NOVEL GENOTYPES IN MIXED CULTURES OF BIOCHEMICAL MUTANTS OF BACTERIA

JOSHUA LEDERBERG[1] AND E. L. TATUM

Hershey has reported (*1*) the occurrence of novel combinations of inherited characters in a bacterial virus. It may not be amiss to describe briefly some experimental fragments, relating to a situation in the bacterium *Escherichia coli,* which may be similar in some respects.

Tatum, in reviewing biochemical mutations in *E. coli* (*6*), has pointed out the advantages offered by these characters for genetic analysis. In particular one may note the facility and certainty with which they can be classified, and their relative stability and independence. Since many of the single biochemical mutants of *E. coli* revert with a measurable frequency of the order of 10^{-7} (*3, 5*) we have used the multiple mutants, obtained by iterated mutations, referred to previously (*6*).

When multiple mutants are grown in mixed cultures in complete (yeast extract-peptone-glucose) medium, there have repeatedly appeared appreciable numbers of prototrophic (*4*), or nutritionally wild-type, cells. Although these have never been found, thus far, in a proportion higher than 10^{-7}, they may be detected readily by plating heavy suspensions of the washed cells into a minimal agar medium, in which only the prototrophs will form macroscopic colonies. Their frequency is very much greater than that anticipated on the hypothesis that the prototrophs result from the coincidental occurrence, in the same clone, of reversions of two or more "loci." Furthermore, single cultures of the same multiple mutants grown and tested under comparable conditions have not been found to contain prototrophs, although small numbers of cells reverted at a single "locus" are, of course, found.

Since it has been established in this laboratory that different biochemical mutants are capable of supplying each others' growth-factor requirements by exchange through the medium, it would appear to be possible that these prototrophs represent heterogeneous aggregations of the different mutants. Attempts to detect or induce the segregation of the components of such putative aggregates by biological and physical means have, as indicated below, been uniformly unsuccessful. Therefore it seems likely that the prototrophs are genotypically unique cells.

TABLE 1. TYPES ISOLATED FROM SINGLE AND MIXED CULTURES. MUTANTS USED ARE INDICATED ON THE LETTERED LINES.

	From single and mixed		From mixed only		From single and mixed
A		$B^-M^-P^+T^+$	and	$B^+M^+P^-T^-$	
	$B^+M^-P^+T^+$		$B^+M^+P^+T^+$ *		$B^+M^+P^+T^-$
	$B^-M^+P^+T^+$				$B^+M^+P^-T^+$
B		$B^-M^-P^+T^+R$	and	$B^+M^+P^-T^-$	
	**		$B^+M^+P^+T^+R$ *		**
			$B^+M^+P^+T^+$ *		
C		$B^-M^-P^+T^+$	and	$B^+M^+P^-T^-R$	
	**		$B^+M^+P^+T^+R$ *		**
			$B^+M^+P^+T^+$ *		
D		$B^-M^-P^+T^+R$	and	$B^+M^+P^-T^-R$	
	**		$B^+M^+P^+T^+R$ *		**
E		$B^-\Phi^-C^-P^+T^+$	and	$B^+\Phi^+C^+P^-T^-$	
	$B^-\Phi^-C^+P^+T^+$		$B^+\Phi^+C^+P^+T^+$ *		$B^+\Phi^+C^-P^-T^+$
	$B^-\Phi^+C^-P^+T^+$		$B^-\Phi^+C^+P^+T^+$		$B^+\Phi^+C^+P^+T^-$
	$B^+\Phi^-C^-P^+T^+$		$B^-\Phi^+C^+P^-T^+$		

* Prototroph.
** See A for biochemical variations.
The letters refer to requirements for essential metabolites as follows:

B = biotin	M = methionine
Φ = phenylalanine	P = proline
C = cystine	T = threonine

R = Resistance to virus T1.

[1] Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

After having passed through a single colony isolation, the prototrophs are quite stable; and of many hundreds of colonies isolated from cultures grown on complete medium, all have proved prototrophic.

Cultures of a prototroph, grown in complete medium, were irradiated with ultraviolet light of such dosage that the number of colonies which appeared on plating in complete agar medium was reduced to $1:10^5$. At this rate of killing, it is evident that most of the supposed aggregates had no surviving representatives. Of the remainder it is likely that only a single cell would survive, in most instances, to form a colony. Nevertheless, each of several hundred colonies that were tested were prototrophic.

Fortunately, the strain with which these experiments were conducted (K-12) is susceptible to the bacterial virus T1 (*2*). In the following experiment multiple mutant strains were used which required biotin and methionine ($B^-M^-P^+T^+$) and threonine and proline ($B^+M^+P^-T^-$) respectively. In addition, mutations, occurring spontaneously at a low rate, for resistance (R) to T1 were selected for by the procedure described by Luria and Delbrück (*2*).

Mixed cultures were plated out as before, and prototrophs isolated and tested for virus resistance. When mixtures of susceptible strains were studied, only susceptible prototrophs were found. Similarly, when resistant strains were mixed the prototrophs obtained were exclusively resistant. When, however, a mixture of ($B^-M^-P^+T^+R$) and ($B^+M^+P^-T^-$) was used, of ten isolated prototrophs, 8 were resistant and 2 sensitive. When ($B^-M^-P^+T^+$) and ($B^+M^+P^-T^-R$) were used, 3 were resistant and 7 sensitive. If the prototrophs consisted of aggregates of the original mutants, each prototroph culture in these cases should have had a large proportion of resistant cells. Yet we have mentioned above the finding of 9 prototroph cultures which were completely lysed by T1. The occurrence of both resistant and sensitive prototrophs is evidence, also, for their internal homogeneity, as one might expect either resistance or susceptibility to be dominant (see Table 1).

Combinations of other mutants have given rise to prototrophs. In particular, isolations have been made from a mixture of proline-threonineless ($B^+\Phi^+C^+P^-T^-$) and biotin-phenylalanine-cystineless ($B^-\Phi^-C^-P^+T^+$) plated into agar containing biotin, phenylalanine and proline. In addition to prototrophs and single reversion types, a biotinless ($B^-\Phi^+C^+P^+T^+$) and a biotin-prolineless ($B^-\Phi^+C^+P^-T^+$) have been isolated.

Note added in proof: Additional experiments and an interpretation of these data have been reported by us. ("Gene recombination in *Escherichia coli*." Nature 158: 558. 1946.)

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