GENETIC RECOMBINATION IN *ESCHERICHIA COLI*.

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ABSTRACT

Wild type or "prototroph" cells were found in a proportion ca. 10^{-6} in mixed cultures of biochemical mutants of *E. coli*. It could be shown that these did not result from spontaneous back-mutation. In addition to wild types, other recombination types were found suggesting the occurrence of a sexual process in *E. coli*. The analysis of the segregation of factors for lactose fermentation and for resistance to bacteriophage led to the conclusion that a single linkage group was present, on which eight factors have been mapped.

Experiments confirming the linear order of genes, and biparental inheritance were performed. It was concluded that *E. coli* is normally haploid and that it undergoes sexual fusion with immediate reduction-division, during which crossing-over takes place. The alternative explanation that the apparent recombinations are due to soluble transforming factors is discussed in detail and shown to be uneconomical. Due to the rarity of the process cytological examination was unfeasible. Attempts to reveal recombination in two other strains of *E. coli* were unsuccessful, as were attempts to induce aberrations in the chromosomal arrangement or the ploidy of the species.

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The study of inheritance in bacteria has, for the most part, been confined to the investigation of mutational changes in the course of clonal reproduction. With the exception of experiments on pneumococcus type transformations there have been few studies on the direct hereditary interaction of one bacterial type with another. The conception that bacteria have no sexual mode of reproduction is widely entertained. This paper will be devoted to the presentation of evidence for the occurrence in a bacterium *Escherichia coli*, of a process of gene recombination, from which the existence of a sexual stage may be inferred.

The genic basis of microbial inheritance does not depend on the demonstrability of a sexual phase in bacteria. However, more powerful genetic methods paralleling classical Mendelian analysis would be available if it were possible to follow the inheritance of characters in the products of a sexual fusion. The few examples of this approach thus far reported have provided no incontrovertible evidence for sexual reproduction in bacteria.

The phenomenon of paragglutination in the colon-typhoid-dysentery group might be regarded as an instance of bacterial hybridization, and was so interpreted by Almquist (1924). As reported by numerous authors, paragglutination refers to the development of new types which react with antisera for each of two distinct strains, when these are grown together in mixed culture (Kuhn and Ebeling 1916; Salus 1925; Wollman and Wollman 1925). The significance of these observations has been attacked by several authors (Breinl 1921, Arkwright 1930, and Kauffmann 1941), chiefly on the grounds that the paragglutination represents a non-specific cross-reactivity characteristic of "rougher" phases of these organisms. Hansen (1929) failed to obtain paragglutination in her experiments. In the light

of more detailed recent information on the antigenic structure of this group, this problem certainly deserves a critical reinvestigation

Sherman and Wing (1937) have described experiments designed to detect recombinations of fermentative characters in mixed cultures of various E. coli and Aerobacter aerogenes strains. While new combinations of biochemical characters were found, similar types were found to an equal extent in pure cultures, so that these authors could not infer the occurrence of a sexual fusion. Their experiments are of the greatest interest, however, since they represent the first attempt to study this problem in bacteria by genetic methods using clearcut characters. Gowen and Lincoln (1942) later performed similar experiments with strains of Phytomonas stewartii using cultures differing in morphological and pigment characteristics. As in Sherman and Wing's studies, these authors were unable to differentiate the new types they found in their mixed cultures from types which arose spontaneously in single cultures. For this reason a definite conclusion could not be drawn from their results.

A discussion of hereditary processes in bacteria must take into account the extensive work on transformation of pneumococcal types, first described by Griffith (1928), and culminating in the isolation of the transorming principle in chemically characterizable form by Avery, MacLeod and McCarty (1944). These studies have revealed that under special experimental conditions, a product isolated from a serologically specific, smooth, pneumococcus culture will convert cells of a non-specific rough culture to the smooth type characteristic of the source of the transforming principle. So far as is known, such transformations can be performed in only one direction (rough to smooth) and only under very special conditions. Boivin (1947) has reported a similar transformation involving the somatic polysaccharide of a strain of E. coli. There have been

reported other instances of varying credibility (Kasarnowsky 1926; Lommel 1926; Legroux and Genevray 1933; Frobisher and Brown 1927; Burnet 1925; Holtman 1939, Cantacuzène and Bonciu 1926). These studies have a direct bearing on recombination experiments, since transformations of this sort might be responsible for the occurrence in mixed cultures of some new types which are interpretable as recombination types. This will be discussed in more detail later.

Morphologically unusual forms of various bacteria have been described by Mellon (1925) as zygospores, and been taken to imply sexual fusion. Smith (1944) and Dienes and Smith (1944) have suggest that the "Large Bodies" observed in some _Bacteroides_ cultures might represent a sexual phase, and Dienes (1946) has made a similar interpretation of Large Bodies in _Proteus._ Star-shaped aggregations of _Phytomonas tumefaciens_ have been studied by Stapp (1942), and more recently by Braun and Elrod (1947) and provoked all of these authors to the suggestion that the stars represented some sort of sexual fusion. As Luria (1947) has pointed out, however, "most of the older material presented in support of the hypothesis of sexuality in bacteria cannot be used as genetic evidence because of the lack of information on the exchange or recombination of discrete hereditary characters in the course of the supposed sexual fusion."

In the absence of zygote-segregation methods, the only techniques available for analysing the genetic structure of bacteria have been mutation analyses. The bacteriological literature is full of reports of bacterial variations, or so-called "dissociations" referring to the development within previously pure clones of new and distinctive types. While these are superficially very similar to the mutations occurring in higher organisms, many bacteriologists (e.g., Rahn, 1937) have rejected a mutational interpretation of bacterial variation, chiefly on the grounds that the variants are

often capable of reverting to the original form. This objection is without substance in view of the commonplace occurrence of reversion both in *Drosophila*, (Timofeef-Ressovsky 1937), and *Neurospora*, (Ryan and Lederberg 1946).

Another distinction that is deeply implanted in classical bacteriology is that the germinal material of bacteria is thought to be capable of direct adaptive modification in response to deleterious conditions, whereas this Lamarckistic viewpoint has now been virtually eradicated from the thinking of students of heredity in higher forms.

Proof that adaptive changes in bacterial populations are due to pre-occurring spontaneous mutations and their subsequent selection by the deleterious environment has not readily been attained. Luria and Delbrück (1943) have, however, studied the occurrence in *E. coli* of phage-resistant variants of which are detected by the application of a specific bacteriophage (T1) to a sensitive population. On the assumption that the resistant variants develop in response to the application of the phage, there should be no greater variation in the number of resistant cells which can be elicited by the phage in similar samples taken from a series of similar cultures than there would be in the case of similar samples taken from the same culture. On the other hand, it was predicted that there would be a wide variation in the number of mutants demonstrable in a series of separate but similar cultures if these mutants arise spontaneously previous to the application of the phage, which acts simply to demonstrate them. This increased variance arises from the fact that the occurrence of a mutation early in the growth of a culture will lead to the occurrence of a large clone of mutant descendants, whereas a mutation which fortuitously occurs later in the growth of the culture will have only a few mutant descendants.

Although an exact mathematical solution of the problem has not appeared, the variance found in Luria and Delbrück's experiments was much larger than could be explained on the direct adaptation hypothesis, and was in good accord with the predictions of an approximate mathematical theory which they developed.

In a similar way, it has been shown that a number of "adaptive" changes in bacteria are the result of spontaneous mutations occurring previous to the application of the agent used to select them. These include, in addition to mutation of *E. coli* to phage resistance as already discussed, resistance to additional phages, (Demerec and Fano, 1945), resistance of *Staphylococcus* to penicillin (Demerec, 1945) and sulfonamides (Oakberg and Luria, 1947), resistance to radiation in *E. coli* (Witkin, 1947), and nutritional adaptations of *Clostridium septicum* (Ryan, et al 1946) and of mutants of *E. coli* (Ryan and Lederberg, unpublished.) Recently published examples of presumably direct adaptive mutations either have not excluded entirely the possibility of previous mutation and selection, as in the adaptation of yeast to pantothenate synthesis, (Lindegren and Raut 1947) or have not full demonstrated the heritable character of the modification, as in the adaptation of *E. coli* to resistance to 2-chloro-p-aminobenzoic acid. (Strandskov, 1947). One may conclude, then, that as in the higher organisms, the germinal determinants of bacteria are not in direct adaptive rapport with the environment.

Additional evidence of the overall similarity of bacterial genes to those of other organisms is contained in the experiments on the induction of phage-resistance mutations by Demerec and Latarjet (1946). They reported that X-rays induced mutations of *E. coli* to T1-resistance, and that the mutations induced were linearly proportional to the X-ray dose. The linear type of response furnishes strong support, on the basis of the "hit-theory" for the

occurrence of a single localized determinant in the cell whose inactivation leads to the mutation.

Finally, by the use of X-rays, and other mutagens, Tatum (1946) has obtained nutritional mutants of *E. coli* which are in every way analogous to such mutants in *Neurospora* where their analysis by orthodox genetic methods has shown them to be effects on single genes. Other investigators have produced similar mutations in *E. coli* (Roepke, et al, 1944) and other bacteria (Burkholder and Giles, 1947; Gray and Tatum, 1944). The mutants obtained by Tatum have subsequently been utilized to demonstrate factor recombination, as will be described in this dissertation.

Materials and Methods

The strain selected for these experiments is Escherichia coli, K-12. It was originally isolated from human feces a number of years ago, and has been carried on agar slants at Stanford University since that time. It was used there as a typical E. coli for demonstrations in student laboratories. It has been found to ferment lactose, to produce indole, and to be susceptible to each of the E. coli phages, T1 to T7, collected by Demerec and Fano (1945). Since it ferments sucrose very slowly, if at all, it would be classified as Escherichia coli "commune". It is moderately motile as determined by hanging-drop observations, and by its slow movement through semi-solid gelatine-agar.

Mutant strains of E. coli characterized by specific growth factor requirements have been obtained after treatment with x-rays, ultra-violet light and nitrogen-mustard. Such strains have been described by Tatum (1945, 1946) and by Roepke (1944). A single nutritional requirement is established at a single mutational step, and on the basis of studies on Neurospora is regarded as based on a change in a single gene. By successive treatments, multiple mutant strains with several genetically and biochemically independent nutritional requirements have been produced. The strains used in these experiments are described in Table 1. In general, the nutritional characteristics of a strain are ascertained by inoculating media consisting of the basal medium plus various supplements; lack of visible growth in the absence of a given growth factor and optimal growth in its presence are the criteria for the determination of the nutritional requirements of a strain.

A mutant strain can be signified by suffixing a "-" sign to the initial of the substance in question; e. g. B-Pa-C- refers to a strain which is deficient in the synthesis of biotin,

phenylalanine and cystine. On the other hand particular emphasis can be placed on the ability of a strain to synthesize a particular growth factor by suffixing a "+". Thus B-Pa-C-T+L+B_1+ would refer to a strain deficient in the three factors mentioned above, but capable of growth in the absence of threonine, leucine, or of thiamin. The representation of a growth factor requirement by a minus sign is justified by the *a priori* consideration that a mutation establishing a growth factor requirement generally represents the loss of a function, and by the experimental finding of Beadle and Coonradt (1944) that wild are dominant to mutant genes in *Neurospora* heterocaryons. Strains which are "+" for all growth factors have been called prototrophs (Ryan and Lederberg, 1946) since this is the nutritional condition of the parental wild type *E. coli* strain from which all the mutants were ultimately derived.

Mutations for resistance to specific bacteriophages or bacterial viruses have proven to be exceedingly useful. They are readily obtained as spontaneous mutants by plating a large number of sensitive bacteria with the particular virus in question; only resistant mutants escape lysis and may be recovered as "secondary" colonies (Fig. 1). Resistant mutants are readily freed from residual virus by serial single colony isolation. Resistance to a given virus may be scored by streaking a loopful of bacteria on an EMB or nutrient agar plate at right angles to a previous streak of the virus suspension (Fig. 1).

It has been found, however, that mutations for resistance to a given virus are not entirely specific, but that resistant mutants display "cross-resistance", i. e., are also resistant to other viruses, Demerec and Fano 1945). For example, most T1-resistant types are also resistant to T5 (For the nomenclature of the bacterial viruses used in this investigation, and a detailed

account of the cross-resistance patterns of another strain of E. coli B, see Demerec and Fano (1945)). The cross-resistance patterns of K-12 are similar to those of E. coli B with the exception that T1-resistant mutants which are sensitive to T5 are not tryptophaneless, as have been reported by Anderson (1946) for the corresponding mutants of E. coli B. In this paper, the designation V_1^r will be used for the more frequent T1-resistant mutant, which is also resistant to T5.

The specificity of fermentation reactions of various bacterial species is a clear indication that the ability to ferment certain sugars has a distinct genetic basis. Because they would be so easily scored on indicator media, mutants unable to ferment various sugars have been looked for. Particular attention was paid to the possibility of isolating "lactose-negative" or "Lac-" mutants, because of the taxonomic significance which has been attached to this character.

The detection of fermentation mutants is readily accomplished by the use of indicator media. The medium "EMB-lactose" used in routine bacteriological work was found to be highly useful. It consists of the following: in G./l., Peptone (or "N-Z-Case") 10, Yeast Extract 1, lactose 10, agar 15, Eosin Y 0.4, Methylene Blue 0.06, sodium chloride 5, dipotassium phosphate 2. On this medium, colonies of bacteria which can ferment lactose (or any other sugar added in its place) rapidly turn a deep purple color, while colonies of non-fermenting organisms remain white or pink but may slowly turn light blue.

Lac-mutations have been recovered in two instances. Among 15,000 colonies of strain Y-10 ($T^-L^-B_1^-$) obtained by spreading a culture previously treated with ultraviolet light, on EMB-lactose agar, a single pink colony was noted. It proved to be the same,

nutritionally, as Y-10 and was therefore regarded as a Lac-mutant and not a contaminant, this stock is labelled Y-53. Among 30,000 colonies of Y-40 (B-M-V_1^r) a single Lac- was recovered following treatment with nitrogen-mustard (Tatum, 1946) and was designated as Y-87. Tests showing that these independent mutations are probably allelic will be described in a later section (see Table 4g). Strains Y-53 and Y-87 differ in the rate at which the Lac-character reverts to the Lac+ condition, but whether this is due to different allelic states or to differences at other loci, cannot be definitely asserted.

Prelimary attempts to obtain maltose, mannitol, and galactose-negative mutants were not successful, presumably because the populations tested were too small. A glycerol-negative strain has been obtained, but the wild type ferments this polyalcohol so poorly to begin with that accurate scoring is difficult; studies in this character have, therefore, not been pursued.

It is particularly fortunate that resistance-tests can be conducted on EMB agar, since this allows the characterization of a strain with respect to virus-resistance and to lactose-fermentation with a single streaking (see Fig. 1).

Morphological variation has occasionally been noted (exceedingly rough or very mucoid colonial form) but is relatively unsuitable for genetic work because the presumably random choice of prototroph recombinants may be influenced.

In additon to the EMB agar already described, a number of other natural or "complete" media have been used. The Difco product "Penassay Broth" has been used most extensively, and is satisfactory for the preparation of inocula, except that it must be supplemented with cystine for the growth of cystineless organisms, such as strain Y-24. Other satisfactory media include a broth consisting of: peptone 5, glucose 5, yeast extract 3, g./l, as well as Difco Nutrient

Broth, and diverse concoctions containing peptone or casein hydrolysates and meat or yeast extract.

The synthetic or minimal medium contains, in g./l.,: NH_4Cl 5, NH_4NO_3 1, Na_2SO_4 2, K_2HPO_4 3, KH_2PO_4 1, glucose 5, asparagine 1.5, $MgSO_4$ 0.1, trace elements (Beadle and Tatum 1945) and $CaCl_2$, a trace.

To avoid flocculation when used with agar, the glucose and agar in solution should be autoclaved separately, and mixed with the other components just before using. Unwashed agar (Difco) is sufficiently free of the growth factors under consideration to be satisfactory for many experiments; the use of washed agar, however, is recommended for the cleanest results.

The detection of recombinants is based upon the inability of biochemical mutant bacteria to proliferate in the absence of their specific growth substances. Plating in minimal agar, therefore, has the effect of a sieve for prototroph cells. To insure against contamination with prototrophs derived by reverse mutation which has been noticed at certain loci, it has been desirable to use multiple biochemical mutants as the parental stocks in recombination studies. Coincidental reversion at two or more loci is ~~the~~ theoretically improbable, and experimentally undemonstrable (see below). For example, plating either B-M-T+L+B_1+ or B+M+T-L+B_1- separately into nominal agar did not lead to the appearance of prototrophs, B+M+T+L+B_1+. When, however, a mixture of these cell types was so "sieved", one prototroph was found for ca. each 10^7 cells inoculated. These have been assumed to arise from the recombination of "+" alleles to form the prototroph.

In early experiments, the two multiple mutants were inoculated together into a complete medium and allowed to grow in mixed culture before plating into minimal agar. This method is not entirely satisfactory because it allows possible selective differentials to alter the relative frequencies of different recomination classes. A modified

procedure was developed, which will now be described in detail.

The mutant stocks are maintained on "complete" agar slants, transferred at intervals of 6-8 weeks. They are inoculated separately into test-tubes containing about 10 ml. of liquid complete medium and incubated overnight at 30° C. with gentle shaking. The following morning, an additional 10 ml. of the same medium is added to each culture, and the tubes are incubated in the same manner for an additional 3-5 hours. These cultures contain from 1-4 x 10^9 cells per ml. They are then washed in the following manner: the cotton plugs are replaced with sterile corks which have been kept in 95% alcohol and the alcohol flamed off just before using. The cultures are then centrifuged at ca. 2500 r.p.m. for 20 minutes, which suffices to pack the cells in the bottom of the test tubes. The supernatant medium is carefully poured off, and the tube is rinsed with ca. 10 ml. sterile distilled water, care being taken not to disturb the pellet. The cells are then resuspended in an additional 15-20 ml. sterile water, and recentrifuged. The supernatant wash water is decanted and replaced with an equal volume of fresh sterile water, in which the cells are suspended. In the meantime, minimal agar plates are prepared. A bottom layer of ca. 15 ml. minimal agar is poured into each Petri plate and allowed to solidify. Cell-suspensions of different mutant stocks are mixed at this time and measured quantities (usually ca. 10^8 - 10^9 cells) are pipetted onto the agar surface. At this time also, one may add such growth factor supplements as are desired to permit the growth of recombination types other than prototrophs. The cell suspensions are then mixed into a layer of ca 10 ml. molten minimal agar (at ca. 45-50° C.) which is poured onto the plates. After the agar has hardened, the plates are incubated at 30° C. for a period of 48 hours. At this time recombinant prototroph colonies will be found distributed throughout the plate, many of them at or near the surface and accessible to picking

for further characterization.

The procedure may be varied in several ways. It is important, however, that the inoculum consist of "young" cells, since cultures of 24 hours or older have given quite inconsistent results. It is possible to store the inoculum in distilled water for at least twenty-four hours without appreciably affecting the yield, which suggests that the aggregation of genetics leading to the recombination process occurs in the molten or in the solidified agar. This occurrence must, however, take place within a few hours, since the recombinant prototrophs are not appreciably slower to appear than wild type cells in a similar physiological state which are streaked on the surface of the plates. Presumably therefore one could increase the yield of prototrophs by making conditions more favorable for the free contact of the cells; as by packing them together in a centrifuge tube in minimal liquid medium. However, the complication of proliferation of prototrophs already formed would interfere with the interpretation of such an experiment. Many physiological factors may interfere with the recombination process, and, for example, the yield may be reduced markedly by inoculating too heavily, or by omitting an under-layer of agar into which, presumably, deleterious metabolic products may diffuse. Instead of mixing the cells in semisolid agar, it is possible to streak the mixture on the surface of slightly dried minimal agar plates. Under these conditions, however, the prototroph colonies are likely to be more heavily contaminated with the residual parental mutant types.

For most purposes, however, this contamination may be ignored, as will be shown in a later section. Prototroph colonies are, then fished and streaked directly on EMB plates, or otherwise tested, to classify them with respect to other factors that may be segregating.

Experimental Results. - Spontaneous mutations of bacteria in pure culture were studied as a preliminary to the investigation of recombi-

nation. The overall frequency of random biochemical mutations in untreated cultures is less than 0.1% (Tatum 1946) although samples totalling not more than 5,000 cells have been studied so that the precision of this measurement is doubtful. In view of the low rate and sporadic, independent occurrence of such spontaneous mutations, however, they may be regarded as a negligible factor in this study.

The spontaneous reversion of biochemical mutants to prototrophs is under continuing study (Ryan and Lederberg) and will be reported on more fully elsewhere. It has been found that many biochemical mutants of _E. coli_, K-12, will revert at a low rate, prototrophs being found in the proportion of 10^{-7} in 24-hour cultures of single mutants. Reversions of different factors are, so far as has been yet ascertained, entirely independent; as predicted from the low rate of reversion of the individual factors, in ca. 10^{-10} cells examined no instance was found where reversion had occurred at both loci of a double mutant. Such a coincidence would have led to the appearance of a prototroph in a culture inoculated with a double mutant such as T-L-. On the basis of these considerations, only double and triple mutants have been used in the study of recombination.

The frequency of spontaneous mutations to virus resistance has the same low order of magnitude as nutritional reversion (Luria and Delbrück, 1943). Mutations from resistance (V_1^r) to susceptibility (V_1^s) have not been described, owing to the lack of efficient techniques for the detection of such reversions.

Prototroph Recombination Types: Since coincidental spontaneous reversion at two or more loci does not occur at a sufficiently high rate to be detected, the presence of prototrophs in mixed cultures of multiple mutants is evidence for gene recombination. Each mutant is capable of synthesizing all the growth factors for which it is not deficient; therefore, different mutants should have "+" alleles for all but the

two or three mutant genes that characterize each strain. The segregation of prototrophic alleles of every gene into one cell would result in a prototrophic cell. It would develop into a visible colony on minimal medium while other mutant cells would be unable to proliferate due to the absence in minimal medium of their nutritional requirements.

When washed samples of mixed cultures of B-M-P+T+and B+M+P-T- were plated into minimal medium, about 100 colonies developed for each billion (10^9) cells inoculated. No colonies appeared after innoculation of samples from the individual double mutants. One interpretation of the occurrence of prototrophs, designated as B+M+P+T+, is that the P+ and T+ genes of B-M-P+T+ and the B+ and M+ genes of B+M+P-T- have segregated into the same cell. This is a recombination hypothesis; alternatives will be discussed in the next section.

The possibility must be considered that the prototrophs do not consist of some sort of association of the unaltered mutants. In a classical illustration of nutritional symbiosis, since designated as syntrophism (Lederberg 1946), Valentine and Rivers (1927) showed that *Hemophilus canis* and *H. parainfluenzae*, which require X and V factor respectively, would grow in mixed culture in media lacking these substances. They concluded that these growth factors, synthesized by the individual bacteria, were exchanged via the medium. While there is no good reason to doubt this conclusion, these authors did not, in fact, conclusively demonstrate that this was the mechanism of the interaction. It is possible that cells were present in their mixed cultures which, as a result of gene recombination, required neither of the two factors. The situation is obscured by the use by these authors of serial transfers of large numbers of bacteria.

Syntrophism has been shown to occur with *E. coli* mutants (Lederberg 1946) (Lampen et al, 1947). It is not likely, however,

that it plays a significant role in the appearance of prototrophic colonies. Washed cells inoculated into minimal medium do not show syntrophism until small quantites of their required growth factors are added. In minimal agar plates heavily inoculated with a washed mixed culture a uniform turbidity does appear which is ascribable to a limited exchange of factors and subsequent syntrophic growth.

Evidence of several sorts has been obtained for the homogeneity and uniqueness of prototrophs isolated from mixed cultures. Most significant, they are quite stable and attemps to detect the original mutants in recombination prototroph cultures by an efficient selective technique (Lederberg and Tatum 1946a, b,) have been unsuccessful. Massive doses of ultra-violet light, killing all but 10^{-5} of the cells in the culture, were no more successful in breaking up the supposed associations. In addition, prototrophs obtained from B-M-P+T+V_1^r and B+M+P-T-V_1^s were studied. Both susceptible and resistant cultures were obtained. Although one of the parental strains is resistant, the susceptible cultures were uniformly lysed upon application of the phage; on the other hand, there was no change in the nutritional behavior of cultures of resistant prototrophs subsequent to the application of the virus, which would be expected, in an association of the original mutants, to lyse the susceptible B+M+P-T-V_1^s cells and leave only B-M-P+T+V_1^r.

A nicotinicless mutant has been obtained by ultra-violet irradiation of a prototroph derived from P-T- and B-M-. The prototroph in which this mutation occurred could have been neither a heterocaryon nor an association of diverse types, since in either case the absence of nic+ genes in the mutant would require the simultaneous mutation of more than one representative of these gene. This coincidence is highly improbable. The microscopic examination of seeded agar supported the conclusion that the cells of strain

K-12 are well dispersed, so that most of the colonies that appear would be derived from single cells when only a few hundred cells are inoculated per plate, as was done subsequent to the initial isolation of prototrophs. Single cell isolations from a "recombination prototroph" strain have been made by Dr. M. Zelle of the National Institute of Health; all of the single-cell cultures tested were of the same nutritional and virus-resistance type as the culture from which they were isolated. Finally, the diversity of recombination types described below is incompatible with the hypothesis that they result from a simple combination of cells.

Transformation, preliminary expts: The evidence just presented points to the conclusion that the prototrophs are a new type of cell, which did not arise by spontaneous changes in a single double-mutant strain. Gene recombination, which was postulated above, is however, not the only interpretation for the origin of these new types which would fit the evidence that has been presented. By analogy with the systems which have been described in pneumococci (Avery et al, 1944) and other strains of E. coli (Boivin 1947) one might postulate that genotypically distinct cells interact not through cell fusion, but through the release of "transforming substances" diffusing through the medium. Such transforming substances would have the property of inducing or directing mutational changes in the cell receiving them so as to lead to what appear to be recombination types.

Since the conditions of the recombination experiments require that any transforming substance be present in the medium, an attempt was made to modify a nutritional mutant with a culture filtrate from another mutant. B+M+P-T-V_1^s was grown in YB broth, and samples of 12- and 36- hour cultures were freed of cells by centrifugation and filtration through an ultra-fine sintered glass filter. The filtrate was diluted with an equal volume of YB and inoculated with

$B-M-P+T+V_1^r$. As a control, $B-M-P+T+V_1^r$ cells were inoculated with $B+M+P-T-V_1^s$ into filtrate broth. After the cultures were incubated for 48 hours, they were analyzed for prototrophs by the methods described above. None were found in the $B-M-P+T+V_1^s$ cultures grown in the presence of $B+M+P-T-V_1^r$ filtrate, indicating the absence of an active transforming principle in the medium under these conditions. On the other hand, the growth in mixed culture of $B-M-P+T+V_1^r$ and $B+M+P-T-V_1^s$ cells resulted in the appearance of numerous prototrophs.

Additional attempts were made to determine whether "transforming activity" could be separated from the living cell. This is tantamount to replacing one of the parental cell types in a recombination experiment with an extract prepared from it, or with comparable materials. Conditions comparable to the plating described on p.12 were used, as well as cell extracts prepared by Boivin's method. (Boivin, 1947).

No activity was found in supernatants of suspensions of Y40 or Y53, together or separately, as tested by plating the supernatants with Y40 or with Y53, into minimal medium and looking for prototrophs. The only manipulation involved here consists of the removal of most of the bacteria from suspension in minimal liquid medium, in which they had been allowed to remain for varying periods up to six hours. "Activity" remained in association with the cells, as tested by plating them with the alternate type. Equally negative results characterized attempts to reveal transforming activity in culture filtrates and cell autolysates prepared, as mentioned, according to Boivin (1947).

Finally, the addition of desoxyribonuclease, (kindly provided by Dr. M. McCarty) in a final concentration of .05 mg./ml. to the mixing and plating medium had no effect on the number of prototrophs which appeared on "crossing" Y40 and Y53.

Additional experiments and considerations of a purely genetic character will be described below; at this point in the experiments, the interpretation of prototrophs as recombination types resulting from a sexual process was adopted as a working hypothesis, and further experiments were designed to elucidate it in detail.

Other recombination types.

If prototrophs arise from the segregation into the same cell of + alleles from its sexual parents, there might, (in a haploid system such as E. coli might well be thought, a priori, to represent,) be found in the same mixed cultures other combinations involving - as well as + alleles. In the first attempts to detect addition segregation types the cultures Y24, B-Pa-C-T+L+B_1+V_1^s and Y 46, B+Pa+C+T-L-B_1-V_1^r were used. There were, thus, available 7 markers some of which might be expected to segregate from the others, and give rise to a variety of recombination types.

Unfortunately, it would not be possible to detect all of the 2^7 or 128 possible recombination types. Only those types could be detected which would grow in a medium in which both of the parents would be suppressed. That is, either biotin, phenylalanine or cystine would have to be omitted to keep Y24 from predominating in the plates, and either threonine, leucine or thiamin to suppress Y46. In fact, it would be preferable to omit at least two factors required by each parent in order that "contamination" by back-mutants at a single locus be eliminated. While this restricts the number of recombination classes that could be isolated, it still leaves a great many. Four markers (two of the - alleles of each parent) would be used up in order to detect the "prototroph" recombinations, but the other three would be free to segregate in such combinations as the genetic system determines.

Since the V_1 locus has not been used for detecting

recombinants, there are a total of nine nutritional double-requirement types - B-T-; B-L-; B-B_1-; Pa-T-; Pa-L; Pa-B_1-; C-T-; C-L-; C-B_1- which could be detected, as well as six single-requirement types and prototrophs. Such types would be found by plating mixtures of Y24 and Y46 into minimal agar containing two supplements such as biotin and threonine. On this medium, both parents would be suppressed, but the recombinant (ex hypothesi) types : B-T-; B-; T-; and prototrophs should be able to grow. Colonies isolated from such a plate would possibly be any of these four classes, and must be classified more fully. This was accomplished by fishing them into small tubes of sterile water, and taking small inocula into a series of tubes of minimal medium supplemented with threonine, with biotin, with neither and with both. The inability of a culture to grow in the absence of a growth factor indicates the - allele, while the tube containing both biotin and threonine serves as a control. Prototrophs, of course, will grow on each of these four media; B- only on the media containing biotin, T- similarly, and B-T- only in the doubly supplemented tube.

The result of such an experiment is summarized in Table 2. In order to determine the proportions of the various types, the number of prototrophs was used as a standard. The total number of prototrophs obtained from those plates which had supplements allowing the development of a given type was compared with the total number of that type isolated. The relative frequency of prototrophs and B_1-, for example, was found using plates supplemented with thiamin, with thiamin and biotin, thiamin and phenylalanine, and thiamin and cystine. The growth requirements of the more interesting "double-requirement" segregants were checked several times using 10 ml. volumes of medium , and isolates purified by serial single colony isolation. V_1 was, of course, also segregating, but was scored only for some of the thiaminless and prototroph isolates.

As indicated by the table (Table 2) several differents recombination types were found. For example, considering the factors B and B_1 only, it will be recalled that the parental arrangements are B-B_1+ and B+B_1- in Y24 and Y46 respectively. In addition to the parental arrangements in such recombinants as B_1- (B+) and B- (B_1+), the non-parental combinations are found in such types as prototrophs (B+B_1+...) and B-B_1-. However, since these can only be detected in the uniform recombination class Pa+C+T+L+ the four types mentioned are not strictly complementary to each other, and comparison of the frequency is not particularly meaningful, as it would be if the cross were simply of the form xy x XY, where xY and Xy should be equally frequent

Beyond the mere existence of many of these types, it will be noted that there are considerable differences in their relative frequencies. However, too much weight cannot be placed on these discrepancies since, in this experiment, the two parental types are grown together in mixed culture in "complete" medium for 48 hours before being washed and plated. Different recombination types formed during growth in liquid might be subject to selective growth differentials which would alter their relative frequencies. It was to counter this objection that the modified procedure described on p.12 was developed.

Although nutritional requirements are not objectionable as markers, and are indispensable for the detection of recombinants, it was considered that such markers as virus resistance and sugar fermentations might be more readily manipulated and scored in large numbers. For further study of segregations, therefore, the behavior of the factors Lac and V_1 have been especially scrutinized.

The character V_1^r has been particularly useful because a selective procedure exists by means of which it can be introduced

mutationally into any desired gene combination. The application of a "cross"heterozygous at this locus to the demonstration that prototrophs are not simply cell-associations has already been mentioned. In the course of those tests, 10 prototrophs each were isolated from the crosses $B-M-P+T+V_1^r$ x $B+M+T-P-V_1^s$, and $B-M-P+T+V_1^s$ x $B+M+P-T-V_1^r$ respectively. In the first case, 8 were V_1^r while 2 were V_1^s. In the second, "reversed" cross, 3 were V_1^r while 7 were V_1^s. The apparent reversal of ratios in reversed crosses, in this small sample, suggested a technique by which the basis of the non-random distribution of recombination classes might be examined. The observations were extended, therefore, to collect more data for this cross and to study other combinations as well.

The segregation of V_1 alleles into prototrophs resulting from three different sets of mutant combinations in which the parents were heterozygous for this locus is shown in table 3. It will be noted immediately that there is a large discrepancy between the frequency with which prototrophs are V_1^r or V_1^s as a result of the "reversal" of the parents in which these alleles are introduced. This discrepancy amounts to a $X^2 \doteq 199$ (for three degrees of freedom) when the values are cumulated. On the other hand comparisons made between the results of "reversed"crosses, the ratios being similarly reversed, show a fairly good fit, a cumulative $X^2 = 9.8$ (for three degrees of freedom) $p = .02$) being obtained. These tests illustrate the combinatorial or Mendelian character of inheritance whereby "gametic frequencies are invariant in respect of any gene substitution applied systematically to the genic content of an organism and of the gametes it produces". (Fisher, 1947) A value of $p \doteq .02$ for goodness of fit under the hypothesis of Mendelian behavior is not as reassuring as one would like, but may perhaps be ascribed to errors in scoring V_1 rather than to a real deviation from the theory. There can, at any rate,

be no doubt as to which of the two modes of comparison gives the better fit.

Subsequent to the completion of the experiments of Table 3, the Lac-mutant stocks Y53 ($T-L-B_1$-Lac-) and Y87 ($B-M-V_1{}^r$ Lac_2-) were obtained as already described. In addition, a $V_1{}^r$ mutant of Y53, Y64, ($T-L-B_1$-Lac-$V_1{}^r$) was readily obtained. The development of these stocks permitted a more critical experiment similar to those summarized in Table 3, but in which the segregations of two factors from various parental combinations could be readily studied. In tables 4, the data from a number of individual experiments are recorded, and analysed for their homogeneity. In tables 5 and 6 the data are summarized, and the results of the different crosses are compared much as in table 4.

Inasmuch as the segregation of B_1+/B_1- was also used in this experiment, a word will have to be said concerning this locus, in anticipation of a further discussion below. It was noted in crosses of $B-M-T+L+B_1+$ x $B+M+T-L-B_1-$ that the yield was approximately ten times as great on thiamin-supplemented as on minimal agar, indicating a B_1-/B_1+ ratio of about 10;1. Rather than separate the 10% B_1+ colonies from the total found on thiamin-supplemented plates, the expedient of comparing the segregations of Lac and V in colonies from minimal and from thiamin-supplemented plates was used. In the latter, B_1- types would be so preponderant that any appreciable deviation from the B_1+ segregation frequencies should be noted among these colonies despite their "contamination" with 10% B_1+.

The significant heterogeneity of the data of Tables 5 and 6 is rather disturbing, but no method has yet been found of avoiding it, and its extent and character are such as to make very laborious any attempts in this direction. The null hypothesis of table 6 must be modified accordingly. It should read: "that the deviations

between the experiments of Table 4 are not greater than can be counted by their intrinsic heterogeneity."

An attempt was made to deal with this problem quantitatively by the variance-ratio method. The X^2 for the various sets of experimental data are given in the tables. The variance of Table 4a is, however, very different from that of the others, and none is available for 4e and 4f as these were the results of single, large experiments. It has not, therefore, been possible to select a characteristic variance for the error or heterogeneity of the data by means of which to test the deviations between the three main experiments. The shift in the type of the rarest class with change in the parental couplings is, however, very striking and affords the clearest qualitative verification of the principle of mechanical recombination.

The conclusions which may be drawn from the analysis are:

1. A far better agreement with a Mendelian hypothesis than with a direct comparison of class frequencies in alternated crosses.

2. No difference in the segregations of Lac and V_1 in the B_1- as against the B_1+ progeny.

3. Non-random segregation of Lac and of V_1 with respect to the nutritional factors, as indicated by deviations from 1:1 ratios of alleles in the prototrophs.

4. Interaction between Lac and V_1 themselves. In this case, for example, the ratio of Lac x: Lac x' is 1389:817 = 1.7 in the subclass V_1 y', but is 828:63 = 13.1 in the subclass V_1 y. (Table 6).

In addition, experiments are recorded in these tables (4g) indicating that the separately obtained Lac- and the V_1^r mutations used in the experiments are indeed allelic, i.e., that no Lac+ or V_1^s segregants, respectively, occur among the progeny of crosses homozygous for Lac- or for V_1^r.

The simplest combinatorial mechanism known is that of random or independent recombination. Since the data do not support this hypothesis, one is led to try the next most simple, and the one characteristic of all other organisms studied: the organization of genes into linkage groups, presumably corresponding to chromosomes.

The first problem which must be solved is: "How many linkage groups can be identified?" The data already presented are not sufficient; the behavior of the various nutritional markers themselves must be studied first.

If one of the five markers in the cross $B-M-T+L+B_1+$ x $B+M+T-L-B_1-$ were independent of the others, we might predict that the class "x"- would be equally frequent with "x"+ in the detectable recombination classes where all the other markers were+ . That is to say, either B-, M-, T-, L- or B_1-, depending on which ones were segregating independently, would occur in a 1:1 ratio with prototrophs.

To test this hypothesis, mixtures of these parental types were plated into agar medium supplemented with one of the five growth factors involved: biotin, methionine, threonine, leucine, or thiamin. On the biotin-supplemented plates for example, the two recombination classes: $B-M+T+L+B_1+$ and $B+M+T+L+B_1+$ are capable of forming colonies. If B is independent, they should be equal in number; if not, there should be a discrepancy from a 1:1 segregation. Colonies were, therefore, picked from such plates and scored by testing them on liquid medium for their growth requirements. The results are in Table 7.

Unfortunately, the B-M- parental type was not entirely suppressed when heavily inoculated into methionine-supplemented plates, due to a low level of contamination with biotin either of the agar, the methionine, or the cell suspensions. This resulted in the crowding out of any recombinants which were formed, so that figures

for the M-:M+ ratio cannot be given. Fortunately, this data turns out not to be critical, as noted below.

It will be seen from Table 7 that none of the other markers have segregated at random, B_1- being more frequent, and the others less frequent than prototroph recombinants.

To account for B- being less frequent than B+ in the M+T+L+B_1+ class, we may postulate that B is linked to one or more of these markers. Comparing the parental couplings: **B+** with M+, T-, L-,B_1- we may conclude that it must be with M that B is linked.

Similarly, we note that B_1- is more frequent than B_1+. The parental couplings are B_1- with B+, M+, T-, L-. Therefore, B_1 must be linked with B or with M, or since these are linked in turn, with both of them. The linkage group: (B_1:B;M) therefore appears, although at this stage the relative order is not determined.

Both L- and T- are rarer than prototrophs. They must therefore be linked with each other, since the parental couplings are: T+ with L+(B_1+;B-;M-), L+ with (B_1+;B-M-), T+ respectively. We are not entitled to infer that the groups (B-;B;M) and (T;L) are or are not independent of each other since an interchange between them is necessary, although insufficient, to produce the detectable prototroph class. With this reservation, the map of Table 6 may be written, for these factors.

The linkage relations of Lac and V may be gotten from Table 6. In the cross (B_1-;B+M+)(T-L-)Lac- X (B_1+;B-M-)(T+L+)Lac+, it may be seen that Lac- is the more frequent, both in the B_1-B+M+T+L+ and the B_1+B+M+T+L+ classes. Lac, therefore, is linked with the (B_1-;B;M;) group although probably not closely with B_1 inasmuch as the B_1 and Lac segregations do not interact. We may, then write: (B_1;B,M;Lac).

In the cross $(B_1-;B+M+)(T-L-)V_1^s$ X $(B_1+;B-M-)(T+L+)V_1^r$, 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+8 or 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 = interchange: no interchange, a value of 10.1% would have been obtained.

The estimation of the distance (B,M) - (T,L) requires a detailed consideration of multiple crossing over. Absolute values for the distances (B.M) - Lac; Lac - V_1 ; V_1-(TL), 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:

$$\frac{\sinh a.\ \sinh b.\ \sinh c}{\sinh x} = .020$$

$$\frac{\sinh a \cdot \cosh b \cdot \cosh c}{\sinh x} = .264$$

$\tanh a = \sqrt{\frac{\alpha\delta}{\beta\gamma}}$ ....

$$\frac{\cosh a \cdot \sinh b \cdot \cosh c}{\sinh x} = .448$$

$$\frac{\cosh a \cdot \cosh b \cdot \sinh c}{\sinh x} = .268$$

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:

$$\frac{\sinh^3 x/3}{\sinh x} = .020, \qquad x = .75,$$

which considering the crudity of the approximation is in good agreement.

On the other hand the application of the uncorrected formula $\frac{(x/3)^3}{x} = .020$ 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; 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.

<u>Linearity</u>

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_1 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 crossover 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 Lac, V_1 and V_6 are congruous between the B_1- and B_1+ classes (the latter in the sense discussed on p. 22). In the totals of "prototrophs" isolated from $B-M-T+L+B_1+Lac+V_1^rV_6^s$ X $B+M+T-L-B_1-Lac-V_1^sV_6^r$, one finds Lac- 78%, V_6^r 82%, and V_1^s 36%, indicating that, as in previous experiments, 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 Lac. Since linkage to B_1 is already eliminated, one would predict from these totals, on the hypothesis of linearity, that Lac and V_6 should be linked, with V_6 to the left of Lac.

The data of Table 8 confirm this prediction. The parental couplings of Lac and V_6 are -r and +s respectively. Of 137 Lac-, 134 were V_6^r; of 39 Lac+, 29 were V_6^s. The order V_6 , 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 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,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 samee 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 megalocephalus* 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 B_1- (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+B_1+ x B+M+T-L-B_1- this would be equivalent to the suppression of prototroph recombinants; B_1- 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-53 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-53 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.

<u>How Many Segregants per Zygote</u>?

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 recomination 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 ($B-M-T+L+B_1+Lac+V_1^r$ x $B+M+T-L-B_1-Lac-V_1^s$) on B_1- containing minimal agar medium. As already noted, about 90% of the colonies from such a cross are $B+M+T+L+B_1-$. The theoretical complementary class would be $B-M-T-L-B_1+$. 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

thiaminless colony could be shown to contain from 10-100 cells capable of forming colonies on the B,M,T,L medium. However, in each case investigated these have been shown to be indistinguishable from the Y-40 parental B-M-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_1- to B_1+. These experiments are, then, inclusive with respect to the occurrence of complimentary geneotypes 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_1- 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 T1-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 a haploid condition of *E. coli*, with reduction 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 egregation, many questions concerning centromere relations, and the umber of strands as meiosis, as well as the dominance or recessive-ess of particular characters, could be studied. Because of the ifficulties of cytological examination, a genetic test was devised hich, it was hoped, would detect stable diploid variants.

In 1913, Penfold, described a peculiar response of E. coli o sodium chloroacetate. Wild type strains appeared to be inhibited y this agent, but gave rise to resistant mutants, which appear as papill-e or button-like projections from the inhibited growth on mutrient 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^r, 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_2 fraction, and formate. The formate is then decomposed to $CO_2 + H_2$ 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^r 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 Coon-radt on Neurospora heterocaryons, it is likely that the ability to perform a reaction will generally be dominant to the inability. Cla^s therefore should be dominant in a diploid heterozygote to Cla^r with respect to enzymatic function. If the correlation between

enzymatic function and resistance is maintained, Cla^s will also be dominant with respect to resistance, i.e., the combination Cla^s/Cla^r will still be sensitive to chloroacetate. Starting from the diploidised wild type, Cla^s/Cla^s, it will require two mutations to produce the resistant type Cla^r/Cla^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 of 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 multisporic 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-B_1-. Lac and V_1 alleles were distributed among these parents in various combinations. For example, the types B-M-Lac-V_1^r ; T-L-B_1-Lac-V_1^s; and T-L-B_1-Lac+V_1^r 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 B_1-, other factors prototrophic, could be produced only by recombination between the B-M-parent and one or the other of the T-L-B_1- parents. By biparental inheritance, in this case, only three of the possible combinations of Lac and V could appear among the prototrophs: Lac-V_1^r; Lac-V_1^s; Lac+V_1^r. If a ménàge à trois were permissible, however, the fourth type Lac+V_1^s 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-methionineless and histidine-p-aminobenzoicless, were obtained from ultraviolet treated material by previously described techniques for the isolation of mutants, Lederberg and Tatum, (1946a) All combinations of the T-L-B_1- 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 estimated 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, although 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 insoculated in the cross B-M-T+L+B_1+ X 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 analagous 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.

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Erratum

References inadvertently omitted from list:

Dienes, L. 1946. Complex reproductive processes in bacteria. Cold Spr. Harb. Symp. Quant. Biol. 11; 38-50.

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Table 1A

Symbols used for various mutations

1. Nutritional requirements. The allele for requirement,i.e.,lack of synthesis of a substance is "-"; the alternative, independence, is "+".

B	biotin	L	leucine	Pa	phenylalanine
B_1	thiamin	M	methionine	T	threonine
C	cystine	P	proline		

2?.Sugar fermentation. The ability to ferment is "+"; the alternative,inability, is "-".

Lactose Lac

3. Bacteriophage resistance. Resistance is designated by the superscript "r"; sensitivity by "s".

V_1 reaction to bacteriophage T1

V_6 reaction to bacteriophage T6.

Table 1B

A Summary of the Mutants Used

Strain No.	Genotype	Origin	Genotype	Agent
K-12	prototroph.	Original wild strain.		
58	B-	K-12	B+	X-ray
58-161	B-M-	58	B-M+	X-ray
58-278	B-Pa-	58	B-Pa+	X-ray
Y-24	B-Pa-C-	58-278	B-Pa-C+	u.-v.
679	T-	K-12	T+	X-ray
679-680	T-L-	679	T-L+	X-ray
Y-10	T-L-B_1-	679-680	T-L-B_1+	X-ray
Y-46	T-L-B_1-${V_1}^r$	Y-10	T-L-B_1-${V_1}^s$	selection
Y-53	T-L-B_1-Lac-	Y-10	T-L-B_1-Lac+	u.-v.
Y-64	T-L-B_1-Lac-${V_1}^r$	Y-53	T-L-B_1-Lac-${V_1}^s$	selection
Y-40	B-M-${V_1}^r$	58-161	B-M-${V_1}^s$	selection
Y-87	B-M-${V_1}^r$Lac-	Y-40	B-M-${V_1}^r$Lac+	N-mustard
Y-25	B-Pa-C-${V_1}^r$	Y-24	B-Pa-C-${V_1}^s$	selection
679-183	T-P-	679	T-P+	X-ray
Y-94	T-L-B_1-Lac-${V_6}^r$	Y553	T-L-B_1-Lac-${V_6}^s$	selection

Table 2.

Biochemical recombination types found in a mixed culture of Y-24 and Y-46.

$B-Pa-C-T+L+B_1+V_1^s$ X $B+Pa+C+T-L-B_1-V_1^r$.

Type:* B Pa C T L B_1	No. isolated	No. of prototrophs	Ratio
- - - + + +	In excess: parental type		
+ + + - - -	In excess: parental type		
+ + + + + +	86	--	--
+ + + + + -	36	37	0.97
+ + + - + +	2	31	0.06
+ + + + - +	4	55	0.07
- + + + + +	5	56	0.09
+ - + + + +	1	52	0.02
+ + - + + +	1	19	0.05
- - + + + +	2	41	0.05
+ + + + - -	3	16	0.19
- + + + + -	3	28	0.11
- + + - + +	isolated in a different run.		
- + + + - +	isolated in a different run.		

* These figures do not include tests of the V_1 character. Of 49 prototrophs tested, 20 (41%) were resistant. Of 2o thiaminless tested, 7 (35%) were V_1^r.

Table 3.

Comparisons of $V_1{}^r$ segregations with alternative parental couplings.

Parents		Prototrophs isolated				
B-Pa-C-T+P+	B+Pa+C+T+P-	$V_1{}^r$	$V_1{}^s$	$\%V_1{}^r$	$X^2(1)$	$X^2(2)$
$V_1{}^r X$	$V_1{}^s$	76	6	92	101	7.9
$V_1{}^s X$	$V_1{}^r$	30	107	22		
B-Pa-C-T+L+B1+	B+Pa+C+T-L-B1-					
$V_1{}^r X$	$v_1{}^s$	80	23	77	65	1.1
$V_1{}^s X$	$V_1{}^r$	53	133	28		
B-M-T+P+	B+M+T-P-					
$V_1{}^r X$	$V_1{}^s$	49	8	86	32	1.8
$V_1{}^s X$	$V_1{}^r$	5	19	21		

$X^2(1)$ refers to a comparison of the classes directly: i. e., to a 2 x 2 table of the data as they stand.

X^2 (2) refers to a comparison in which the gametic output of $V_1{}^r$ is inverted in the same manner as was done by changing the parental coupling. In both cases, n = 1 for each table The cumulative X^2_3 are 198 and 10.8 respectively.

Table 4a.

Data of individual experiments. Lac, V_1 scores on colonies isolated from minimal agar plates in the cross: Y40 x Y53

$B-M-T+L+B_1+Lac+V_1^r \times B+M+T-L-B_1-Lac-V_1^s$

Class recovered: $B+M+T+L+B_1+\ldots$

Exp. #	$Lac-V_1^r$	$Lac-V_1^s$	$Lac+V_1^r$	$Lac+V_1^s$
1.	51	11	23	4
2.	28	21	21	0
3.	21	18	11	0
4.	189	44	129	3
5.	52	39	39	2
6.	17	10	9	0
7.	35	30	35	0
8.	20	22	10	3
9.	37	23	16	1
10.	20	13	15	2
11.	26	9	11	3
12.	19	10	11	2
13.	27	12	30	1
14.	27	13	13	1
15.	33	28	14	0
Total	602	303	387	22
%	45.8	23.1	29.4	1.7

Homogeneity: $X^2 = 101.$, $n = 42.$ $p \ll .001$

Table 4 b.

Data of individual experiments. Lac, V_1 scores on colonies isolated from thiamin-supplemented plates in the cross Y 40 x Y53.

$B-M-T+L+B_1+Lac+V_1^r$ x $B+M+T-L-B_1-Lac-V_1^s$

Class recovered: $B+M+T+L+B_1-$ ca. 10% B_1+.

Exp.	$Lac-V_1^r$	$Lac-V_1^s$	$Lac+V_1^r$	$Lac+V_1^s$
1.	30	21	17	1
2.	73	46	50	4
3.	27	12	30	1
4.	59	34	31	3
5.	16	12	13	0
6.	21	19	5	0
7.	18	12	13	1
Total	244	156	159	10
%	42.9	27.4	27.9	1.8

Homogeneity: $X^2 = 16.9$ n = 18, p = .5

Homogeneity of B_1+ totals with B_1- totals (Tables 4a & 4 b)

602	303	387	22	1314
244	156	159	10	569
846	459	546	32	1883

$X^2_3 = 4.4$

With a normal distribution of X^2, p would be 0.2. In view of the heterogeneity of the B_1+ data however, this result is artificially low.

Table 4 c.

Data of individual experiments. Lac, V scores on colonies isolated from minimal agar in the cross: Y 64 x 58-161.

$B-M-T+L+B_1+Lac+V_1^s$ x $B+M+T-L-B_1-Lac-V_1^r$

Class recovered: $B-M-T-L-B_1-$.....

Exp.	$Lac-V_1^r$	$Lac-V_1^s$	$Lac+V_1^r$	$Lac+V_1^s$	Sum
1.	37	55	5	19	116
2.	42	53	1	23	119
3.	8	9	1	2	20
4.	13	16	1	9	39
5.	8	12	1	8	29
Total	108	145	9	61	323
%	33.4	45.0	2.8	18.9	

Homogeneity: $X^2 = 5.6$ n = 12. p = .95.

Table 4d.

As above, scores on colonies isolated from thiamin-supplemented agar

Class recovered: $B+M+T+L+B_1-$ + ca. 10% B_1+

Exp.	$Lac-V_1^r$	$Lac-V_1^s$	$Lac+V_1^r$	$Lac+V_1^s$	Sum
1.	109	125	8	70	312
2.	20	21	1	9	51
3.	4	5	0	1	10
Total	133	151	9	80	373
%	35.6	40.5	2.4	21.4	

Homogeneity: $X^2 = .65$ n = 3, p = .85 (expts. 1 & 2 only)

Comparison of 4c and 4d totals (B_1+ with B_1-)

4d	133	151	9	80	373
4e	108	145	9	61	323
	241	196	18	141	696

$X^2 = 1.7$ n = 0.6

Table 4e

Lac, V_1 scores on colonies isolated from minimal agar in the cross: Y87 X Y10. B.

$B-M-T+L+B_1+Lac-V_1^r$ x $B+M+T-L-B_1-Lac+V_1^s$

Class recovered: $B+M+T+L+B_1+\ldots$

Single Experiment.

	$Lac-V_1^r$	$Lac-V_1^s$	$Lac+V_1^r$	$Lac+V_1^s$	Total
	28	6	46	37	117
%	23.9	5.1	39.3	31.6	

Table 4F

As above. Isolates from thiamin agar.

Class recovered: $B+M+T+L+B_1-$ + 10% B_1+

	102	7	201	91	401
%	25.4	1.7	50.1	22.7	

Comparison of 4e and 4f (B_1+ with B_1-

28	6	46	37	117
102	7	201	91	401
130	13	247	128	518

$\chi^2 = 8.2$, $n = 3$, $p = .04$.

Table 4g

Tests of allelism of mutations at Lac and V loci.

1. Test of allelism of Lac- in stocks Y53 and Y87.
$B+M+T-L-B_1-Lac-$ x $B-M-T+L+B_1-Lac_2-$. 134 prototrophs isolated: all Lac-.

2. Test of allelism of V_1^r in stocks Y40 and Y-46
$B-M-T+L+B_1+Lac+V_1^r$ X $B+M+T-L-B_1-Lac-V_1^r$
161 prototrophs isolated: all V_1^r

128 Lac- ; 33 Lac+.

Table 5
Summary of Tables 4.

The segregation of Lac and V into "prototrophs" derived from various crosses. Scores of B_1+ and B_1- classes have been pooled in view of their homogeneity.

Parents				Recombinants: B+M+T+L+B_1±			
B-M-T+L+B_1+	B+M+T-L-B_1-		Lac-V_1^r	Lac-V_1^s	Lac+V_1^r	Lac+V_1^s	Total
A. Lac-V_1^r	Lac-V_1^s		846	459	546	32	1883
		%	44.9	24.4	29.0	1.7	
B. Lac-V_1^s	Lac-V_1^r		241	296	18	141	696
		%	34.6	42.5	2.6	20.3	
C. Lac-V_1^r	Lac-V_1^s		130	13	247	128	518
		%	25.1	2.5	47.7	24.7	
							3097.

The3x4 contingency tables of Table 5 gives the following X^2 Value (for 6 degrees of freedom.)

$X^2 = 777.3$

Table 6.

The data of Table 5. are rearranged so as to bring corresponding interchange classes in the same column.

B-M-T+L+B_1+Lac $\underline{x}$ $V_1\underline{y}$ X B+M+T+L+B_1-Lac $\underline{x'}$ $V_1\underline{y'}$

Parents					Class recovered: B+M+T+L+...				
	x	y	x'	y'	x'y'	xy'	xy	x'y	Total
ab	+	r	-	s	546 (29.)	846 (44)	459 (24)	32 (2)	1883
cd	+	s	-	r	141 (20)	296 (42)	241 (35)	18 (3)	696
ef	-	r	+	s	130 (25)	247 (48)	128 (25)	13 (2)	518
Totals					817	1389	828	63	3097
%					26.4	44.8	26.8	2.0	
s					.270 : a	.458 : b	.273 c	---	

X^2 = 40.7, n = 6. No true value of p can be calculated because of the inconsistent variance of the different populations. Compare, however, with the value of X^2 = 777 of Table 5.

The figures in parentheses are the percent contribution of each class to the row total. "s" are the proportions of the single interchanges according to the map:

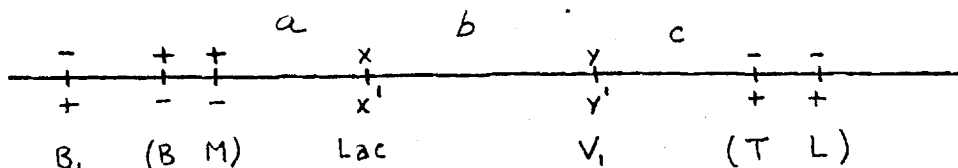


Table 7

RELATIVE FREQUENCY OF VARIOUS BIOCHEMICAL RECOMBINATION CLASSES IN THE CROSS

$B-M-T+L+B_1+$ x $B+M+T-L-B_1-$ *

From plates supplemented with	Number of colonies tested	Recombination classes found					
		Type	Number	Type	Number	Ratio	χ^2
Biotin	70	B-	10	B+	60	0.17	36
Threonine	46	T-	9	T+	37	0.24	17
Leucine	56	L-	5	L+	51	0.096	38
Thiamin	87	B_1-	79	B_1+	8	9.88	56

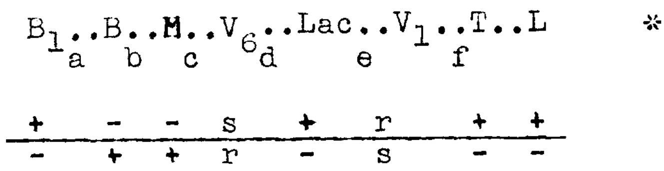
* Cells of the parental types were mixed and plated into agar supplemented with the growth factor indicated. On this medium, the two recombination classes indicated on each line of the table could form colonies. Contrasting alleles only are specified; other loci, unless otherwise specified, have the "+" configuration. The χ^2 for the ratio of single biochemicall deficient types , types to prototrophs is calculated for a comparison with the 1:1 expectation of a random segregation. As can be seen from the χ^2 values, the probability that the deviations are due solely to chance is, in each case, less than .001.

Table 8

SEGREGATION OF Lac, V_1 and V_6

$B-M-T+L+B_1+Lac+V_1^rV_6^s$ x $B+M+T-L-B_1-Lac-V_1^sV_6^r$

B-M-T-L-	Lac:	-	-	-	-	+	+	+	+	Total
	V_1:	r	s	r	s	r	s	r	s	
	V_6:	r	r	s	s	r	r	s	s	
...B_1+		24	16	1	0	2	1	10	2	56
...B_1-		52	42	2	0	6	1	16	1	120
Total		76	58	3	0	8	2	26	3	176
%		43	33	1.7	0	4.6	1.1	15	1.7	
Crossover region		e	f	cde	cdf	d	def	c	ced	



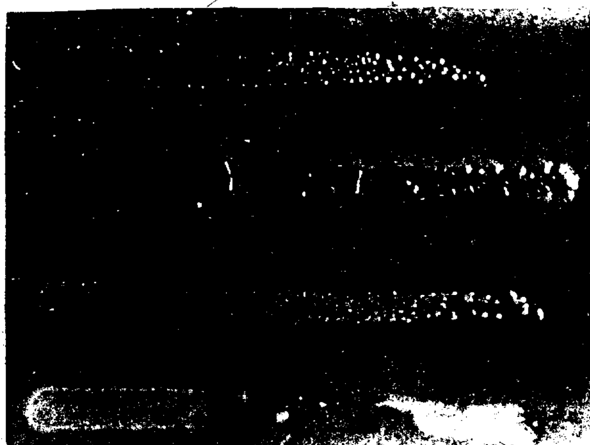
*This map is not intended to represent the map distances, merely the linear order.

Table 9

Pairwise Occurrence of Recombination in Mixtures of Three Components

Parental Types		Recombinant Prototrophs				
B-M-T+L+B_1+	B+M+T-L-B_1-	B+M+T+L+B_1- or B_1+				
		Lac-V_1^r	Lac-V_1^s	Lac+V_1^r	Lac+V_1s	Tot
Lac-V_1^r	Lac+V_1^r Lac-V_1^s	173	49	4	0	226
Lac+V_1^s	Lac+V_1^r Lac-V_1^s	0	136	37	40	213
Lac+V_1^s Lac-V_1^r	Lac-V_1^s	65	48	0	25	138
Lac+V_1^r	Lac-V_1^r Lac+V_1^s	16	0	7	28	51

Total................ 628.



Captdon for Fig. 1.

Fig. 1. The phenotypes of the four combinations of Lac and V are illus trated. In order they are: Lac+V_1^r ; Lac+V_1^s; Lac-V_1^r; Lac-V_1^s. An EMB-lactose agar plated was first streaked vertically with the virus T1. Subsequently, each of the bacteria was streaked, from left to right, perpendicularly across the virus streak. After 16 hours incubation, both the Lac and V_1 phenotypes are well developed. Developin in the zone where Lac-V_1^s has been lysed can be seen two colonies of resistant mutants: Lac-V_1^r.