534. *Induced and Other Variations in Bacterial Cultures. Part VI.* A Comparison of the Efficiency of the Methods for Isolating Nutritional Mutants.*

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The efficiency of the penicillin and " screening " methods for the isolation of nutritional mutants is compared. About 1 in every 8 strains isolated by the penicillin method was a nutritional mutant. With the " screening " method the frequency was about 1 in 19 and when no procedure for concentrating nutritional mutants was used the frequency was about 1 in 73.

It made little difference whether a period of growth in a complete medium between irradiation and plating was or was not included in the isolation technique.

Within the limits of the experiments the degree of irradiation did not appear to influence the percentage of mutants among the surviving cells.

Various techniques have been devised for facilitating the isolation of nutritional mutants. It is now possible to make a comparison of the efficiency of these methods on the basis of experiments with *Bact. coli* which are on a more extensive scale than the earlier experiments with *Bact. lactis aerogenes* (*J.*, 1951, 1159).

The methods in question may be summarised thus : after irradiation the suspension may be : (1) plated out directly on a complete agar medium ; (2) plated out on a minimal agar medium, then incubated, layered with a complete agar medium, then re-incubated (*i.e.*, " screening ") ; and (3) treated with penicillin and then plated out on a complete agar medium.

In method 1 a mixture of colonies derived from mutant and non-mutant cells is obtained. In method 2 the colonies which appear after the addition of the complete agar layer are the mutants, and with method 3 all the colonies should be mutants, the penicillin having destroyed the non-mutants. In methods 1 and 2 a period of growth in a complete medium may be allowed before plating. In the penicillin experiments growth in a complete medium was always included in the technique.

The " screening " method and the penicillin method are those which have been reported on most favourably in the literature (see Lederberg, *Ann. Rev. Microbiol.*, 1949, **3**, 1 ; Plough, Young, and Grimm, *J. Bact.*, 1950, **60**, 145). Since at this stage we are concerned only with the efficiency of the two methods in separating out the nutritional mutants, it is unnecessary to consider the other variables, such as the duration of the irradiation, and whether growth in a complete medium was included in the technique. These two methods are compared in Table 1.

Table 1.

Technique	No. of strains which technique indicated as mutants	No. of strains unable to grow in standard synth. medium	%
" Screening "	56	3	5·4
Penicillin	527	68	12·9

From these results it will be seen that only about one strain out of every 19 which the screening method indicated as nutritional mutants was unable to grow in the standard synthetic medium. In the penicillin method the frequency was 1 in about 8. For comparison, in the experiments where no procedure was used for concentrating mutants (method 1), 7 strains unable to grow in the standard synthetic medium were obtained out of 509 tested, *i.e.*, about 1 in 73.

The penicillin method appears to be the most efficient, and from the purely practical point of view it is the method of choice, since it involves less labour. Davis, the originator of the penicillin method (*J. Amer. Chem. Soc.*, 1948, **70**, 4267) has claimed that over 80% of the colonies of *Bact. coli* which survived the drug treatment were mutants, whilst Lederberg and Zinder (*ibid.*, p. 4267), using a similar technique with strains of *Salmonella*,

* Part V, preceding paper.

found that approximately one-half of the survivors were unable to grow in minimal medium. In the present experiments the frequency was about 1 in 8. It is possible that the question of standards to be adopted for mutants, which has already been discussed in Part II (*J.*, 1951, 1159), may also be involved here. It will be of interest to consider the lags of the survivors from the various experiments in which the penicillin technique was used. They are given in Table 2.

TABLE 2. *Lags in the standard synthetic medium of strains isolated by the penicillin method after ultra-violet irradiation.*

	Lag (days)					No growth	
	0—1	1—2	2—3	3—4	over 4	in 2 wks.	Total
No. of strains	346	26	23	13	51	68	527

From these results it will be seen that, according to the standards adopted, the frequency of mutants could be increased. In these experiments the most rigid standards were used, namely, the strains were incubated for up to 2 weeks before it was assumed that growth would not take place. Growth did not begin in any of the experiments after 10 days.

Table 2 includes all the strains isolated in the various experiments in which the penicillin method was used. Some of these experiments were carried out by irradiating a strain of *Bact. coli* which had not been trained to grow with optimal efficiency in the standard synthetic medium. Although, as has been mentioned previously (Part V), when this untrained strain was plated out (without irradiation) and 200 colonies tested, the lags in the standard synthetic medium were all less than 15 hours, it is possible that the survivors after penicillin from an irradiated culture of an untrained strain might have longer lags in the standard synthetic medium than the survivors from a trained strain. That this was not the case is shown in Table 3 in which the figures of Table 2 have been rearranged.

TABLE 3.

	Lag (days)					No growth	
	0—1	1—2	2—3	3—4	over 4	in 2 wks.	Total
No. of strains :							
Trained	150	24	20	11	31	64	300
Untrained	196	2	3	2	20	4	227

With regard to the screening method, Lederberg and Tatum, in the original paper (*J. Biol. Chem.*, 1946, **165**, 381), state that in the case of *Bact. coli*, most, but not all, of the colonies which appear after the addition of the complete agar layer have been demonstrably mutants, but there are indications that highly unstable mutants occur, which would behave like the parent strain after their initial isolation. This may explain why in these experiments only one strain out of 19 which the technique indicated as mutants was unable to grow in the standard synthetic medium. There is also the difficulty in any screening technique of deciding how long to leave the cells in the minimal medium before adding the complete agar layer. If too long a time is allowed some of the nutritional mutants may die; if the time is too short the lag phase of some cells, which might have grown in the minimal layer, may not be overcome, and hence they would be isolated as mutants. In the experiments described here a compromise was reached by allowing 24 hours' growth in the minimal agar layer. The lags of the survivors from the screening experiments are reported in Table 4.

TABLE 4.

	Lag (days)					No growth
	0—1	1—2	2—3	3—4	over 4	in 2 wks.
No. of strains :						
Trained	25	4	1	0	0	3
Untrained	23	0	0	0	0	0

Comparison of Tables 3 and 4 will show that the survivors from the screening experiment either grew in the standard synthetic medium within 3 days, or did not grow at all, whereas in the experiments in which penicillin was used some of the survivors had lags of longer than

4 days, but eventually grew in the synthetic medium. Actually some of these cells grew after 8—9 days. These longer lags may be due to the treatment with penicillin. For comparison, in the experiments in which no method for concentrating mutants was used, 7 strains were unable to grow in the synthetic medium and the remaining 502 strains grew within 1 day.

TABLE 5. *The influence of growth in a complete medium, between irradiation and plating, on the number of mutants.*

Expt.* No.	Method of isolation	No. of strains tested	Lag (days) 0—1	Lag (days) 1—2	No growth in 2 wks.
3	Screening : (*a*)	2	1	1	0
	(*b*)	4	3	1	0
4	Direct plating : (*a*)	80	79	0	1
	(*b*)	75	74	0	1
5	Direct plating : (*a*)	60	60	0	0
	(*b*)	6	4	0	2
	Screening : (*a*)	5	5	0	0
	(*b*)	1	1	0	0

* These numbers correspond to the Experiment nos. in Part V, Table 1.
(*a*) A period of growth in a complete medium was included.
(*b*) No period of growth in a complete medium was included

It has been claimed by some workers (Demerec and Latarjet, *Cold Spring Harb. Symp.*, 1946, **11**, 38; Demerec, *Proc. Nat. Acad. Sci.*, 1946, **32**, 36) that, after irradiation and before plating, the bacterial suspension should be grown in a complete medium. In some of the experiments reported here this procedure was adopted; in others it was not. A comparison can therefore be made. To rule out the possibility that the results may have been influenced

TABLE 6. *The effect of the degree of irradiation on the production of mutants.*

Fraction surviving	Trained strain No. tested	Trained strain Mutants	Untrained strain No. tested	Untrained strain Mutants
Penicillin method.				
$1{\cdot}6 \times 10^{-1}$	—	—	89	1
10^{-1}—10^{-2}	91 *a*	16	45	0
10^{-2}—10^{-3}	50 *b*	15	49	0
10^{-3}—10^{-4}	50 *b*	17	16	0
10^{-4}—10^{-5}	—	—	28	3
10^{-5}—10^{-6}	75 *a*	16	—	—
10^{-6}—10^{-7}	19 *a*	0	—	—
10^{-7}—10^{-8}	—	—	—	—
$6{\cdot}0 \times 10^{-9}$	15 *a*	0	—	—
Direct plating.				
10^{0} (no irradiation)	—	—	200	0
10^{-6}—10^{-7}	75	1	—	—
10^{-7}—10^{-8}	163	2	131	3
Growth in a complete medium and direct plating.				
10^{-6}—10^{-7}	80	1	—	—
10^{-7}—10^{-8}	60	0	—	—
Screening.				
10^{-6}—10^{-7}	18	0	—	—
10^{-7}—10^{-8}	1	0	23	0
10^{-8}—10^{-9}	4	2	—	—
Growth in a complete medium and screening.				
10^{-4}—10^{-5}	3	1	—	—
10^{-6}—10^{-7}	2	0	—	—
10^{-7}—10^{-8}	5	0	—	—

a, First experiment. *b*, Second experiment.
In the penicillin and screening methods the strains tested were only those which the technique had indicated as mutants.

by other factors it will be safer to consider only those experiments in which the sole difference between the techniques was a period of growth in a complete medium. There were 3 such experiments, and they are summarised in Table 5. It appeared that it made

little difference whether a period of growth in a complete medium was or was not included in the isolation technique.

The Effect of the Degree of Irradiation on the Production of Mutants.—Hollaender and Emmons (*Cold Spring Harb. Symp.*, 1941, **9**, 179) have claimed that in fungi the production of mutants with increasing doses of ultra-violet radiation rises, reaches a plateau, and then falls off again. The experiments described previously should show whether this factor is involved, since in them the time of irradiation was progressively increased until the number of survivors was reduced from 10^9 to less than 10 per ml. In Table 6 the results of all the irradiation experiments are summarised with this end in view. Since, as has been shown earlier, the methods for the isolation of mutants are not equally effective, the experiments have been classified into groups in which the same method of isolation was used.

The general impression to be gained from the experiments in which the penicillin method was used is that the frequency of mutants when trained bacteria are irradiated increases as the fraction surviving is reduced from 10^{-1} to 10^{-4}, and then decreases. However, these experiments were not all carried out on the same suspension, and it is possible that they are not strictly comparable. In the first experiment the frequency of mutation when the fraction surviving was between 10^{-1} and 10^{-2} was 17·6%, and when the fraction surviving was between 10^{-5} and 10^{-6} it was 21·4%. In the second experiment at a fraction surviving of between 10^{-2} and 10^{-3} the percentage of mutants was 30·0 and in the range of 10^{-3} to 10^{-4} it was 34·0. In contrast, in the experiments on untrained bacteria, which were all carried out on the same suspension, it was at the lowest fraction surviving investigated that the frequency was greatest. Since in these experiments there was a period of growth in a complete medium, these comparisons will only be valid if the mutants have similar lags and grow at the same rate in each test, or if they behave like non-mutants in a complete medium.

The results with the other techniques are so varied that when taken into consideration along with the results from the penicillin experiments it is difficult to point out any influence of the degree of irradiation on the number of mutants isolated.

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