261. *Induced and Other Variations in Bacterial Cultures. Part III. Recovery of Normal Growth Rates by Slow-growing Mutants of* Bact. lactis aerogenes.

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The recovery of ultra-violet light-damaged mutants of *Bact. lactis aerogenes* to normal growth rate in a simple synthetic medium is studied quantitatively and the course of events compared with that expected on the basis of different theories.

It has been shown that all the strains of *Bact. lactis aerogenes* isolated after irradiation with ultra-violet light are capable of growth in the synthetic medium with varying growth rates (Part II, Table I). The interesting question arises whether these growth rates are constant for the particular strain, that is, whether the damage wrought by the ultra-violet light is irreparable, or whether the cells can be retrained to behave like normal organisms. The latter alternative proves to be true, since on serial sub-culture in the synthetic medium all the strains (23 in number) were eventually trained to the normal mean generation time, and when they were so trained their biochemical reactions were those of a normal *Bact. lactis aerogenes*. The quantitative study of the recovery process has been undertaken.

The experiments are summarised in Table I. Between 20 and 60 sub-cultures were usually necessary for training, during which the growth rate slowly increased. This recovery is, according to one point of view, suggestive of the repair and expansion of a damaged enzyme system. The alternative hypothesis is that spontaneous genetic mutation (reversion) may take place at some stage during the training process, resulting in cells with the normal mean generation time, and that these then outgrow the rest.

The effect of such spontaneous mutation on the shape of successive growth curves has been calculated for comparison with experiment.

Let n represent the number of slow-growing cells, m the number of back mutants, and ρ be the rate of back mutation (reversion). It is assumed that the formation of the back mutants does not appreciably reduce the number n at any time. We then have the equations

$$\mathrm{d}n/\mathrm{d}t = k_1 n$$
$$\mathrm{d}m/\mathrm{d}t = k_2 m + \rho \mathrm{d}n/\mathrm{d}t$$

Therefore
$$\mathrm{d}m/\mathrm{d}t = k_2 m + \rho k_1 n$$

and
$$\mathrm{d}m/\mathrm{d}t = k_2 m + \rho k_1 n_0 e^{k_1 t}$$

where n_0 is the value of n when $t = 0$.

The solution of the last equation is

$$m e^{-k_2 t} - m_0 = \frac{\rho k_1 n_0}{k_1 - k_2} \{e^{(k_1 - k_2)t} - 1\}$$

whence
$$m = \frac{\rho k_1 n_0}{k_2 - k_1} \{e^{k_2 t} - e^{k_1 t}\} + m_0 e^{k_2 t}$$

$$N_{\text{total}} = n + m$$

and, if $m_0 = 0$, we have
$$N_{\text{total}} = n_0 e^{k_1 t} + \frac{\rho k_1 n_0}{k_2 - k_1} \{e^{k_2 t} - e^{k_1 t}\}$$

Let
$$N_{\text{total}}/n_0 = \nu \text{ and } \rho k_1/(k_2 - k_1) = A$$

Then
$$\nu = (1 - A)e^{k_1 t} + A e^{k_2 t}$$

In typical cases the mutant grows about half as fast as the original or the reverted form : we may therefore for purposes of illustration take $k_2 = 2k_1$. We shall also take the absolute values of k_1 and k_2 to correspond to mean generation times of 60 and 30 minutes respectively. The values of ν have been calculated as functions of time for various values of ρ, the magnitude of the latter being suggested by statements that reversion rates for *Bact. coli* mutants lie in this sort of range. (In Fig. 1 the curves A, B, and C correspond respectively to values of 10^{-5}, 10^{-6}, and 10^{-8} for ρ.)

TABLE I.

Training of irradiated strains. Mean generation times.

No. of subcultures in normal medium.	No.:	Type 1. 16.	25.	26.	28.	31.	Type 2. 20.	27.
2		—	65	—	—	44	—	—
3		—	—	58	44	—	39	—
4		—	—	—	—	—	42	41
5		—	37	44	—	—	40	—
6		—	—	—	—	44	41	38
7		—	38	—	40	—	—	—
9		72	—	41	—	—	—	—
10		—	—	—	—	—	43	34
11		—	—	—	45	—	—	—
13		—	—	—	—	—	—	35
14		—	—	47	—	—	—	—
17		—	42	—	—	—	—	—
20		—	—	60	—	*34*	35	*33*
21		—	—	—	36	—	*32*	—
22		46	—	—	—	—	—	—
27		—	35	—	—	—	—	—
31		—	—	52	—	—	—	—
32		33 *	—	—	47	—	—	—
38		—	*33*	38	—	—	—	—
39		48	—	—	—	—	—	—
44		—	—	*33*	*31*	—	—	—
		34	—	—	—	—	—	—

	No.:	Type 3a. 32.	33.	38.	Type 3b. 21.	22.	23.	24.	29.	30.	34.	35.	36.	39.	41.	42.
1		78	48	—	—	—	—	—	—	—	—	—	—	—	—	49
2		—	—	—	—	78	—	—	46	46	31/94 †	—	—	—	—	—
3		—	—	—	47	—	56	50	—	—	—	47/150 †	44	42	39	—
4		*33*	41	—	44	—	—	—	—	—	—	—	—	—	—	—
5		—	—	*28*	—	79	—	45	42	51	—	—	—	—	—	—
6		—	—	—	42	—	38	—	—	—	—	—	—	—	—	46
7		—	—	—	—	44	—	—	—	—	—	—	—	—	—	—
8		—	*30*	—	—	—	—	—	45	—	—	—	57	—	—	—
9		—	—	—	—	44	34	—	—	41	—	—	—	49	41	—
10		—	—	—	—	—	—	—	43	—	44	57	—	—	—	—
12		—	—	—	—	39	—	38	—	—	—	—	—	—	—	—
13		—	—	—	36	—	36	—	—	—	—	—	—	—	—	—
14		—	—	—	—	—	—	—	—	—	*31*	—	—	—	—	—
15		—	—	—	45	42	*33*	*33*	—	—	—	—	72	—	57	—
18		—	—	—	35	42	—	—	—	—	—	59	—	—	—	—
19		—	—	—	—	—	—	—	—	46	—	—	—	—	—	—
20		—	—	—	—	—	—	—	49		—	—	—	—	—	*29*
22		—	—	—	*32*	55	—	—	—	—	—	—	—	—	—	—
25		—	—	—	—	—	—	—	—	—	—	—	56	42	41	—
27		—	—	—	—	—	—	—	—	—	—	41	—	—	—	—
32		—	—	—	—	—	—	—	—	—	—	—	—	—	44	—
33		—	—	—	—	54	—	—	—	—	—	—	—	38	—	—
36		—	—	—	—	—	—	—	47	65	—	45	—	—	—	—
38		—	—	—	—	47	—	—	—	—	—	—	—	—	—	—
42		—	—	—	—	—	—	—	—	—	—	—	39	—	—	—
43		—	—	—	—	57	—	—	37	*31*	—	—	—	—	40	—
44		—	—	—	—	—	—	—	—	—	—	—	—	39	—	—
46		—	—	—	—	—	—	—	—	—	—	36	—	—	—	—
50		—	—	—	—	—	—	—	—	—	—	—	48	—	36	—
52		—	—	—	—	—	—	—	—	—	—	—	—	46	—	—
53		—	—	—	—	42	—	—	—	—	—	—	—	—	—	—
54		—	—	—	—	—	—	—	—	—	—	*34*	—	—	—	—
55		—	—	—	—	—	—	—	35	—	—	—	—	—	—	—
62		—	—	—	—	*32*	—	—	—	—	—	—	—	—	*34*	—
63		—	—	—	—	—	—	—	*34*	—	—	—	—	—	—	—
64		—	—	—	—	—	—	—	—	—	—	—	—	*33*	—	—
65		—	—	—	—	—	—	—	—	—	—	—	—	—	*32*	—

* Sugar reactions still incorrect. † Composite growth curve.

Italicised figures denote correct m.g.t. and correct sugar reactions.

In a normal growth curve there is during the period of active multiplication a linear relation between the time and the logarithm of the number of bacteria. The presence of reverse mutants leads to the bending of the growth curve as these outgrow the unchanged cells, and the result is a rather rapid fall in the mean generation time from 60 to 30 minutes. The value assumed for ρ affects the time at which the increase of slope occurs (Fig. 1), but not the rate at which it occurs when it once sets in. In the figures ν is plotted in such a way as to imply that unlimited multiplication of the culture is allowed to take place, without the interruption of serial subcultures. In fact, of course, the growth is interrupted by the serial transfers, a multiplication ratio of about 10^2 representing one subculture, as indicated by the horizontal

FIG. 1.

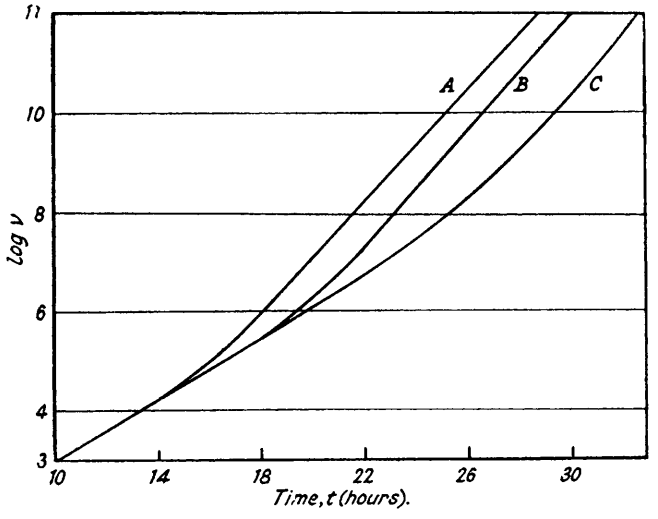
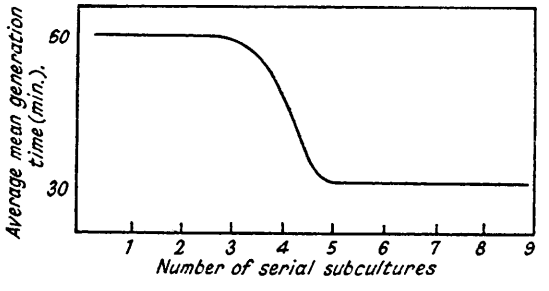


FIG. 2.



lines in Fig. 1. If a formal mean generation time is calculated from the mean slope of the curve of log ν against *t*, then the relationship of this mean generation time to the serial subculture number will be of the form shown in Fig. 2.

It appears that the change from the longer to the shorter mean generation time should take place in the course of a single subculture. To detect the transformation it is necessary to obtain growth curves for every subculture during the training process.

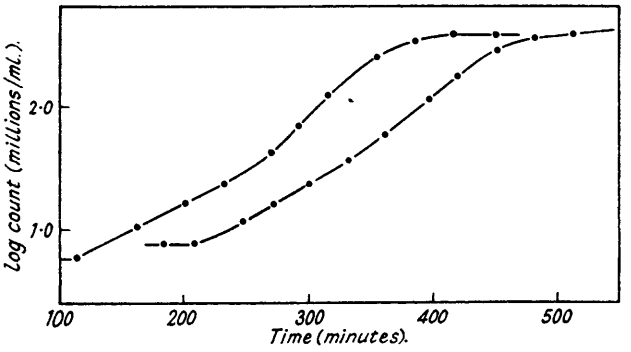
This experiment was carried out on strain No. 14 and the entire growth curve determined for every subculture up to 70 when training was complete. It will not be feasible to reproduce all the curves, but it may be stated that they were all of the normal logarithmic type or else of the type shown in Fig. 3. The mean generation times during training are given in Table II.

At first sight the curves in Fig. 3 are strongly suggestive of the theoretical form shown in Fig. 1, but the total picture of the retraining phenomenon does not accord with this conclusion. If there were a single reverse mutation which restored the normal cells, the transition from the slower to the more rapid growth would declare itself in the course of a given subculture and then be permanent. Actually there is a slow and gradually developing effect of training,

observable over very many subcultures (Table II). The composite growth curves appear at certain stages and then disappear, and whatever they indicate they do not represent an irreversible re-emergence of a fast-growing strain at one given stage.

If we assume that there are competing metabolic routes used by the cell, and that damaged enzyme systems gradually repair themselves during growth, a generally plausible idea of the retraining can be formed. The lags characteristic of the competing reaction systems vary in a rather random way from one subculture to another owing to the lack of control of the inoculum ages : hence the fluctuations in rate and the occasional appearance of the segmented curves (see " Chemical Kinetics of the Bacterial Cell," Oxford, 1946, p. 161).

FIG. 3.



Repair of damaged enzyme systems is thus a possible explanation of the retraining process. A single reverse mutation would not explain the quantitative picture.

On the other hand, a complex series of mutations which were reversed one by one at various stages of the training would give a good explanation of the very slow and erratic character of the phenomenon. Such an assumption, however, does not represent the most economical hypothesis. Nor does it explain the frequent relapses in the return of the mean generation time to the standard value. According to the hypothesis of spontaneous reverse mutation each stage of recovery (so long as no fresh exposure to the ultra-violet light occurred) should at least be permanent.

TABLE II.

Mean generation times during serial subculture of strain No. 14 *in the normal synthetic medium.*

Sub-culture no.	M.g.t. (mins.).	Sub-culture no.	M.g.t. (mins.).	Sub-culture no.	M.g.t. (mins.).	Sub-culture no.	M.g.t. (mins.).
1	45	19	41	36	37	53	38
2	45	20	39	37	44/30	54	37
3	44			38	36	55	36
4	49	21	38	39	39	56	32
5	43	22	42	40	37	57	36
6	45	23	38			58	36
7	40/52	24	38	41	35	59	35
8	37/51	25	37	42	35	60	36
9	38/43	26	37	43	39		
	35/43	27	37	44	33	61	35
		28	35	45	32	62	31
11	38	29	38	46	40	63	37
12	39/46	30	38	47	47	64	39
13	38			48	32	65	34
14	38/46	31	37	49	33	66	33
15	35/45	32	38	50	34	67	36
16	40	33	38			68	32
17	36	34	35	51	43	69	30
18	40	35	33	52	36	70	32

Where two figures are given a composite growth curve is indicated.

Mean values of the m.g.t. for the successive ranges of 10 sub-cultures :

No.	1—10	11—20	21—30	31—40	41—50	51—60	61—70
M.g.t. (mins.)	44	40	38	37	36	37	34

According to the hypothesis that damaged enzyme systems gradually repair themselves the fluctuations are natural. Growth is characterised by lag and by mean generation time. Lag is a function of age, and therefore if we have a makeshift metabolic route (used by the damaged cell) competing with a gradually more practicable route, the lags of the one or the other will vary according to the exact age of the culture when it is tested. The one or the other method of growth may, therefore, at intermediate stages be the one followed on any given occasion.

The chief difficulty about this view is to explain the extreme slowness of the retraining (so easily accounted for if a long wait for a favourable mutation is postulated). The explanation which the hypothesis can offer is as follows. The damaged cell can in fact grow by a makeshift metabolic route, but as long as this is functioning it by-passes processes in which the repair of the enzyme systems would occur through linking of function and reproduction of enzyme systems. Retraining occurs only when the makeshift mechanism lags long enough to allow the damaged normal mechanism to be utilised (however slowly) and so repair itself.

This view predicts an experimentally verifiable effect. If serial subcultures are carried out continuously without interruption the retraining should be slower than it is when the cultures are allowed to age considerably in each cycle. With mutant 14 retraining required 70 subcultures when transferred at 24-hour intervals, but 117 when subcultured at twice this frequency. Mutant No. 20 when subcultured daily showed a broken growth curve with mean generation times of 72/39 at the 6th subculture, while it showed a mean generation time of 30 minutes at the 6th subculture when transferred at intervals of three days. It seems reasonable to infer that when the conditions are such as to allow mechanisms with a long lag (impaired enzyme systems) to compete, the retraining process is favoured.

On the whole, therefore, we may conclude that, as far as the present set of experiments on the quantitative picture of the recovery phenomenon goes, the hypothesis of a direct retraining involves fewer difficulties and assumptions than the alternative.

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