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** Contribution No. 2701.

[1] Meselson, M., F. W. Stahl, and J. Vinograd, these PROCEEDINGS, **43**, 581 (1957). Their treatment also neglects the small pressure dependence of $(\partial\mu_2/\partial m_2)_P$, equation (2).

[2] Young, T. F., K. A. Kraus, and J. S. Johnson, *J. Chem. Phys.*, **22**, 878 (1954).

[3] Williams, J. W., K. E. Van Holde, R. L. Baldwin, and H. Fujita, *Chem. Revs.*, **58**, 715 (1958).

[4] Baldwin, R. L., these PROCEEDINGS, **45**, 939 (1959).

[5] Goldberg, R. J., *J. Phys. Chem.*, **57**, 194 (1953).

[6] Refer to reference 12 for the definition of the term "free solute."

[7] The superscript zero is used throughout this paper to designate variables at atmospheric pressure. The density $\rho^\circ(m_2)$ is a function only of m_2 and should be thought of as a composition variable. The actual density ρ is a measure of physical density and is a function of m_2 and pressure.

[8] Ifft, J. B., D. H. Voet, and J. Vinograd, *J. Phys. Chem.* (in press).

[9] Pohl, F., Dissertation, Rheinische Friedrich Wilhelms Universität, Bonn, Germany (1906).

[10] κ_2 was evaluated with the aid of the following equation derived from equation (4) and with the aid of the intercept method for determining partial specific quantities:

$$\kappa_2 = \kappa + \frac{1}{\rho^\circ \bar{v}_2^\circ}(\kappa_i - \kappa)$$

The quantity κ_i is the intercept at $Z_2 = 1$ of the tangent to the κ versus weight fraction of CsCl' Z_2, curve.

[11] All subsequent error approximations are based on a pressure of 130 atmospheres.

[12] Hearst, J. E., and J. Vinograd, these PROCEEDINGS, **47**, 999 (1961).

[13] Equation (13) is equation (22) in reference 12. The subscript s refers to the solvated species; a_1^0 is the activity of the solute at atmospheric pressure.

[14] Hearst, J. E., and J. Vinograd, *Arch. Biochem. Biophys.*, **92**, 206 (1961).

[15] Margenau, H., and G. M. Murphy, *The Mathematics of Physics and Chemistry* (Princeton: D. Van Nostrand Co., 1956), p. 515.

[16] Hearst, J. E., and J. Vinograd, these PROCEEDINGS, **47**, 1005 (1961).

[17] Jacobson, B., *Arkiv för Kemi*, **2**, 177 (1951).

[18] Szybalski, W. (private communication).

AMBIVALENT rII MUTANTS OF PHAGE T4

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The *r*II mutants of phage T4 differ from the standard type in being inactive on strains of *Escherichia coli* lysogenic for phage λ. While the mutants attach to the cells and inject their DNA, progeny fail to appear. In this paper it is shown that one or another subset of the *r*II mutants can become active as a result of modification of the host, either by mutation or by the action of 5-fluorouracil. The effects are characterized by extreme specificity, applying only to certain *r*II mutations at certain points within the two *r*II cistrons. The phenomenon resembles what has been called, in many other systems, "allele-specific suppression,"[1] in which a muta-

tion outside a genetic region controlling a particular function suppresses the effects of some of the mutations within the region.

An advantage of the phage system is that a given genetic message, in the form of a phage mutant, can be readily inserted into different hosts. The stimulus for the present investigation derives from the possibility that modifications of the host affecting the expression of specific phage mutations might be a key to the detection of alterations in the components of the cellular system for translating genetic information.

Materials and Methods.—Bacterial strains: For crosses and for non-selective plating *E. coli* B was used. *E. coli* KB is the strain previously designated[2] as K. It was derived from K12(λ), which was cured of lysogenicity to produce the sensitive strain K12S (Lederberg and Lederberg[3]), and transmitted through Luria to Benzer, who relysogenized it with phage λ obtained from K12(λ). *E. coli* KT is otherwise known as 112–12 (λh #3). It was derived from K12(λ) by treatment with ultraviolet light to produce a cured strain 112-12 (Wollman[4]), was later lysogenized with a mutant of λ and found its way through Epstein to Tessman to us.

Designation of rII mutants:[5] All were derived from phage T4B, with the exception of those designated by ED, which are spontaneous mutants derived from T4D. *Spontaneous mutants* have either no prefix, or the prefixes SN or SD. A single letter prefix from A through J designates spontaneous mutants derived from revertants of spontaneous *r*II mutants. *Mutagen-induced mutants* are prefixed as follows: NA, NB or NT induced by nitrous acid; EM by ethyl methane sulfonate; HB by hydroxylamine; N or M by 5-bromouracil or 5-bromodeoxyuridine; AP by 2-aminopurine; DAP by 2,6-diaminopurine; BC by 5-bromodeoxycytidine; P by proflavine; PT, PB by heat at low pH; UV by ultraviolet light. Many of the mutants were contributed by J. Drake, R. Edgar, E. Freese, M. Meselson and I. Tessman.

Genetic mapping of rII mutants was done by techniques previously described.[5] Strain KB was used as the selective indicator except when crosses involved *r*II mutants that were too active on KB, in which case strain KT was employed. For conventional crosses, as used in the analysis of *r*EM64, the procedure of Chase and Doermann[6] was followed.

Media: Unless otherwise noted, the medium was broth (1% Difco bacto-tryptone plus 0.5% NaCl). For plates, 1.2% agar was added for the bottom layer and 0.7% for the top layer. In experiments involving 5-fluorouracil, a synthetic medium (M9S) was used containing, per liter of solution, 5.8 gm Na_2HPO_4, 3.0 gm KH_2PO_4, 0.5 gm NaCl, 1.0 gm NH_4Cl, 0.25 gm $MgSO_4 \cdot 7H_2O$, 2.7 mg $FeCl_3 \cdot 6H_2O$, 4.0 gm glucose, 20 mg L-tryptophan, and 2.5 gm Difco vitamin-free casamino acids. M9 buffer is the same medium minus the carbon compounds. *5-fluorouracil* was kindly donated by the Hoffman-LaRoche Company.

Burst size measurements were made in broth at 37°C using exponentially growing cultures at a cell concentration of 2 to 3 × 10^8/ml. Phage was added in the presence of 5 × 10^{-3} *M* NaCN at a multiplicity of less than 0.01 phage per bacterium. After allowing 15 min for adsorption, a sample was diluted 10^4-fold. (If a measurement required the elimination of unadsorbed phage, T4 antiserum was added to the adsorption tube 10 min after infection.) After 60 min of growth the cells were lysed with chloroform and the phage yields were assayed on *E. coli* B. The number of cells infected by *r*II mutants cannot be readily assayed, but, since adsorption under the conditions used was virtually complete, the burst size was calculated using the titer of the phage stock.

Treatment of bacteria with ethyl methane sulfonate (EMS): Induction of mutations in the bacterial strains was done according to a method communicated by E. Lin. One drop of EMS (Distillation Products Industries) was dissolved in 5 ml of an overnight aerated culture of bacteria in broth. After 2 hours' incubation at 37°C, a tenfold dilution was made into fresh broth and aerated overnight. This culture was treated with EMS, the cycle being repeated a total of six times. To measure the effectiveness of the treatment, the proportion of bacteria resistant to phage T6 was measured. This proportion increased more than 100-fold in the treated series and reached a maximum by the third cycle, but it remained almost unchanged in controls unexposed to EMS.

Results.—Ambivalent rII mutants: *r*II mutations have been located in a small portion of the genetic map of the phage T4, at more than three hundred distinct

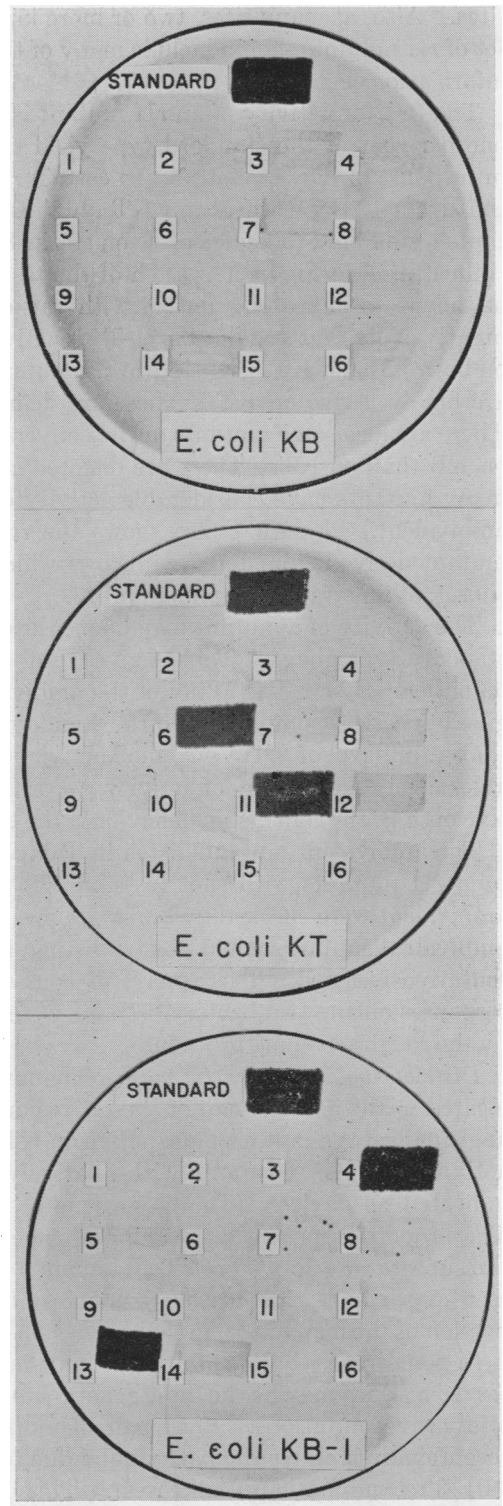


FIG. 1.—Spot tests for activity of various *r*II mutants on three bacterial strains. Each plate was seeded with about 10^8 bacteria in a top layer of soft agar. Streaks of standard type T4 and sixteen different *r*II mutants were added (to the right of each number), by means of a sterile paper strip dipped into a stock of the mutant phage, and the plates were incubated overnight at 37°C.

Standard type (r^+) is active on all three bacterial strains. Ambivalent mutants of subset 1 are illustrated by spots 6 and 11. Spots 4 and 13 are ambivalent mutants of subset 3. The occasional plaques are due to revertants present in the mutant stocks. The mutants are:

(1) *r*131,
(2) *r*1272,
(3) *r*289,
(4) *r*AP129,
(5) *r*859,
(6) *r*NT332,
(7) *r*744,
(8) *r*607,
(9) *r*NT341,
(10) *r*BC24
(11) *r*C204,
(12) *r*1011,
(13) *r*N38,
(14) *r*UV272,
(15) *r*N84, and
(16) *r*P58.

sites. Also, at many sites, two or more kinds of mutations are known. This large set of *r*II mutants should include many of the various possible changes in the genetic information elements.

The test for activity of an *r*II mutant is a sensitive one, since a small fraction of one per cent of the standard type yield can be readily detected. The difference in response of bacterial strains to certain *r*II mutants was first discovered between the strain KB and the strain KT obtained from Irwin Tessman. Particular mutants were found to be defective on strain KB but to be active on strain KT. This is illustrated in Figure 1. Each of the test plates shown was seeded with bacteria of the strain indicated, spotted with various phage mutants, and incubated overnight. Note that standard type T4 is active (lyses the bacteria producing clearing) on both KB and KT. Of sixteen *r*II mutants inactive on KB, two (spots 6 and 11) are highly active on KT. These are defined as ambivalent mutants of subset 1. There are also some mutants of the converse type (not shown) that are more active on KB than on KT. These are denoted as ambivalent subset two. (In addition, many mutants have considerable activity on both KB and KT but are not clearly ambivalent.) Figure 1 also shows the response of the bacterial strain KB-1, a mutant derived from KB (see below) which serves to identify a third ambivalent subset, as illustrated by spots 4 and 13.

The activity of a mutant may be measured by its burst size as compared with the standard type phage. Table 1 gives results for various *r*II mutants when adsorbed on either KB or KT. Many of the ambivalent mutants of the class active on KT give burst sizes comparable to the standard type. This does not necessarily mean that a full level of "*r*II$^+$ enzyme" activity is produced, since the burst size may be an exaggerated measure of activity. For the class of ambivalent mutants more active on KB, the contrast in behavior of the two bacterial strains is less pronounced.

The ambivalent mutants listed in Table 1 were found by screening several thousand *r*II mutants with spot tests on KB and KT. Those which appeared to be ambivalent were checked by burst size measurements. In cases where two or more ambivalent mutants could not be distinguished by recombination, only one representative is listed. There may, of course, be other ambivalent mutants whose responses on the two bacterial strains were not sufficiently different to be detected by the rough screening procedure.

Distribution in the genetic map: The ambivalent mutants listed in Table 1 are located in the genetic map of the *r*II region at the sites shown in Figure 2. A triangle indicates a mutant more active on KT and a square indicates one with greater activity on KB. Mutants with relatively low activity on both KB and KT are indicated as circles. (The stars denote a third ambivalent subset—see below.) The proportion of sites at which ambivalence has been noted is about 1 in 23 for the subset more active on KT and about 1 in 39 for the reciprocal type. It can be seen at a glance that neither kind of ambivalence is restricted to any particular portion of the map or to one cistron.

In some cases, both ambivalent and non-ambivalent mutations have been observed at what may be the same genetic site. Table 2 lists the data for such possibly alternative mutations. Each pair of mutants, when crossed, gave no observable recombination above the background due to spontaneous reversion (less than about 0.01% recombination in all cases). This does not necessarily prove that the sites

TABLE 1

BURST SIZES OF VARIOUS rII MUTANTS ON *E. coli* STRAINS KB AND KT

	Bacterial Host	
	E. coli KB	*E. coli* KT
Standard type phage		
r^+	100.	100.
Non-ambivalent rII mutants		
r131	0.00	0.00
r1272	0.00	0.03
Ambivalent rII mutants of subset 1		
rHB118	0.00	98.
rHB122	0.00	42.
rC204	0.06	104.
rN11	0.00	127.
rEM64	0.00	99.
rHB32	0.00	19.
rHB35	0.00	100.
rHB80	0.00	6.3
rHB129	0.00	102.
rHB84	0.00	106.
r2074	0.00	20.
rEM84	0.00	79.
rHB74	0.00	72.
rNT332	0.00	72.
rB94	0.00	81.
rHB232	0.00	108.
Ambivalent rII mutants of subset 2		
rSN86	37.	2.8
rHB309	40.	3.0
r154	41.	3.8
rNB3830	5.0	0.3
rN40	10.	0.03
rHB33	16.	1.1
rN89	7.2	0.6
rHB8	50.	4.0
rDAP35	13.	1.1

Burst sizes are expressed as per cent of the burst size of standard type, which was several hundred on each host strain. A zero in the last decimal place indicates a value of less than one for that digit. The values (particularly low ones) are subject to fluctuations from one experiment to another and some are quite sensitive to temperature. Differences less than a factor of two are probably not significant.

are identical. It shows clearly, however, that ambivalence is not merely a property of certain small regions of the map.

A feature not shown in Figure 2 is the number of apparent recurrences at each site. At some of the sites many independent mutations have been observed which may or may not all be ambivalent. For example, four other mutations occurred at the site rHB129 and all were ambivalent in the same way as rHB129. At other sites, ambivalence provides a further criterion (in addition to degree of activity, reversion rate, and response to mutagens) for discriminating between alternative mutations at the same apparent site.

Back cross test of an ambivalent mutant: Since ambivalent mutants are in the minority, one possibility which must be considered is that they owe their properties to additional mutations (outside of the rII region) which affect the behavior of the phage on different bacterial strains. For example, a given rII mutant might have the potential to be fairly active on both strains KB and KT but might happen to harbor a second mutation, having no relation to rII, which prevents the adsorption of the phage to one of them. This would make the mutant appear to be ambivalent. While a mutation affecting adsorption could, of course, be detected directly, other more elusive mechanisms might be imagined. From the fact that at some sites several (and in some cases all) recurrences are ambivalent, it would seem most un-

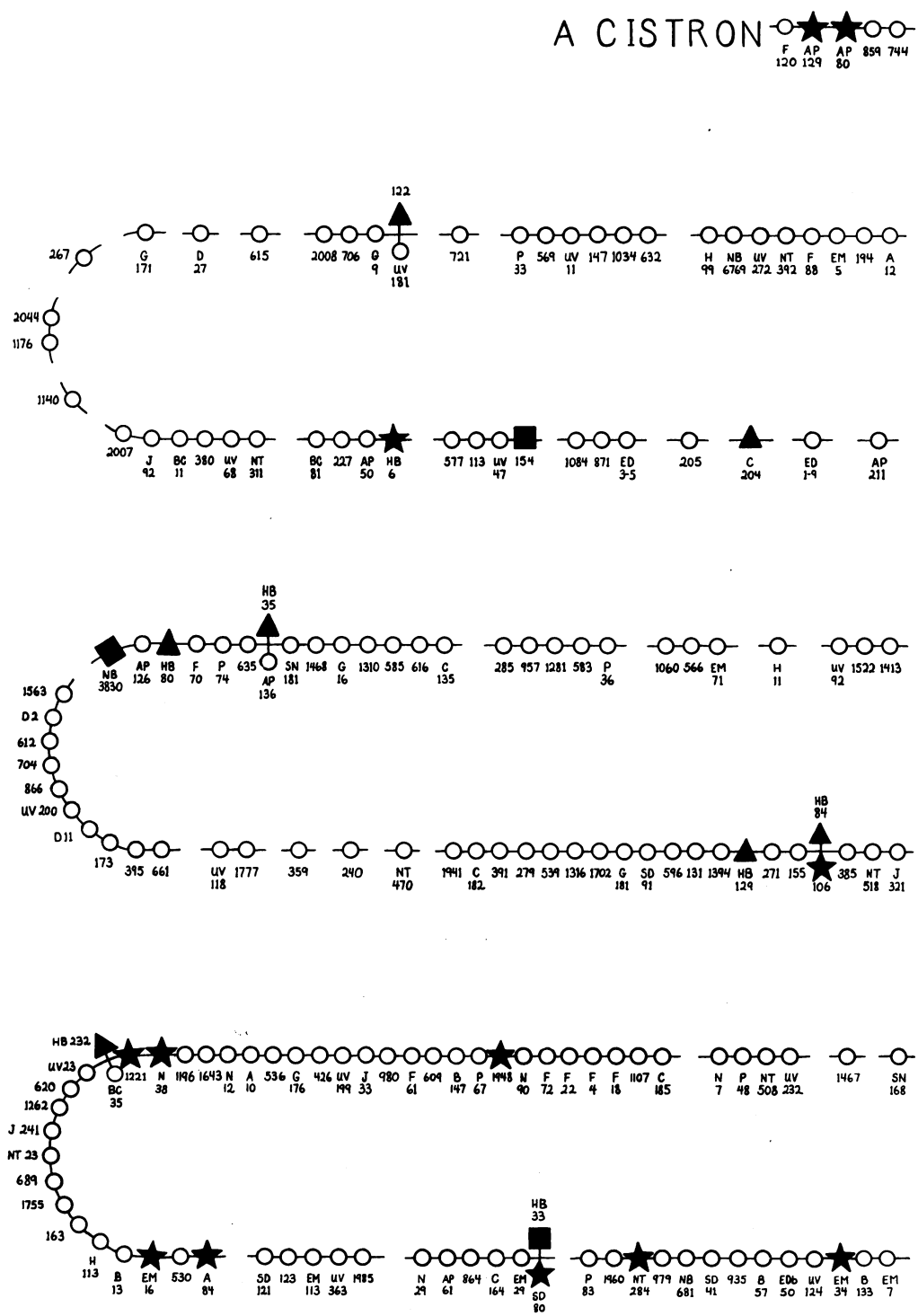
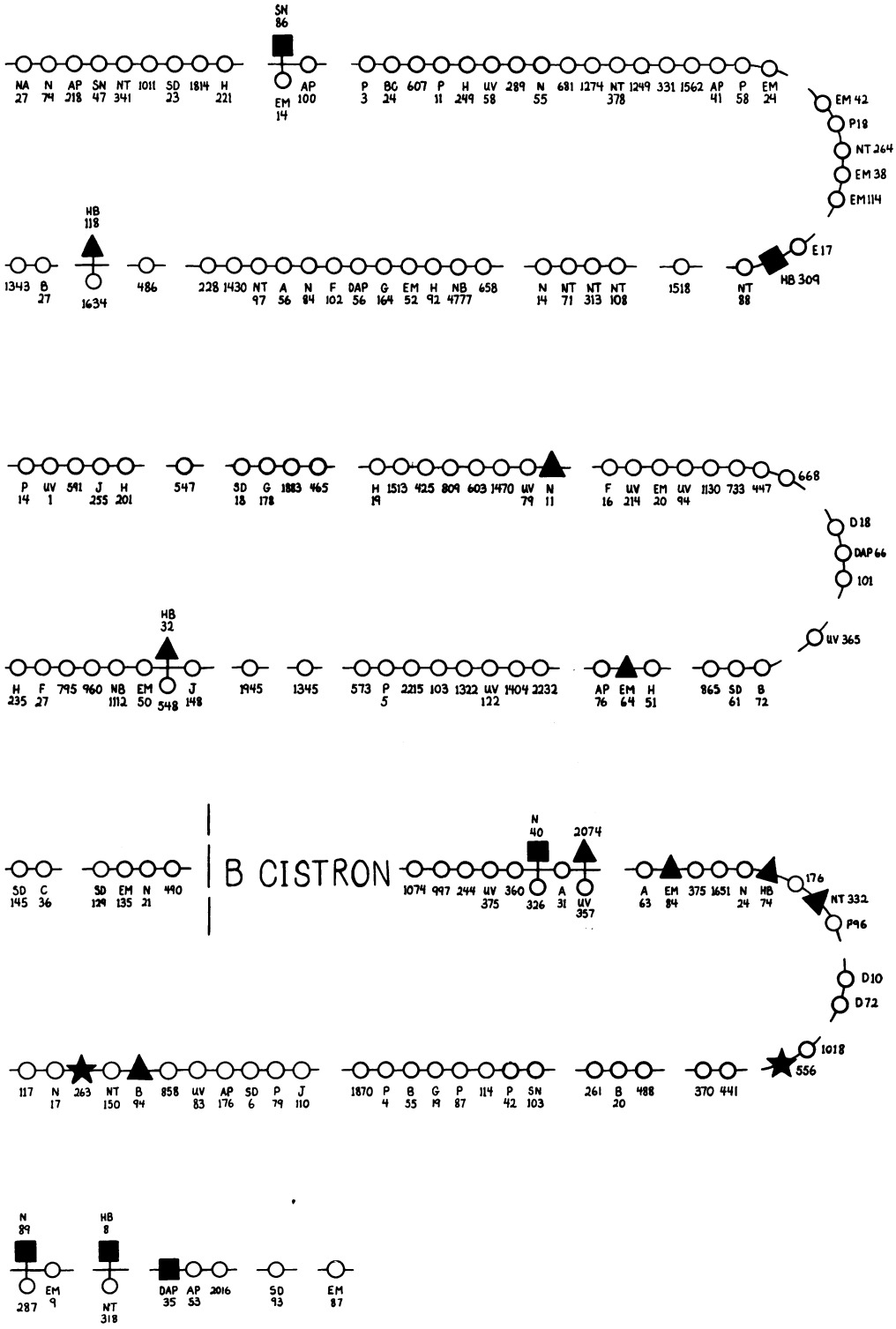


FIG. 2.—Genetic map of the *r*II region, showing the locations of sites at which ambivalent mutants have been found. The breaks in the map indicate segments as defined by the ends of deletions.[6] The order of the segments has been determined as shown. The order of mutations within any one segment has not been determined, but all give recombination with one another.


B CISTRON
▲ Ambivalent of subset 1, more active on E. coli KT than on E. coli KB.
■ Ambivalent of subset 2, more active on E. coli KB than on E. coli KT.
★ Ambivalent of subset 3, more active on E. coli KB-1 than on E. coli KB.
○ No pronounced ambivalence noted.

likely that ambivalence can, in general, be attributed to independent extraneous mutations. If such a mutation exists, however, it should be possible to separate it from the *r*II mutation by a back cross to the standard type.

TABLE 2

PAIRS OF AMBIVALENT AND NON-AMBIVALENT MUTANTS FOR WHICH RECOMBINATION HAS NOT BEEN DETECTED

*r*II mutant	Bacterial Host: *E. coli* KB	Bacterial Host: *E. coli* KT
*r*SN86	37.	2.8
*r*EM14	0.00	0.05
*r*HB118	0.00	98.
*r*1634	0.00	0.4
*r*HB122	0.00	42.
*r*UV181	0.04	0.08
*r*HB32	0.00	19.
*r*548	0.00	0.04
*r*HB35	0.00	100.
*r*AP136	0.00	0.00
*r*HB84	0.00	106.
*r*106	0.4	0.1
*r*N40	10.	0.03
*r*326	0.00	0.00
*r*2074	0.00	20.
*r*UV357	0.00	0.00
*r*HB232	0.00	108.
*r*BC35	0.00	0.00
*r*HB33	16.	1.1
*r*SD80	0.00	0.00
*r*N89	7.2	0.6
*r*287	0.02	0.04
*r*HB8	50.	4.0
*r*NT318	0.00	0.04

The mutants of each bracketed pair cannot be distinguished by recombination, but behave differently on the two bacterial strains. Burst sizes are given as per cent of the burst size of the standard type phage.

With this aim, *r*EM64 was crossed with standard type and the progeny plated on B; 348 *r*-type plaques were picked, purified by replating, and tested on KB and KT, as shown in Figure 1. All but one had the same ambivalent character as *r*EM64. This one exception was inactive on both KB and KT and proved to be a double mutant with one mutation at the site of *r*EM64 plus a second mutation in the B cistron. It therefore must have arisen from *r*EM64 by mutation and does not represent a loss of ambivalence by recombination. The proportion of *r*II progeny that lost ambivalence by recombination was thus less than 0.3 per cent. In contrast, a cross of *r*EM64 with *r*C41, a mutant near the beginning of the A cistron (at the same site as *r*AP80), yielded 1.5 per cent r^+ recombinants. The distance from *r*EM64 to either end of the *r*II region is at least as great as to *r*C41. Therefore, the results show that the ambivalence of *r*EM64 cannot be due to any mutation outside the *r*II region.

The effect of 5-fluorouracil on rII mutants: Naono and Gros and their collaborators[7-9] have reported that 5-fluorouracil (5FU), a pyrimidine analog which is incorporated into RNA,[10] causes changes in the rates of incorporation of certain amino acids as well as modification of the properties of β-galactosidase and alkaline phosphatase of *E. coli*. This suggests that the analog may cause specific errors in

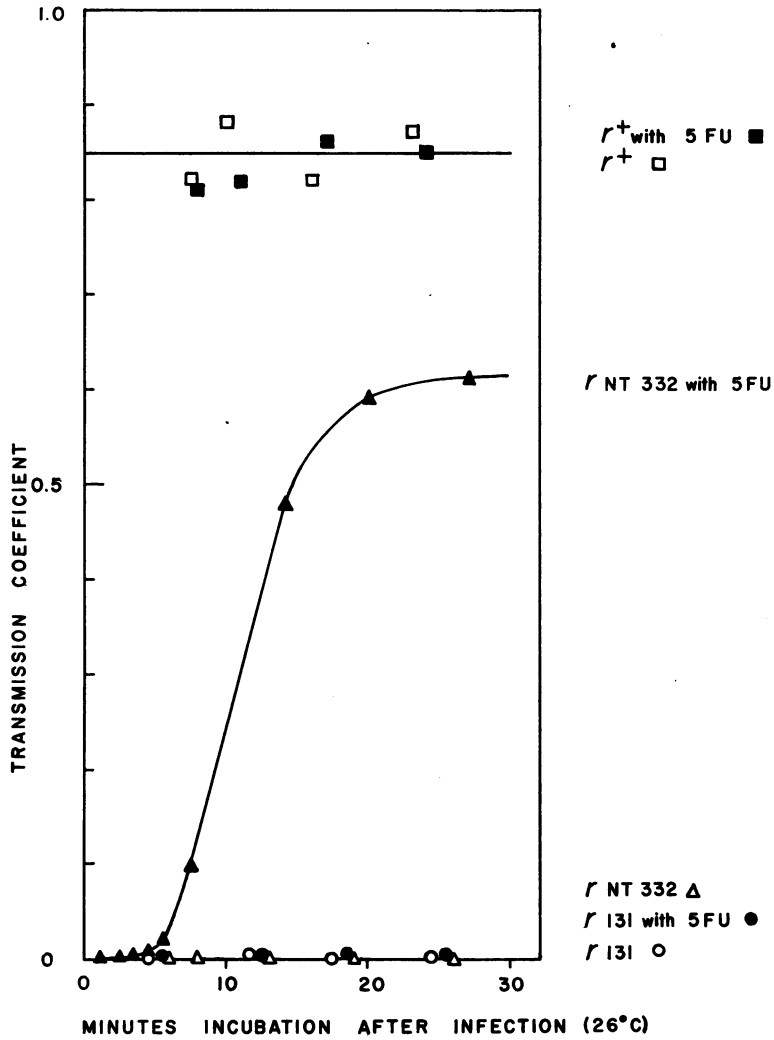


FIG. 3.—Effect of 5-fluorouracil on the activity of *r*II mutants on E. coli KB. The transmission coefficient is the fraction of infected cells yielding at least one progeny particle.

In this experiment a culture of KB growing exponentially in M9S medium was used at a cell concentration of 3.7×10^8/ml. Phage was added in the presence of 0.01 *M* NaCN at a multiplicity of 0.3 per bacterium. After allowing 10 min for adsorption at 37°C anti-T4 serum was added, and 5 min later a sample was diluted 100-fold in *M*9 buffer, then 20-fold at 26°C into M9S + 20 γ/ml thymidine with and without 10 γ/ml 5FU. This is time zero on the graph. At various times thereafter, samples were plated on *E. coli* B using broth plates (this arrests the action of 5FU) to determine the fraction of cells producing at least one progeny particle. The latent period under these conditions was 100 min for the standard type phage in the absence of 5FU.

the translation of the genetic information of the cell. If this idea is correct, certain mutants (having the proper genetic errors) should be stimulated by 5FU.

Many *r*II mutants have been tested for their response to 5FU and some have been found to respond strikingly. Figure 3 shows the effect of 5FU on a responsive and a non-responsive mutant. A convenient index to the activity of a mutant is the

"transmission coefficient," i.e. the fraction of infected cells (strain KB in this case) giving rise to at least one *r*II progeny particle, as assayed by plating on *E. coli* strain B, on which *r*II mutants produce plaques. In this experiment KB cells were infected with the mutant phage (in the presence of cyanide to prevent development) and at time zero, diluted into growth medium with or without 5FU. At various times thereafter, samples were plated on strain B. The transmission coefficient for the responsive mutant, *r*NT332, increased rapidly during the first 20 min of exposure to 5FU. By contrast, *r*131 showed no detectable response.

Under the conditions given in Figure 3, uracil, at a concentration of 20 γ/ml, completely prevented the stimulation of *r*NT332 by 5FU while thymidine, at the same concentration, had no detectable effect. This suggests that the stimulating effect of 5FU is not due to an interference with DNA synthesis. The effect of 5FU on *r*NT332 is a physiological one and not due to mutagenesis, since the progeny phage yielded by KB (in the presence of 5FU) are no more active (in the absence of 5FU) than their parents. 5-Bromouracil at the same molar concentration and under the same conditions produced no increase in the activity of either of these mutants on KB.

5FU causes some inhibition of phage growth in general, as shown by a decreased burst size of the standard type phage. (This is not evident in the figure, since the transmission coefficient still remains close to unity.) However, the increased activity observed for the responsive mutant is over and above this general inhibition. Both the general inhibition and the specific stimulation are insensitive to the concentration of 5FU above about 1γ/ml.

Table 3 shows data for the response to 5FU of various mutants tested on KB. A comparison with Table 1 shows that 12 of the 16 ambivalent mutants of subset 1 are responsive to 5FU on KB. By sharp contrast, a survey of mutants (not belonging to subset 1) chosen arbitrarily from 55 different sites revealed only one (*r*N24) which was responsive to 5FU. Difficulties arise in testing the ambivalent mutants of the subset more active on KB since they generally have considerable activity on KT even without 5FU.

Alteration of the bacterial response pattern by mutation: Strains KB and KT are derived from the same original strain, but they have passed through various treatments in different hands and have had opportunities to accumulate changes. Each has at one time been "cured" of its prophage and re-lysogenized with another strain of λ. Since the presence of prophage λ is necessary for the block against *r*II mutants it was important to determine whether the response pattern is related to the prophage specificity.

This was tested by transferring the prophage from one strain into the other. A culture of KT was induced with ultraviolet light and the resulting phage was used to lysogenize strain K12S, the same non-lysogenic strain that had been used to manufacture the lysogenic strain KB. Two independently-arising lysogenic strains were tested with five ambivalent mutants of subset 1 and five of subset 2 and gave results typical of strain KB. Thus, the identity of the prophage *per se* does not determine the reponse pattern.

The response pattern of the bacteria can, however, be altered by mutation. Such mutants have been isolated from cultures subjected to treatment by ethyl methane sulfonate (see *Methods*). Two kinds of changes were looked for: (1) acquisition

of ability to support the development of an inactive *r*II mutant, and (2) loss of ability to permit development of an active *r*II mutant. Both types can be screened for by spreading bacteria on plates to form colonies in the presence of a limited number of *r*II phage particles (about 10^5 per plate). Under these conditions, most bacteria divide before encountering a phage particle and proceed to produce colonies. If the phage is inactive on a particular strain, the killing effect of the phage particles is negligible and most colonies develop smoothly. Mutant clones which permit the phage to multiply appear as nibbled colonies and are chosen for further testing. In looking for changes of type 2, the situation is reversed and the occasional solid colonies are chosen as suspects. Some of the variants isolated in this way are simply ones which have lost the block against all *r*II mutants (which can occur due to the loss of prophage λ) or ones which adsorb the phage poorly, but others show altered patterns of response specific for certain *r*II mutants.

In Table 4, a comparison is given of KT with a strain derived from it by treating with ethyl methane sulfonate and screening for resistance to *r*NT332, an ambivalent mutant of subset 1. KT-5 differs from KT in having lost the ability to support the multiplication of all ambivalent mutants of subset 1 but the remains similar to KT with respect to subset 2.

The behavior of a bacterial variant obtained by the converse procedure is illustrated in Figure 1 and in Table 5. KB-1 was obtained by treating KB with ethyl methane sulfonate and screening for sensitivity to *r*AP129, a mutant which is not active on either KB or KT. The result is a variant bacterium on which an entire set of *r*II mutants is active, thus revealing a third ambivalent subset. The mutants of this subset are identified in Figure 2 by stars. This subset numbers

TABLE 3

SPECIFICITY OF THE 5-FLUOROURACIL EFFECT ON THE ACTIVITY OF *r*II MUTANTS ON *E. coli* KB

	Transmission Coefficient on *E. coli* KB		
	Control	5FU	Response
Standard type			
r^+	0.95	0.88	−
Non-ambivalent *r*II mutants			
*r*131	0.000	0.000	0
*r*1272	0.000	0.000	0
54 mutants at different genetic sites			0
*r*N24	0.000	0.43	+
Ambivalent *r*II mutants of subset 1			
*r*HB118	0.002	0.65	+
*r*HB122	0.002	0.005	0
*r*C204	0.000	0.09	+
*r*N11	0.000	0.10	+
*r*EM64	0.000	0.78	+
*r*HB32	0.000	0.006	0
*r*HB35	0.000	0.37	+
*r*HB80	0.000	0.002	0
*r*HB129	0.000	0.31	+
*r*HB84	0.000	0.41	+
*r*2074	0.001	0.000	0
*r*EM84	0.000	0.47	+
*r*HB74	0.000	0.16	+
*r*NT332	0.000	0.63	+
*r*B94	0.000	0.27	+
*r*HB232	0.000	0.59	+

Measurements of transmission coefficient were made as described in the legend for Figure 3. The assays were made at t = 25 min at which time the maximum effect of 5-FU is reached.

TABLE 4

ALTERATION OF THE RESPONSE PATTERN OF STRAIN KT BY MUTATION

	Bacterial Host Strain	
	E. coli KT	*E. coli* KT-5
Standard type phage		
r^+	100.	100.
Non-ambivalent mutants		
*r*131	0.00	0.00
*r*1272	0.03	0.00
Ambivalent *r*II mutants of subset 1		
*r*HB118	98.	0.00
*r*HB122	42.	0.00
*r*C204	104.	0.00
*r*N11	127.	0.00
*r*EM64	99.	0.00
*r*HB32	19.	0.00
*r*HB35	100.	0.00
*r*HB80	6.3	0.00
*r*HB129	102.	0.00
*r*HB84	106.	0.00
*r*2074	20.	0.00
*r*EM84	79.	0.00
*r*HB74	72.	0.00
*r*NT332	72.	0.00
*r*B94	81.	0.00
*r*HB232	108.	0.00
Ambivalent *r*II mutants of subset 2		
*r*SN86	2.8	1.5
*r*HB309	3.0	1.5
*r*154	3.8	3.2
*r*NB3830	0.3	0.00
*r*N40	0.003	0.00
*r*HB33	1.1	0.4
*r*N89	0.6	0.4
*r*HB8	4.0	1.6
*r*DAP35	1.1	0.2

The table gives the burst size, relative to standard type phage, for various phage mutants on the two hosts. Bacterial strain KT-5 was isolated from KT by treating with ethyl methane sulfonate and screening for resistance to *r*NT332.

14 mutants, or approximately one per 25 sites. It remains to be seen how many subsets can be identified by an exhaustive survey.

Discussion.—The most striking aspect of the effects described is their extreme specificity. Since all *r*II mutants within a given cistron are defective in a unitary physiological function, the change in the host bacterium cannot concern its response to the function *per se*, or it would affect all the *r*II mutants of a cistron similarly. This does happen, for instance, if the bacterium loses its prophage λ, in which case all *r*II mutants become active. Bacterial variants are also known[17] in which the block against *r*II is partially removed, but again this applies to all *r*II mutants.

A review of the literature on suppression and a discussion of various proposed mechanisms has recently been given by Yanofsky and St. Lawrence.[1] One of their suggestions is that specific suppression might, in some cases, be explained by minor changes in the specificities of the system[11, 12] for activating amino acids and attaching them to acceptor RNA. Suppose, for example, that one of the activating enzymes were altered, so that it had some tendency to couple the wrong amino acid to a given acceptor RNA. The result could be the occasional insertion of an amino acid *a* in positions in a protein where amino acid *b* belongs. While such a fault in the translation of the genetic information would tend to reduce to some degree the activity of all enzymes, it could greatly increase the activity of an in-

TABLE 5

ALTERATION OF THE RESPONSE PATTERN OF STRAIN KB BY MUTATION

	Bacterial Host Strain	
	E. coli KB	*E. coli* KB-1
Standard type phage		
r^+	100.	100.
Non-ambivalent mutants		
*r*131	0.00	0.00
*r*1272	0.00	0.00
Ambivalent *r*II mutants of subset 3		
*r*AP129	0.2	45.
*r*AP80	0.2	6.9
*r*HB6	0.03	5.0
*r*106	0.1	2.2
*r*556	0.00	1.0
*r*263	0.02	7.6
*r*1948	0.5	108.
*r*N38	0.2	53.
*r*1221	0.05	5.8
*r*EM16	0.3	32.
*r*A84	1.3	97.
*r*SD80	0.00	7.9
*r*NT284	0.08	14.
*r*EM34	0.00	11.

Strain KB-1 was isolated from KB by treating with ethyl methane sulfonate and screening for sensitivity to *r*AP129. The table gives the burst size, relative to standard type phage, for various phage mutants on two hosts. The behavior of ambivalent mutants of subsets 1 and 2 is unaffected by the mutation from KB to KB-1.

active one in which the defect were due to a mutation of just the right kind, namely, one which has caused amino acid *b* to replace the normal *a*. A similar result might also be effected by modification of one of the acceptor RNA's. This theory would readily explain the specificity of suppression, as well as the case studied by Crawford and Yanofsky[13] in which a mutant synthesized an inactive protein, while the suppressed strain was shown to synthesize both the inactive variety and some of the active form.

As shown here, modification of the bacterium by mutation can lead to an altered response with respect to an entire subset of phage mutations, and the presence of 5FU leads to a similar effect. The high degree of coincidence between the specificity of action of 5FU and a bacterial mutation implies that closely related mechanisms may be involved in both cases. In the light of the observations of Naono and Gros on the alteration of proteins found in the presence of 5FU, it would seem not unlikely that the differences between strains KB and KT and the other bacterial mutants described may reside in slight differences in their mechanisms for translating genetic information. A prediction of the model would be that suppressor mutations, while specific in action on only certain mutations within a cistron, might nevertheless be capable of affecting some mutations in various other unrelated cistrons. This can be easily tested with *r*II mutants on strains of *E. coli* known to contain specific suppressors for one or another bacterial mutation.

In an attempt to obtain definitive evidence for this mechanism, the bacterial strains are being examined for changes in their activating enzymes and acceptor RNA. If changes in the specificity of activating enzymes or acceptor RNA can indeed be identified between bacteria differing by single mutations, experiments of the kind described in this paper may offer a promising approach to a study of the genetic control of the components of the cellular translation mechanism.

Differences in specificity for the acceptor RNA's and activating enzymes of different organisms are known to exist. Berg *et al.*[14] showed that if the acceptor RNA of *E. coli* is first saturated with methionine, using an activating enzyme from yeast, subsequent addition of activating enzyme from *E. coli* leads to attachment of still more amino acid. This experiment shows not only that the *E. coli* and yeast enzymes are different, but also that *E. coli* contains at least two types of RNA molecules capable of accepting methionine. For some of the other amino acids, the differences between *E. coli* and yeast are even more striking: the activating enzyme from either organism will attach amino acid only to its homologous acceptor RNA. This is known to be the case for at least leucine,[15] and for arginine and tyrosine.[16] It does not follow that the different acceptors for a given amino acid also behave differently in pairing with a given template. Nevertheless, in view of these facts, the possibility must be seriously considered that the rules for translation of a specific base sequence in hereditary nucleic acid into a corresponding sequence of amino acids in protein , i.e. the genetic code, may be different in different organisms.

Summary.—The set of *r*II mutants defective on one strain of *Escherichia coli* contains subsets having activity on other strains. The ability to grow, of the various subsets of the *r*II phage mutants, may be altered by mutation of the bacterium. Three distinct subsets have been identified so far, containing, respectively, one per 23, one per 39, and one per 25 *r*II sites. In addition, the phenotypes of certain *r*II mutants can be reversed by the action of 5-fluorouracil, an analog known to be incorporated into RNA. These phenomena are discussed in terms of possible modifications in the cellular mechanism for translating genetic information.

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[1] Recently reviewed by C. Yanofsky, and P. St. Lawrence, *Ann. Rev. Microbiol.*, **14:** 311 (1960).

[2] Benzer, S., these PROCEEDINGS, **41,** 344 (1955).

[3] Lederberg, E. M., and J. Lederberg, *Genetics*, **38,** 51 (1953).

[4] Wollman, E. L., *Ann. inst. Pasteur*, **84,** 281 (1953).

[5] Benzer, S., these PROCEEDINGS, **47,** 403 (1961).

[6] Chase, M., and A. H. Doermann, *Genetics*, **43,** 332 (1958).

[7] Naono, S., and F. Gros, *C.R. Acad. Sci. (Paris)*, **250,** 3527 (1960).

[8] *Ibid.*, 3889

[9] Bussard, A., S. Naono, F. Gros, and J. Monod, *C.R. Acad. Sci. (Paris)*, **250,** 4049 (1960).

[10] Horowitz, J., and E. Chargaff, *Nature*, **184,** 1213 (1959).

[11] Hoagland, M. B., *Brookhaven Symposia in Biol.* **12,** 40 (1959).

[12] Crick, F. H. C., "The Biological Replication of Macromolecules," *Symposium Soc. Expt Biol.*, **12,** 138 (1958).

[13] Crawford, I. P., and C. Yanofsky, these PROCEEDINGS, **45,** 1280 (1959).

[14] Berg, P., F. H. Bergmann, E. J. Ofengand, and M. Dieckmann, *J. Biol. Chem.* (in press).

[15] Rendi, R., and S. Ochoa, *Science*, **133,** 1367 (1961).

[16] Benzer, S., and B. Weisblum, unpublished.

[17] Mahler, I., personal communication.