, have been measured in F medium and in complex media at maximal growth rates in the breeder. In addition, using the chemostat and a complex medium with tryptophan as the limiting growth factor, these rates were determined at very short generation times and at generation times similar to those at which F medium chemostats were operated.

Methods

The organism used in this work, B/1t, a tryptophan-requiring bacteriophage T_1–resistant strain of *Escherichia coli* B, is the same as that studied by Novick and Szilard in the chemostat (3). Two mutations from sensitivity to resistance with respect to the bacteriophages T_5 and T_6 were studied. All mutation rates were measured at 37°C.

For any given experiment, overflow samples from the growth tube were collected in a vessel held at 0°C. (an ice bath) at given time intervals, and the population assayed for total numbers and for numbers of T_5-resistant mutants and T_6-resistant mutants. Total numbers were measured by making appropriate dilutions in nutrient broth, adding an aliquot to 2.5 ml. of soft nutrient broth agar at 45°C., then pouring on nutrient broth agar Petri dishes and counting colonies after about 20 hours' incubation at 37°C. In addition turbidity was checked in a Beckman spectrophotometer at 350 mμ for F medium and at 575 mμ for the complex media. The numbers of T_5- and T_6-resistant mutants were scored by adding to about 1 ml. of the collected culture sample (about 1 to 2 × 10^8 bacteria) a large (tenfold) excess of the respective bacteriophage in soft nutrient broth agar at 45°C., pouring on nutrient broth agar Petri dishes, and counting the number of resistant colonies that appear after 20 hours' incubation at 37°C.

The number of mutants in the population (the population density remaining constant) is plotted as a function of time. Under conditions where there is no selection for or against the mutants under observation, this plot defines a straight line whose slope is the mutation rate. The mutation rate will be given as the increase in the number of mutants per 10^8 bacteria per hour.

F Medium Experiments

The breeder was operated on F medium[1] supplemented with 1 gm./liter asparagine and an excess of 20 mg./liter of tryptophan. The generation times

[1] F medium is made up of the following components:

	gm./liter distilled water
Ammonium chloride (anhydrous)	1.0
Magnesium sulfate "	0.1
Potassium phosphate monobasic (anhydrous)	1.5
Sodium phosphate dibasic (anhydrous)	3.5
Lactic acid (85 per cent)	10.6
Sodium hydroxide	3.5

in this medium were somewhat variable, being 76 minutes and 96 minutes in two experiments. The mutation rates to resistance to the bacteriophages T_5 and T_6, under these conditions, were not significantly different from those observed in a chemostat with F medium operated at a generation time of about 4 hours (see Table I). This indicates that the tryptophan limitation in the chemostat has no effect on the mutation rate, and that the mutation rate in reciprocal hours does not vary between bacterial generation times of 76 minutes and 228 minutes. Novick and Szilard (3) and Lee (4) have established the generation time independence of mutation rates in the chemostat between generation times of 120 and 600 minutes.

TABLE I

F Medium Experiments

Machine	Generation time	Mutation rate ($\times 10^8$ hr.)		F medium plus supplement
		T_5 resistance	T_6 resistance	
	min.			
Breeder 134	96	1.3	0.7	+20 mg./liter tryptophan
Breeder 135	76	1.4	0.6	+1 gm./liter asparagine
Chemostat 133	230	1.1	0.4	+ 0.5 mg./liter tryptophan + 1 gm./liter asparagine
Chemostats (3, 4)	120–600	1.4	0.3	+ 0.5 mg./liter tryptophan

Complex Medium Breeder Experiments

The mutation rates were subsequently studied at shorter generation times in the breeder operated on Difco nutrient broth (8 gm./liter) with 1 gm./liter sodium chloride, and 0.05 per cent tween 80. Under these conditions the generation time was about 42 minutes and the mutation rates were $3.7/10^8$ bacteria/hour to T_5 resistance and $3.1/10^8$ bacteria/hour to T_6 resistance (see Table II).

Since these mutation rates were, respectively, 2.5- and 10-fold above those observed in the F medium experiments, the broth concentration was varied to determine the possible presence of a mutagen in the broth. For 2.5- and 5-fold reductions in broth concentration, the generation time remaining the same, the mutation rates were unaffected (see Table II).

It also seemed possible that the high mutation rates observed in broth resulted from an additional mutation, selected against in F medium but not in broth. If such were the case, the mutation rate measurement in F medium would not include this mutation and would therefore be lower than the rate observed in broth. To explain in this way the differences observed, at least half of the T_5-resistant, and about 90 per cent of the T_6-resistant mutants

appearing in the broth breeder would have had to be selected against in F medium.

To exclude this possibility, the following reconstruction experiment was carried out in F medium in a chemostat. Ten T_5-resistant and ten T_6-resistant mutants were isolated from a broth breeder that had been running sufficiently long so that more than 90 per cent of the mutant population had originated

TABLE II

Nutrient Broth Experiments

Machine	Generation time	Mutation rate ($\times 10^8$ hr.)		Medium
		T_5 resistance	T_6 resistance	
	min.			
Breeder 10	42	4.0	3.4	8 gm./liter broth
18	—	3.2	3.0	" " " "
26	38	5.9	4.2	" " " "
27	40	3.5	2.7	" " " "
109	38	3.2	3.5	" " " "
80	46	3.1	2.4	8 gm./liter broth + 0.01 M glutamic acid
79	40	4.7	3.1	8 gm./liter broth + 0.01 M cysteine
76	39	3.2	2.4	" "
		Average..3.9	3.1	
Breeder 38	42	3.2	1.8	3.2 gm./liter broth
47	46	2.7	2.0	" " " "
48	42	4.1	2.2	" " " "
57	41	4.2	1.8	" " " "
58	43	4.9	2.0	" " " "
68	40	4.0	1.5	3.2 gm./liter broth + 400 units/liter catalase
		Average..3.8	1.9	
Breeder 19	40	4.3	3.3	1.6 gm./liter broth
35	44	4.2	2.3	" " " "
		Average..4.2	2.8	

in the breeder. The mutants were restreaked three times on broth agar to free them from phage. Approximately equal numbers of each of these 20 isolates were added to a population of the parent strain B/1t so that, in all, they constituted about 2 per cent of the population, and this mixture was grown in an F medium chemostat. Daily assays of the population showed less than 0.3 per cent selection per generation for the T_5-resistant and about 0.2 per cent for the T_6-resistant fractions of the population during a period of over 80 generations, indicating that selection plays no significant role relative to

the mutation rate differences between F medium and broth. A similar reconstruction experiment was carried out using mutants isolated from a breeder

TABLE III

Nutrient Broth Experiments

Machine	Generation time	Mutation rate ($\times 10^8$ hr.)		Medium
		T_6 resistance	T_6 resistance	
	min.			
Breeder 82	31	3.8	—	8 gm./liter broth 5 gm./liter glucose
Breeder 92	33	2.8	2.6	2 x F phosphate*
Breeder 132	30	3.2	3.2	8 gm./liter broth 5 gm./liter glucose
Anaerobic breeder 131	37	2.0	2.4	1/2 F sodium lactate‡ F phosphate
Anaerobic breeder 81	36	3.1	2.3	8 gm./liter broth 5 gm./liter glucose 2 x F phosphate
Breeder 84	45	2.3	1.4	8 gm./liter broth
Breeder 113	43	3.2	2.4	150 mg./liter theophylline
Breeder 109	58	2.2	3.5	8 gm./liter broth 50 mg./liter adenosine
Breeder 108	31	3.1	4.0	8 gm./liter broth 5 gm./liter glucose 1/2 F sodium lactate F phosphate 50 mg./liter adenosine
Breeder 29	35	3.5	6.3	8 gm./liter broth 50 mg./liter adenosine

* F phosphate 3.5 gm./liter sodium phosphate dibasic; 1.5 gm./liter potassium phosphate monobasic.

‡ F sodium lactate 14 gm./liter.[1]

run with the casein hydrolysate medium (to be described later) with similar results.

It was considered possible that the high mutation rates in the breeder were due to excessive production of peroxides associated with rapid aerobic growth. To exclude peroxide production the breeder was operated anaerobically. Glucose and phosphate were added to the broth and a nitrogen plus 2 to 4

per cent carbon dioxide gas mixture was bubbled through the growth tube and used in the nutrient reservoir to maintain pressure. There is no significant difference in the mutation rates between aerobic and anaerobic growth in glucose-phosphate broth medium and the rates are the same as in broth without glucose and phosphate (see Table III).

Since the effects of purine type mutagens such as theophylline, and antimutagenic compounds including adenosine have been measured in F medium with the chemostat (3–5), some of these compounds were added to broth breeders to see whether they might exert any effect on these high mutation rates. The data in Table III show that recrystallized theophylline had no significant effect at concentrations which, in the chemostat, gave an increase from 1.5 to $14/10^8$ bacteria/hour in the mutation rate to T_5 resistance (6). Adenosine, at a concentration of 50 mg./liter, had no significant effect on the T_5 mutation rate, as compared with a suppression to $0.5/10^8$ bacteria/hour at the same concentration in the F medium chemostat even in the presence of purine type mutagens (5). The high mutation rates to T_6 resistance in the presence of adenosine remain unexplained.

Experiments with Casein Hydrolysate Medium

In order to make a direct comparison of the rapidly growing populations in the breeder with populations at similar growth rates in the chemostat a complex casein hydrolysate medium lacking tryptophan was devised.[2]

[2] Casein hydrolysate medium consists of:

	per liter distilled water	
Casein hydrolysate (Difco vitamin-free acid hydrolyzed casein)	5	gm.
Magnesium sulfate (anhydrous)	0.1	"
Lactic acid (85 per cent)	1.06	"
Sodium hydroxide	0.35	"
Potassium phosphate monobasic (anhydrous)	0.15	"
Sodium phosphate dibasic (anhydrous)	0.35	"
Guanine	10	mg.
Adenine	10	"
Thymine	10	"
Cytosine	10	"
Inositol	2.9	"
Calcium pantothenate	20	μg.
Niacin	200	"
Ascorbic acid	4	"
Riboflavin	8	"
Vitamin B_6	1.5	"
Thiamine	3	"
Folic acid	0.6	"
p-Aminobenzoic acid	1	"
Biotin	0.4	"

The medium is adjusted to a pH of 7.0 and autoclaved.

Using this medium, supplemented with an excess of tryptophan (20 mg./liter) in the breeder, the organism grows at the same rate as in broth. Under aerobic conditions, the mutation rates are also about the same (see Table IV), the rate to T_5 resistance is $3.7/10^8$ bacteria/hour and to T_6 resistance is $2.4/10^8$ bacteria/hour. Under anaerobic conditions (see Table IV), there seems to be some reduction in the mutation rate, but not down to the rates observed in the chemostat with F medium. Adenosine appears to reduce

TABLE IV

Casein Hydrolysate Experiments

Machine	Generation time	Mutation rate ($\times 10^8$ hr.)		Medium: 5 gm./liter casein hydrolysate + vitamins, purines, pyrimidines 20 mg./liter tryptophan
		T_5 resistance	T_6 resistance	
	min.			
Breeder 48A	42	3.5	2.9	1/25 F phosphate*
49	44	3.8	2.5	" " "
102	41	4.0	1.4	5 gm./liter glucose, 1/2 F sodium lactate‡
103	38	3.3	1.9	F phosphate
125	32	3.7	3.0	Same medium as preceding one, but without use of tween or hot wire
126	34	3.8	2.6	
Anaerobic breeder 128	39	2.5	—	
Anaerobic breeder 94	40	1.6	2.6	5 gm./liter glucose, F phosphate
Breeder 95	32	3.7	—	50 mg./liter adenosine
Breeder 83	47	8.3	2.7	150 mg./liter theophylline, 1/25 F phosphate
Breeder 85	43	8.4	2.1	

* F phosphate 3.5 gm./liter sodium phosphate dibasic; 1.5 gm./liter potassium phosphate monobasic.

‡ F sodium lactate 14 gm./liter.[1]

the mutation rate to T_5 resistance (no antimutagenic effect has yet been demonstrated with respect to mutation to T_6 resistance), but here again there is not so great a reduction as is demonstrated in the F medium chemostat where the rate is $0.5/10^8$ bacteria/hour with 50 mg./liter of adenosine (6). In the presence of theophylline there is demonstrated an effect like that observed in the F medium chemostat, but again it is not as great. As already mentioned, 150 mg./liter recrystallized theophylline increases the mutation rate to T_5 resistance nearly tenfold in the F medium chemostat (6), while in the casein hydrolysate breeder the mutation rate only increased from 3.7 to $8.4/10^8$ bacteria/hour.

TABLE V

Chemostats with Casein Hydrolysate

Machine	Generation time	Mutation rate ($\times 10^8$ hr.)		Medium 5 gm./liter casein hydrolysate + vitamins, purines, pyrimidines F phosphate* 1/2 F sodium lactate‡ 5 gm./liter glucose
		T_6 resistance	T_6 resistance	
Chemostat 105	37 min.	3.8	1.7	1 mg./liter tryptophan
122	" "	4.6	1.8	1 mg./liter tryptophan No tween No hot wire
98	42 min.	3.3	3.5	1 mg. liter tryptophan
104	44 "	4.4	2.5	" " " "
121	54 "	2.9	1.9	0.75 mg./liter tryptophan No tween No hot wire
119	58 min.	4.5	2.1	0.75 mg./liter tryptophan
120	65 "	2.2	1.8	0.75 mg./liter tryptophan No tween No hot wire
118	74 min.	3.8	1.7	0.75 mg./liter tryptophan
123	86 "	2.7	1.3	0.5 mg./liter tryptophan No tween No hot wire
117	98 min.	2.8	1.0	0.5 mg./liter tryptophan
124	150 "	2.5	1.1	0.5 mg./liter tryptophan No tween No hot wire
110	3.4 hrs.	1.6	1.0	0.5 mg./liter tryptophan
115	4.5 hrs.	1.7	1.0	No tween
111	4.6 hrs.	2.0	1.1	No hot wire
Anaerobic chemostat 97	50 min.	2.4	2.3	1 mg./liter tryptophan 50 mg./liter adenosine No sodium lactate
Anaerobic chemostat 129	64 min.	1.9	1.6	1 mg./liter tryptophan No tween No hot wire
Anaerobic chemostat 130	99 min.	1.4	1.0	0.75 mg./liter tryptophan No tween No hot wire

* F phosphate 3.5 gm./liter sodium phosphate dibasic; 1.5 gm./liter potassium phosphate monobasic.

‡ F sodium lactate 14 gm./liter.

This same casein hydrolysate medium was then used in chemostats run with tryptophan as the controlling growth factor. It will be noted (see Table V) that at short generation times there is no significant difference between mutation rates in the chemostat and in the breeder. It may also be noted that anaerobic conditions and adenosine have a small effect on these rates.

As may be seen from Fig. 1, which is based on Table V, for both the T_5 and T_6 mutations in casein hydrolysate medium, there is a decrease in the mutation rate with increasing generation time. The T_5 rate decreases from a value of about $4.0/10^8$ bacteria/hour at generation times of about 30 to 40 minutes to $2.0/10^8$ bacteria/hour at generation times of 3 to 4 hours. The T_6 rate falls from about $2.0/10^8$ bacteria/hour to about $1.0/10^8$ bacteria/hour. In both cases most of this reduction in the mutation rates occurs at generation times of less than 1.5 hours. This dependence of the mutation rate on generation time is also observed under anaerobic conditions (see Table V).

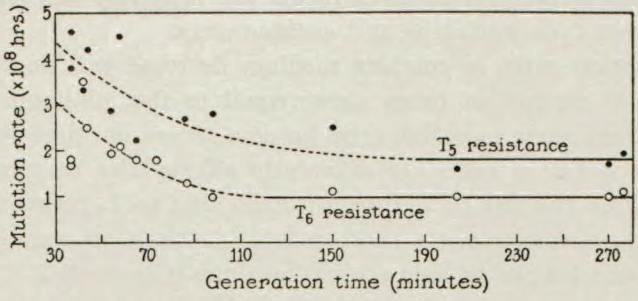

FIG. 1. Mutation rates in casein hydrolysate chemostats.

Controls

Since it was necessary to use 0.05 per cent tween 80 in the medium and also a hot platinum filament to break the foam resulting from aeration, a series of controls were done to demonstrate that neither expedient was responsible for the high mutation rates found in complex media. A number of experiments in Tables IV and V were run without either tween or the hot wire and the results obtained at short generation times were not significantly different from those in which the hot wire and tween 80 were used. At longer generation times there may be a suggestion of somewhat higher mutation rates to T_5 resistance in Experiments 119, 118, and 117 in Table V. There was also noted in some of these experiments a slight discoloration (yellowing) of the medium. These experiments without tween and the hot wire were necessarily of short duration as compared to others described here, since wall growth often developed. They were followed long enough to determine the mutation rates before wall growth appeared, though with somewhat reduced accuracy. Breeder 58 in Table II was run with three times the normal tween 80 concentration, 0.15 per cent, with no effect on mutation rates.

In order to determine the possible mutagenic effect of the visible light from the projector which was used to determine turbidity, breeder 57 (Table II) was run in a manner such that the time of exposure of the culture to the light beam was increased threefold. This had no effect on the observed mutation rates. In addition, it may be noted that chemostats not exposed to light showed the same mutation rates as did corresponding breeders which were exposed to light.

DISCUSSION

The mutation rates observed in complex media in the experiments reported here differ from those observed with F medium chemostats in which tryptophan was the controlling growth factor.

1. The mutation rates in complex media are higher, even at long generation times.

2. The mutation rates in complex media are relatively insensitive to the actions of purine type mutagens and antimutagens.

3. The mutation rates in complex medium decrease with increasing generation time. At generation times about equal to the minimum generation time in F medium, these mutation rates become generation time–independent.

4. The two mutation rates are differently affected by complex medium. The ratio between the rate to T_5 resistance and that to T_6 resistance is about 4 in F medium chemostats. At short generation times in complex medium this ratio is about 1.4 and at long generation times it is about 2.

The high mutation rates in broth are probably not due to some unknown mutagenic agent in the broth since these rates do not vary with broth concentration. In addition, these same rates are obtained in the casein hydrolysate medium in which the components are reasonably well defined and in all likelihood include no known mutagen. The insensitivity of these rates, in broth, to the addition of either theophylline or adenosine suggests the presence of an excess of an adenosine-like antimutagen in the growing culture, either as a component of the broth or as a product of the growing bacteria. In the casein hydrolysate medium which presumably does not contain such a compound, theophylline increases the mutation rate when added to the breeder, but not as much as it does in the F medium chemostat. If the bacteria make some antimutagenic compound, it is possible that they produce less in casein hydrolysate medium than they do in broth.

It is conceivable that the high mutation rates in complex media, as compared with F medium, are associated with a medium-dependent lag between the phenotypic expression and normal multiplication of the newly appeared mutant. Such a lag would result in a temporary selection against the mutant and the observed mutation rate would require a correction factor equal to e^n in which n is the average duration of the lag expressed in numbers of genera-

tions (time duration of lag divided by the generation time of the steady-state population).

If such a lag were a nuclear segregation lag, different in F medium and complex medium, the rates of two different mutations would be altered by the same factor e^n/e^m in which n and m would be the number of generations of lag for the respective media. Since the mutation rate to T_6 resistance is 8 to 10 times higher in complex medium than in F medium,[3] while the rate to T_5 resistance is only about three times higher, e^n/e^m is not constant and such an explanation is inadequate.

Other kinds of lag in division of the newly appeared mutants cannot be excluded. In any case, such a lag would not only have to be different in different media, but would have to be differently affected for the two mutations. In addition, to explain the generation time dependence of the mutation rates for fast growth in casein hydrolysate chemostats, the factor n for a given mutation rate would have to change with generation time.

The generation time dependence of the mutation rates in casein hydrolysate, tryptophan-controlled chemostats at short generation times can, perhaps, be attributed to a change in the chemical environment of the bacteria. Over the range of generation times from about 40 minutes to 90 minutes where the mutation rates change, the steady state concentration of tryptophan in the growth tube varies from about 300 μg./liter to about 30 μg./liter. At longer generation times, where the mutation rate is generation time–independent, the concentration is even lower. In F medium chemostats, the growth tube concentration of tryptophan necessary to maintain a generation time of about 120 minutes is about 1 μg./liter (2). The very high growth tube concentration of tryptophan required in casein hydrolysate chemostats suggests that the other amino acids interfere with the uptake of tryptophan by the bacteria.

The higher mutation rates at short generation times are however, not due to the correspondingly high tryptophan concentrations because the mutation rates in the breeder with F medium and 20 mg./liter tryptophan are about the same as in F medium chemostats with 0.005 per cent of this concentration.

There have been observed, when comparing complex media with F medium, a number of differences. In complex media, the mutation rates are higher, they are less sensitive to mutagens and antimutagens, they are generation time–dependent, and the relative rates for the two mutations are different. Unless the observed mutation rates in complex media and F medium are influenced by grossly different division lags, the difference must be attributed

[3] The difficulty of accurately measuring low mutation rates did not make it seem worth while to further define the rate to T_6 resistance in F medium. This rate has been very carefully measured in F medium chemostats by Lee (4) and it is this value, $0.3/10^8$ bacteria/hour, that has been compared with the rate observed in complex media.

to the difference in chemical environment. The environment might affect various as yet unknown agencies of mutagenic action, for example by influencing a cellular mutagen-antimutagen balance or by direct modification of mutability.

SUMMARY

The breeder and the chemostat have been used to measure mutation rates for two mutations under a variety of steady state growth conditions. These rates have been found to be higher in complex medium than in minimal (F) medium. The effects of changes in nutritional conditions on these high rates have been described. In addition, the mutation rates at short generation times, in complex medium, have been shown to decrease with increasing generation time.

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