In the cross \((B_1-;B^+M^+)(T-L-)V_1^S \times (B_1^+;B^-M^-)(T+L+)V_1^R\), \(V_1^R\) is more frequent both in \(B_1^-\) and in prototrophs. It is linked therefore to \((T,L)\). However, it is also linked to Lac, in the other linkage group. There is, therefore, but a single linkage group, of which the only suitable arrangement is: \(B_1..(B,M)\) Lac .. V_1..(T,L) .., the order of the factors within the parentheses being indeterminate from the present data.

Crossover theory

In an attempt to compute map distances from the available data, a crossover theory must be used in which, unfortunately, no correction for chiasma interference can be made. Such interference conceivably may result in large discrepancies between true and estimated values of map distance, particularly in the region \((B,M)\) to \((T,L)\).

As can be seen from the map, Table 6, an interchange between \(B_1\) and \((B,M)\) results in \(B_1+B^+M^+\) ...; lack of interchange in \(B_1-B^+M^+\) ...

The ratio between these two types is 8:79, indicating a proportion of interchange to total of \(8/79 = 9.2\%\). With so small a distance, correction for double or multiple crossovers would be negligible compared to the experimental error. With the formula developed below:

\[
\tanh x = \frac{\text{interchange}}{\text{no interchange}} = \frac{8}{79+8} = 9.2\%
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The estimation of the distance \((B,M) - (T,L)\) requires a detailed consideration of multiple crossing over. Absolute values for the distances \((B,M) - \text{Lac} ; \text{Lac} - V_1 ; V_1 - (T,L)\), or \(a, b, c\), respectively, are not available but only their relative proportions as given by the ratios of the single-crossover types in Table 6.

The values of \(a, b, c\) can however be estimated from their ratios, \(r_a, r_b, r_c\) and the proportion of the "triple crossover" class of Table 6, \(r_d\), since the frequency of multiple crossing over will depend on the absolute map distances.
A recovered prototroph chromatid will fall into the classes a...d according to the distribution of crossover "breaks" in its various segments. Since interchange between B,M and T,L is required to produce a prototroph, only those chromatids with an odd number of breaks in the region a+b+c will be recovered.

The map distance, x, may be defined as (100 X) the mean number of crossover "breaks" in a segment. In the absence of interference, there should be a Poisson distribution of chromatids with varying numbers of breaks, the frequencies of 0,1,2,n, breaks being given by successive terms of the expression: $e^{-x} (1, x, x^2/2!, x^n/n!)$. In this case, only chromatids representing the odd terms of this expansion can be considered, their sum being $e^{-x} (\sinh x)$ which is equivalent to $\frac{1 - e^{-2x}}{2}$. The sum of the even terms is $e^{-x} (\cosh x)$ whence the expression $\tanh x$ for the ratio of interchanges to non-interchanges. It can be shown from the addition formula for $\tanh x_1 + x_2$ that this formulation is equivalent to Haldane's addition formula $x_{12} = x_1 + x_2 - 2x_1x_2$.

The expression $e^{-x}\sinh x$ also applies to the chances of interchange in any segmental part of $x$, e.g., a, b, c. We have then, the four expressions following:

\[
\frac{(e^{-a}\sinh a)(e^{-b}\sinh b)(e^{-c}\sinh c)}{e^{-x}\sinh x} = r_d = .020
\]

\[
\frac{(e^{-a}\sinh a)(e^{-b}\cosh b)(e^{-c}\cosh c)}{e^{-x}\sinh x} = r_a = .264
\]

and so forth.

Since $e^{-a}.e^{-b}.e^{-c} = e^{-x}$ appears in both numerator and denominator of each of these expressions, they can be cancelled out leaving only the hyperbolic terms:

\[
\frac{\sinh a. \sinh b. \sinh c}{\sinh x} = .020
\]
\[
\begin{align*}
\sinh a \cdot \cosh b \cdot \cosh c &= \frac{.264}{\sinh x} \\
\cosh a \cdot \sinh b \cdot \cosh c &= \frac{.448}{\sinh x} \\
\cosh a \cdot \cosh b \cdot \sinh c &= \frac{.268}{\sinh x}
\end{align*}
\]

While the solution of this system of equations would provide a theoretically exact solution for \(a, b, c\) and \(x\), we are here concerned primarily with the estimation of \(x\), and this can be more readily obtained with the help of certain approximations. In particular, \(a\) may be taken as \(s_a x\), where \(s_a\) is \(r_a/r_a + r_b + r_c\), i.e., the fraction of the "single" crossover types represented by \(a\). This is not exact insofar as the proportions of the types \(a, b, c\) will not be directly related to the distances \(a, b, c\) in all cases, the contribution of the triple-crossover, single-interchange, types being as the 3rd powers of the distances, etc., rather than the first power.

The result of this approximation is the equation in one variable:

\[
\frac{\sinh s_a x \cdot \sinh s_b x \cdot \sinh s_c x}{\sinh x} = .020,
\]

which can be solved by successive approximations. The solution is \(x = .80\), or 80 units (morgans). \(a, b, c\) are then 21\(+, 36\(+, and 22 units respectively.

As a check on the approximation used, the result of substituting \(a = b = c = x/3\) may be considered. This leads to the equation:

\[
\frac{\sinh^3 x/3}{\sinh x} = .020, \quad x = .75, \text{ which considering the crudity of the approximation is in good agreement.}
\]

On the other hand the application of the uncorrected formula \(\left(\frac{x}{3}\right)^3 = .020\) gives the result, still lower, \(x = .73\). This corresponds to counting single-crossovers only in the \(a, b, c\) types, and taking the three regions as equal in length.
The values already cited: 21+, 36+, and 22+ will be taken as representing the best available estimate of the three regions.

Interference, by shifting the distribution of crossover types towards the lower values, would be expected to diminish \( r_d \) and therefore lead to a low estimate of \( x \). However, interference would also tend to cause a spacing of crossovers, increasing the likelihood that where there are, for example, three crossovers, one shall be found in each segment and lead to a "d" type. With a random distribution of crossovers, there is only about 1 chance in 5 that 3 crossovers will be distributed 1:1:1:1; by interference this figure could conceivably be increased to 1/1, which would compensate for a fivefold bias against triple-crossovers compared to single-crossovers for a given value of \( x \). Interference has not yet been sufficiently analysed in other organisms to permit of any more direct evaluation of the extent to which these effects will cancel each other. It would clearly be desirable to find other means of estimating these distances, perhaps by the use of biochemical markers located in the left hand region of the map. A comparison of the results might give direct information concerning the possible role of interference.

Linearity

In constructing a map, and calculating distances, it has been taken for granted that there is in \( E. coli \) a system of linear linkage, such as has been demonstrated quite conclusively in \( Drosophila \), and inferred in all higher organisms. What direct evidence may one bring to bear on this question?

The method which one is forced to employ in hybridizing this bacterium introduces certain complications. The classical proof of linearity is based on the additive character of distances, expressed in morgans, between loci occurring within the same linkage group. The determination of map distances is based upon a comparison between
parental and new combinations of linked genes, as determined in the progeny of zygotes selected at random. In *E. coli*, on the other hand, one is limited to the recovery of that recombination class in which there has necessarily been an interchange between certain biochemical loci, in the cases here discussed, between (B,M) and (T,L).

The data analysed above, concerning the segregations of Lac and V cannot be used for a demonstration of linear order without an error of circular reasoning. This is shown by the indeterminacy of interference which affects very vitally the linear additive properties of adjoining crossover segments.

The bearing of the reversed crosses tabulated in Tables 3 and 4 has already been mentioned. They illustrate the combinatorial character of the segregation mechanism but do not specify it more closely. For example, one might postulate that genes of bacteria are embedded in an n-dimensional matrix, which is ordinarily conserved, but which occasionally permits of a gene-for-gene interchange. This is equivalent to the "Konversion" theory once proposed by Winkler (1932) as an alternative to crossing-over theory in higher organisms, and which has been revived most recently, in modified form, by Lindegren (1947). The Konversion theory is made untenable by evidence for the interaction of different interchanges, for example, between Lac and V as already cited. The Konversion theory can be made to fit such results only by making it experimentally indistinguishable from the classical crossing-over theory.

The interactions between interchanges also exclude similar matricial theories where the units are perhaps not single genes, but blocks of them - for example a multilinear radial arrangement. At least for the genes involved in such interactions, one is forced to conclude that they are in a continuous segment. Other genes, as yet unstudied, of course might be shown to be placed on branches or
other bizarre modifications of the chromosome, but thus far no need for such exceptions has arisen.

Additional, perhaps more direct support for the linear order of genes is provided by data on the segregation of \( V_6 \) summarized in Table 8. It will be noted that the segregations of \( \text{Lac}, V_1 \) and \( V_6 \) are congruous between the \( B_1^- \) and \( B_1^+ \) classes (the latter in the sense discussed on p. 72). In the totals of "prototrophs" isolated from \( B-K-T+L+B_1+\text{Lac}+V_1^T V_6^S \times B+M+T-L+B_1^-\text{Lac}-V_1^S V_6^P \), one finds \( \text{Lac}^- 78\% \), \( V_6^P 82\% \), and \( V_1^S 36\% \), indicating that, as in previous experiments, \( \text{Lac} \) is linked to \( (B,M) \) and \( V_1 \) to \( (T,L) \). In addition, \( V_6 \) is linked to \( (B,M) \) somewhat more intensely perhaps than is \( \text{Lac} \). Since linkage to \( B_1 \) is already eliminated, one would predict from these totals, on the hypothesis of linearity, that \( \text{Lac} \) and \( V_6 \) should be linked, with \( V_6 \) to the left of \( \text{Lac} \).

The data of Table 8 confirm this prediction. The parental couplings of \( \text{Lac} \) and \( V_6 \) are \(-r \) and \( +s \) respectively. Of \( 137 \) \( \text{Lac}^- \), \( 134 \) were \( V_6^P \); of \( 39 \) \( \text{Lac}^+ \), \( 29 \) were \( V_6^S \). The order \( V_6, \text{Lac}, V_1 \) is also supported, since the four most frequent types are those corresponding to single-crossovers on this basis, while they would include multiple-crossover classes with any other order.

Since \( \text{Lac} \) and \( V_1 \) are segregating, the totals for the four combinations of these two factors can be compared with those of previous experiments. A 4x2 table comparison with the corresponding cross, Table 5, row 1, gives \( \chi^2 = 19.6 \) for three degrees of freedom, while this would be an exceedingly poor agreement if a normal distribution obtained, an analysis of variance by means of the variance ratio shows that \( p = .05 \) that the discrepancy can be accounted for in terms of the variance of the replicated experiments.

The first 8 factors tested, \( B,M,T,L,B_1,\text{Lac}, V_1, \) and \( V_6 \) have been shown to belong to the same linkage group. It is, there-
fore, extremely likely that there is but one linkage group in E. coli, the chances that another of the same magnitude exists being 2⁻⁷, or .008. There is no cytological evidence to suggest more than one chromosome in E. coli. No other genetically investigated organism has so few linkage-groups. Cytologically, the nearest analogue is perhaps the compound chromosome of Ascaris megalcephalus v. univalens, 2n = 2.

Attempts to Induce Aberrations

Using a chromosomal theory as a working hypothesis, it was hoped that some verification could be found by the study of types in which the normal order of genes was disturbed. Since there is only one chromosome (from the genetic evidence), the only types of rearrangements would be changes leading to a series of inversion types. It was thought that such types might be detected by genetical procedures, by virtue of their effect on crossing over. In particular, the occurrence of an inversion in the region B₁⁻ (B,M) would be expected to have the effect of eliminating the recombination classes involving interchanges in this region. In the cross B-M-T+L+B₁+ x B+M+T-L-B₁⁻ this would be equivalent to the suppression of prototroph recombinants; B₁⁻ types, however, would be recoverable, and allow the investigation of the extent of the changes.

Preliminary attempts to find such aberration types have, to date, been unsuccessful. The procedure was as follows:

Following treatment with nitrogen mustard or 20,000 r of x-rays, cells of Y-40 and of Y-55 were incubated separately for 24 hours, to allow the separation of cells or nuclei that might have been associated at the time of treatment. The cultures were then streaked out on nutrient agar plates. Single colonies of Y-40 were picked and streaked across a nutrient agar plate. Streaks of similarly treated Y-55 colonies were made from the opposite direction,
so that in the center of the plate, cells of the two types were mixed, treated colony by treated colony. The occurrence of colonies which would not interact to produce prototrophs, as detected by plating into minimal medium, would be an indicator that the combination was heterogeneous for an aberration. Since in these experiments, both "parents" were exposed to treatment, each plating was equivalent to the testing of two chromosomes, for the occurrence of an aberration. No marked variation in the yield of prototrophs was noted in tests involving 121 mustard and 28-X-ray-treated chromosomes. This can scarcely be regarded as an adequate sample in view of the stringent selection imposed by the technique, which might be expected to eliminate any aberration types which are even slightly less vigorous than the normal. This consideration is especially relevant in view of the "hemizygous" condition of any aberrations in the probably haploid vegetative cells. These studies will be continued.

**How Many Segregants per Zygote?**

In the experiments detailed in this paper, recombinants were obtained from different cell types which were first exposed to each other in an agar medium. Therefore, each prototroph recombinant colony seen by the experimenter marks the site of formation of a zygote. The question may immediately be raised whether there are at that site other recombination classes which, by virtue of their biochemical deficiencies, may not proliferate within the prototroph colony on the minimal selective medium. This is equivalent to inquiring whether there is but a single viable product of meiosis (as in megasporogenesis in many higher plants) or more than one, as in ascomycetes. The solution to this problem would be of special interest in relation to the possible occurrence of four-strand crossing over. In addition, if an appreciable proportion of prototroph colonies consisted of two distinct segregation types,
it would be necessary to isolate these types for the collection of segregation data.

There are at least three ways in which a zygote might yield more than one haploid recombinant. Firstly, the zygote might be capable of proliferation in the diplophase (or sporophyte), leading to the occurrence of several diploid cells, each of which might undergo meiosis independently, and by chance yield several segregation types. Secondly, a single zygote might produce, after meiosis, in addition to the prototroph, the complementary multiple mutant class. Thirdly, in a system of four-strand crossing-over, there might be two supplementary prototroph recombinants differing in the segregation of factors such as Lac and $V_1$ for which the diploid was heterozygous.

Obviously, the proper investigation of these possibilities requires that one stringently avoid contamination of one colony with another. For this reason, the cell-suspensions used were diluted so as to yield only about 5-10 recombination colonies per plate.

Crosses were made between Y-40 and Y-53 ($B\cdot M\cdot T\cdot L\cdot B_1\cdot Lac\cdot V_1^P$ x $B\cdot M\cdot T\cdot L\cdot B_1\cdot Lac\cdot V_1^S$) on $B_1^-$ containing minimal agar medium. As already noted, about 90% of the colonies from such a cross are $B\cdot M\cdot T\cdot L\cdot B_1^-$. The theoretical complementary class would be $B\cdot M\cdot T\cdot L\cdot B_1^+$. Because of its nutritional deficiencies, it could not be expected to proliferate on the minimal medium even had it been produced after meiosis. The possibility remains, however, that a few cells of this constitution might still be present among the $10^8$ or so $B_1^-$ cells of the predominant type in a colony. By plating such colonies into medium lacking $B_1$ but containing biotin, methionine, threonine and leucine, the $B_1^-$ cells would be suppressed, while the postulated multiple mutant type could form colonies and be recovered.

The experiment just described was carried out, testing 52 colonies for their content of other cell types. In general, a
thinless colony could be shown to contain from 10-100 cells capable of forming colonies on the D.W.T.I. medium. However, in each case investigated these have been shown to be indistinguishable from the Y-40 parental B-\(\Phi\)-type, and must be presumed to arise from a surprisingly low degree of contamination of the colony with these cells from the heavily seeded plate. A few colonies were found which could be characterized as reversions from \(B^-\) to \(B^+\). These experiments are, then, inclusive with respect to the occurrence of complimentary genotypes in the same colony. With appropriate stocks, not as yet available, it should eventually be possible to manipulate the situation so that the complementary type could be recovered selectively, excluding both parents and the dominant recombination class.

A search for supplementary types was conducted with the same crosses, except that colonies appearing on \(B^-\) agar were streaked out directly on EMB-lactose agar to determine whether any of them were heterogeneous for this factor. In some cases, a number of isolated colonies from each EMB-test plate were then also tested for homogeneity with respect to Ti-resistance. About 90 colonies were so tested; only 1 colony was found containing both Lac+ and Lac- cells. It is impossible to be certain that, with this low frequency, the single colony which was picked actually was derived from two distinct zygotes. These experiments cannot be considered as bearing critically on the question of the occurrence of two- or four-strand crossing over because of the absence of information concerning the viability of more than one meiotic product.

**Diploidy.**

The segregation of characters observed between prototroph recombinants strongly suggests the haploid condition of *E. coli*, with recombination immediately following the zygotic fusion. If this condition could be modified so as to yield stable diploid variants, which,
using with haploid cells, might yield zygotes showing tri-somic segregation, many questions concerning centromere relations, and the number of strands as meiosis, as well as the dominance or recessiveness of particular characters, could be studied. Because of the difficulties of cytological examination, a genetic test was devised which, it was hoped, would detect stable diploid variants.

In 1913, Penfold, described a peculiar response of *E. coli* to sodium chloroacetate. Wild type strains appeared to be inhibited by this agent, but gave rise to resistant mutants, which appear as papillary or button-like projections from the inhibited growth on nutrient agar containing 0.1% sodium chloroacetate. The resistant mutants were peculiar insofar as they lacked the capacity to form gas from glucose, although abundant acid was formed.

These findings were confirmed with *E. coli*, K-12. In addition, it was found that while the resistant mutant, Cla<sup>R</sup>, could form gas from formate, it could not from pyruvate. The presently accepted scheme for the formation of gas from carbohydrates by *E. coli* involves the splitting of pyruvate to acetate, or similar C<sub>2</sub> fraction, and formate. The formate is then decomposed to CO<sub>2</sub> + H<sub>2</sub> by the "formic hydrogenlyase" complex. Since the capacity to form gas from formate is intact, while that from pyruvate is impaired, it may be assumed that there is a correlation between Cla<sup>R</sup> and the enzymatic splitting of pyruvate. While other interpretations are perhaps not ruled out, this was adopted as the most likely explanation, and is the basis for what follows.

On a priori grounds, and from the work of Beadle and Coonradt on *Neurospora* heterocaryons, it is likely that the ability to perform a reaction will generally be dominant to the inability. Cla<sup>S</sup> therefore should be dominant in a diploid heterozygote to Cla<sup>R</sup> with respect to enzymatic function. If the correlation between
enzymatic function and resistance is maintained, Cla\textsuperscript{S} will also be dominant with respect to resistance, i.e., the combination Cla\textsuperscript{S}/Cla\textsuperscript{R} will still be sensitive to chloroacetate. Starting from the diploidised wild type, Cla\textsuperscript{S}/Cla\textsuperscript{S}, it will require two mutations to produce the resistant type Cla\textsuperscript{R}/Cla\textsuperscript{R}. On this basis, a diploidised E. coli should yield mutations to the phenotype of resistance to chloroacetate only with extreme infrequency compared to the normal haploid form. Since one need merely streak out cells of a suspected diploid type on chloroacetate agar, and record the development of papillae, this working hypothesis provides a possible tool for the detection of diploids. Unfortunately, none have yet been found among some dozens of tests of camphor- or acenaphthene- treated material. This matter was discussed primarily to illustrate a possibly very fruitful line for further research, particularly from the point of view of the possibility of cytogenetical correlations.

Transformation. Experiments designed to extract transforming factors from cells of E. coli were mentioned in an earlier section. The failure or such experiments is in line with the genetical properties of the recombination system, linkage, etc., but cannot be regarded as conclusive for the exclusion of diffusible transforming factors. The methods used may have been too delicate to extract appreciable quantities or too rough to preserve what was extracted. There is, however, a further type of genetic experiment which bears on the possibility of transformation via soluble substances.

A glance at Table 7 shows that some "multiply-transformed" classes are more frequent than those involving changes of but one or two loci of one of the parents. On the transformation hypothesis, this might be interpreted in terms of the non-uniform susceptibility of different cells to transformation, so that wherever it takes place at all, it is likely to affect several genes. Under these
conditions, one would anticipate that susceptible cells might be influenced simultaneously by a mixture of transforming factors. On the transformation hypothesis for the exchanges described in this paper, it would be equivalent to predicting tri-parental recombinations in mixtures of three genetic cell-types.

A sexual mechanism has rather different consequence. Among other sexual organisms, biparental inheritance is the rule, barring the dubiously relevant exception of certain multiporic embryo-sac types in the angiosperms. In mixtures of three genetic types, only those types of zygotes may be inferred which result from pairwise fusions of cells. Zygote formation is so infrequent that the coincidence of successive fusions of the segregants of a Type 1 × Type 2 zygote with a Type 3 gamete has a negligible likelihood.

This critical point of difference was subjected to experimental test in the following way. The same biochemical parents were used as before, namely B-M- and T-L-B₁-. Lac and V₁ alleles were distributed among these parents in various combinations. For example, the types B-M-Lac-V₁⁺; T-L-B₁-Lac-V₁⁺; and T-L-B₁-Lac⁰V₁⁺ were used. Suspensions of these types were prepared and all three mixed together in a manner analogous to that already described for normal, pairwise crosses. From such combinations, prototrophs or B₁⁻, other factors prototrophic, could be produced only by recombination between the B-M-parent and one or the other of the T-L-B₁- parents. By biparental inheritance, in this case, only three of the possible combinations of Lac and V could appear among the prototrophs: Lac-V₁⁺; Lac-V₁⁺; Lac⁰V₁⁺. If a ménage à trois were permissible, however, the fourth type Lac⁰V₁⁺ should be found also.

By using different combinations of alleles, the experiment may be varied so that a different class becomes the exceptional in each case. In Table 9, the results of four experiments, so constructed
that a different class should be lacking on the basis of biparental inheritance in each experiment, are set down. It will be seen that no exceptional types appeared in a total of 628 tests. It may be concluded that genetic factors from different cells are not freely miscible, as would be demanded by the simplest versions of transformations. On the other hand, gene recombination is restricted in any instance to exchanges of genetic material between two cell types.

The results of this experiment are also a check on spontaneous mutation as the source of what have been claimed to be recombinations. On the spontaneous mutation hypothesis there should be no discrimination against the exceptional types which were not found in these experiments.

The genetics of bacterial transformations is still in an exceedingly primitive state, and there is no information concerning the occurrence of interactions in bona fide transforming systems. If transformation is to account for the results of the present experiments it will have to fulfill the following conditions: a) non-independence of factors, simulating a linkage group; b) potential capacity of carrying all the genetic factors of the donor in a single parcel; c) immiscibility of parcels derived from different cells. From a genetic point of view, such a transforming factor would be indistinguishable from a gamete, and its definition would be based on chemical properties only. It will be recalled, however, that Muller (1947) has interpreted the pneumococcus transformation in similar terms: "still viable bacterial chromosomes or parts of chromosomes floating free in the medium.....these have penetrated the capsuleless bacteria and, in part at least, taken root there, perhaps after having undergone a kind of crossing-over with the chromosome of the host."

Further genetic work on transforming systems will be required to substantiate this interpretation, particularly in view of the
relatively low molecular weight, 500,000, which has been ascribed to
the pneumococcus factors. (Avery et al. 1944).

Other E. coli strains

In an attempt to find how generally the ability the recombine
is distributed among bacteria, studies were made on two other strains
of E. coli. These were designated B/r and L-15. B/r, obtained through
the courtesy of Dr. E. Witkin, is a radiation-resistant mutant of
strain B. Both B and B/r have been used extensively in studies on
mutation from phage-sensitivity to phage-resistance. L-15 is a strain
used by Roepke, Libby and Jones (1944) for the production of biochemical
mutations. A variety of biochemical mutants of L-15 were obtained through
the courtesy of Dr. Roepke. Double mutants of B/r, arginine-methio-
nineless and histidine-p-aminobenzoateless, were obtained from ultra-
violet treated material by previously described techniques for the
isolation of mutants, Lederberg and Tatum, (1946a). All combinations of
the T-L-B1- mutant, Y53, of K-12, and of the mutants of B/r and of L-15
were made and plated into minimal medium as already described. In no
case was there any suggestion of the formation of prototrophs within
L-15 or B/r mutants, between them, or with K-12. It is recognized that
the conditions for recombination in those strains may differ from K-12,
or that there may be genetic conditions of mating type. It was esti-
mated that recombination would have been detected had it occurred with
a frequency of not less than 10^-3 of that found in K-12 mutants.

Serious cytological studies seeking to identify the zygote
in K-12 have not been attempted in view of the futility of attempts
to characterize and verify so rare an occurrence. The burden of this
investigation has been the verification of the recombination of genes
in a bacterium, and the elucidation of some of its genetic properties.
The way is open for considerable further work, using recombination as
a tool of genetic analysis, and to the more detailed picturization of
process in space and time.

Discussion

Although a transformational interpretation of these experiments has not been excluded beyond any shadow of doubt, it makes little difference for most purposes whether one adheres to invisible zygotes as against unextractable transforming factors. The techniques described should be useful in either case toward the solution of genetic problems in bacteria. Many of these are discussed in the Cold Spring Harbour Symposium for Quantitative Biology, Volume 11, 1946, which deals with the genetics of microorganisms. These problems include the genetic nature of phenotypically complex mutations, the verification of reverse mutation as the basis of genotypic reversion, the site of interaction of certain mutations involving glycolytic enzymes, the genetic basis of antigenic variations, the verification of the "one-to-one" theory of the relationship between genes and enzymes in bacteria, and in general, any instance where it is required to test the allelism of two or more genetic variations.

Genetic recombination has, of course, a far broader meaning in biology than as a laboratory tool. The recombination of mutations is a source of variation that may be of crucial importance in the evolution of new "adaptive peaks". While this statement, in its general terms is indubitable (see Dobzhansky 1941) recombination can only effect the reshuffling of preexistent mutations. Concerning the natural historical significance of the latter for bacteria, we are in a state of woeful ignorance, so that we are hardly in a position to discuss the significance of bacterial recombination in concrete terms. To this must be added the caution that genetic combination was found, luckily, in one E. coli strain, and not in two others which were studied.
It may be wondered at that the apparent recombination rate is so low. It will be recalled that about $10^{-6}$ of the cells insculated in the cross $B-M-T+L+B_1^+ \times B+M+T-L-B_1^-$ showed interchange in the region $(B,M) - (T,L)$. Since the estimated map distance is 80 units this is also the correct order of magnitude of the fusion process. However, this is possibly not to be ascribed to any sexual imperfections of the colon bacterium, but to the method of enumeration. It seems likely that an analogous comparison of the number of somatic and generative cells in a higher plant, or the ratio of perithecia to total nuclei in a fruiting culture of Neurospora would not give very different ratios. It is also possible that the optimal conditions for zygote formation and germination have not yet been achieved and that by special procedures the rate of fusion may be accelerated to the level where there might be some hope of trapping it in the field of the microscope.

Since the mutants used in the recombination experiments were derived from the same wild-type strain, there can be no question of genotypic mating type determination. The failure of two *E. coli* strains to exhibit recombination might conceivably be ascribed to genetic heterothallism, such as has caused many fungi to be classified as "imperfect". The chances of finding the appropriate mates are of course very slim, but should not be entirely overlooked.