²¹ Sueoka, N., these PROCEEDINGS, **45**, 1480 (1959).

²² Meselson, M., personal communication.

²³ Meselson, M., Ph.D. thesis, California Institute of Technology (1957).

²⁴ DeLamater, E. D., *Stain Tech.*, **26**, 199 (1955).

²⁵ Watson, J. D., and F. H. C. Crick, *Cold Spring Harbor Symp. Quant. Biol.*, **18**, 123 (1953).

²⁶ Delbrück, M., and G. S. Stent, in *The Chemical Basis of Heredity*, ed. W. D. McElroy and B. Glass (1957), p. 699.

GENETIC STRUCTURE OF RECOMBINANT CHROMOSOMES FORMED AFTER MATING IN *ESCHERICHIA COLI* K12*

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Genetic studies on bacteria and bacteriophage have demonstrated the presence of a linear array of hereditary determinants.¹⁻³ Experiments on bacterial transformation and phage infection identify DNA (deoxyribonucleic acid) as the carrier of genetic information.⁴⁻⁵ Replication of DNA has been studied with various organisms.⁶⁻⁸ Experimental results suggest that DNA molecules are composed of two units, which separate from each other during duplication, to form two daughter molecules, each composed of a parental unit and of a newly synthesized one, and that the units themselves remain intact through succeeding duplications. They are consistent with the model of DNA duplication proposed by Watson and Crick.⁹

In chromosome duplication, the two units of the parental DNA, which are assumed to have equivalent genetic information, transfer the genetic characters of the parent to each of the two daughter chromosomes. However, when two chromosomes recombine, the DNA units of the two parents, which may not be identical, participate in the formation of recombinant chromosome(s). Therefore, the genetic structure of recombinant chromosomes may have characteristics different from those formed in a duplication process not involving recombination. In the present paper the genetic characteristics of recombinant chromosomes formed after mating in *E. coli* K12 are analyzed.

The process of bacterial recombination can be summarized as follows.¹⁰⁻¹¹ When a culture of *Hfr* cells (male, yielding a high frequency of recombination) and a culture of *F*⁻ cells (female) are mixed, some *Hfr* cells pair with *F*⁻ cells, forming an intercellular bridge. The *Hfr* chromosome is introduced into the *F*⁻ cell unidirectionally through the bridge. If in the course of transfer the chromosome breaks, the *F*⁻ cell receives only a fragment of the *Hfr* chromosome. After the transfer of genetic material, genetic recombination between the fragment of *Hfr* chromosome and the *F*⁻ chromosome takes place in the zygote and is followed by segregation.

Let us consider several models of the recombinant chromosome(s) formed after mating and examine the predictions made by each model as to the patterns of segregation to be observed in the descendants of a zygote. A bacterial chromosome

is assumed to be composed of two units with equivalent genetic information. The units separate during duplication, each unit going to one of the two daughter chromosomes. If recombination is to occur, at least one of the single units must contain information from both parents. Such a recombinant unit could be formed either by the direct incorporation of part of the *Hfr* fragment into an F^- chromosome or by a shift of template from the F^- chromosome to the *Hfr* fragment during the formation of the unit. If recombination occurs during duplication of an F^- chromosome at least one of the four units produced must be recombinant. We will consider three models by which these units could combine to form the recombinant chromosomes after mating. In model A two recombinant units are produced which pair to form a homozygous recombinant chromosome. In model B only one recombinant unit is produced and emerges as a heterozygous recombinant chromosome. In model C two recombinant units are produced and each of them combines with one of the parental units to form a heterozygous recombinant chromosome.

If certain assumptions are introduced, each model predicts a different segregational pattern. The following assumptions are made:

(a) Each nucleus contains one complete chromosome, its duplication resulting in nuclear division.

TABLE 1
NUMBER AND GENETIC PURITY OF RECOMBINANTS AS PREDICTED BY
MODEL A, B, AND C IN FIGURE 1

Generation	Number and purity of recombinants		
	A	B	C
0	1-Mixed	1-Mixed	1-Mixed
1	1-Mixed	1-Mixed	1-Mixed
2	1-Pure	1-Mixed	2-Mixed
3	2-Pure	1-Pure	2-Pure
4	4-Pure	2-Pure	4-Pure

(b) The increase in the number of F^- cells results from division of tetranucleated cells into binucleated cells. At cell division, the nuclei assort in a specific and regular way, so that two sister nuclei pass into the same daughter cell.

(c) The chromosome fragment derived from the *Hfr* parent does not engage in recombination more than once.

(d) Recombination does not take place between established chromosomes of an F^- cell.

(e) All the descendants of a zygote are viable.

As will be described later, the majority of recombinants in our mating system give segregation results that justify these assumptions.

On the basis of the above assumptions, the three possible models of the segregational patterns of a zygote are diagrammatically presented in Figure 1. The number and genetic purity of recombinant cells predicted by the three models are presented in Table 1.

Attention may be called to the following differences between the predictions of the three models. In models A and B a mixed clone does not multiply as such, but gives rise to a pure recombinant clone and nonrecombinant clone. Segregation leading to the production of a pure recombinant clone occurs at the second division after genetic recombination in model A and at the third division in models B and C.

FIG. 1.—Segregational patterns predicted by the three models. A chromosome is represented by two adjacent rectangles, which stand for two units. The chromosomes of an *F*⁻ cell are represented by a pair of separate rectangles. A short rectangle represents a chromosome fragment derived from the *Hfr* parent. The *Hfr* fragments are omitted from the figure after the first generation. A filled square is a genetic marker different in the *Hfr* and *F*⁻ parents. The descendants of a chromosome that does not participate in recombination are omitted after the second generation.

Model C predicts an increase in the number of mixed clones prior to the division that produces two pure recombinant clones.

These models describe the segregational patterns of single zygotes. The models can be subjected to experimental tests on mass culture if the formation and segregation of zygotes are sufficiently synchronized. The tests consist in plating at intervals the progeny of the zygotes on agar media of such composition that the relevant character of each cell is identified by the character of the corresponding colony.

We shall first describe the properties of our mating system and the kinetics of segregation of recombinants, and then some experimental justification of the assumptions.

Experimental.—The lactose utilization character was selected as the genetic marker to be scored. This character is easily identified by the color of the colonies produced on EMB lactose medium¹²; a pure *Lac*⁺ colony is purple, a pure *Lac*⁻ colony is light pink, and a mixed colony *Lac*⁺ and *Lac*⁻ is variegated (*Lac*^v).¹³ The system is especially suitable because of the ease with which the cells producing

pure clones or mixed clones can be identified. *E. coli* K12, strain CS 101, a derivative of the original *Hfr* isolated by Cavalli,¹⁴ was used as a donor. Strains derived from W1 were used as *F*⁻ partners.¹⁵ Their relevant genetic characters are as follows: CS 101 (*Hfr Lac*⁺ *T*⁺ *L*⁺ *Pro*⁺ *T5*^r *T6*^s *Strep*^s), W1 (*F*⁻ *Lac*⁻ *T*⁻ *L*⁻ *Pro*⁺ *T5*^s *T6*^s *Strep*^s), W1-3 (*T6*^r *Strep*^r mutant of W1), and W1-4 (*T*⁺ *L*⁺ *Pro*⁻ derivative of W1-3).¹⁶

Kinetics of segregation of Lac⁺ *recombinants*: The crosses were performed in the following manner, with minor modifications as noted. The *Hfr* and *F*⁻ cells were grown to about 1×10^8 and 2×10^8 cells per ml respectively in Difco Penassay broth, centrifuged, and resuspended at a density of about 5×10^8 cells per ml in fresh broth. Equal volumes of the two suspensions were mixed and incubated at 37°C with gentle shaking. After 30 minutes, 2.5×10^{10} particles per ml of ultraviolet-irradiated phage T6 were added to eliminate the *Hfr* cells.¹⁷ Although the *T6*^s locus is transferred to most zygotes in this cross, at the time of phage treatment all zygotes still have the *T6*^r phenotype. At the 37th minute, antiphage serum was added to neutralize unadsorbed phage. At the 40th minute the culture was diluted 10⁵-fold into broth and incubation was continued. At various times thereafter aliquots of the mating culture were diluted and spread on EMB lactose medium. The medium was always supplemented with 100 µg per ml of streptomycin to suppress any surviving *Hfr* cells.

After 18 to 20 hours incubation, the colonies were classified as *Lac*⁺, *Lac*⁻, or *Lac*[°]. All *Lac*⁺ colonies were picked and streaked on EMB lactose plates in order to test the genetic purity of the *Lac* character. Usually several percent of these colonies originally classified as *Lac*⁺ produced both *Lac*⁺ and *Lac*⁻ colonies on the streak plates; they were reclassified as *Lac*[°]. Several colonies classified as *Lac*[°] were also tested by streaking; these invariably produced both *Lac*⁺ and *Lac*⁻ colonies.

The results of a cross CS 101 (*Lac*⁺ *T6*^s *Strep*^s) and W1-3 (*Lac*⁻ *T6*^r *Strep*^r) are presented in Figure 2. The significant features of this experiment may be summarized as follows. The total number of the *F*⁻ cells increased from the time of mixing. At first the apparent generation time (doubling time) of the total *F*⁻ cells was about 40 minutes, but the cells did not attain exponential growth until about 130 minutes when the generation time had decreased to about 20 minutes. The initial slow growth rate appears to result from the treatment received by the cells before mating and from the initial high concentration of cells in the mating culture, since a similar growth curve was obtained when an unmated *F*⁻ culture was treated in the same way.

The number of recombinants that formed *Lac*⁺ or *Lac*[°] colonies remained constant from the 40th to the 100th minute. Afterwards it increased at the same rate as the number of *F*⁻ cells. Initially most of the recombinants were *Lac*[°]. At about the 80th minute the number of *Lac*[°] cells began to decrease, reaching about 50 per cent of the original number at the 100th minute and 10 to 20 per cent at the 130th minute. The decrease of *Lac*[°] cells was matched by a corresponding increase of *Lac*⁺ cells until the 100th minute. Zygotes formed by CS 101 and several other strains derived from W1 showed similar kinetics of segregation.

Efficiency of detection of Lac⁺ *recombinants*: It is especially important for the present experiment to know the efficiency of detection of recombinant clones, that

FIG. 2.—Kinetics of segregation of *Lac*⁺ recombinants. Cultures of CS 101 (*Lac*⁺ *T6*⁺ *Strept*⁺) and W1-3 (*Lac*⁻ *T6*⁺ *Strept*⁺) were mixed at time zero and incubated. After 30 minutes phage T6 was added to eliminate *Hfr* cells. The mixture was then treated with antiphage serum at the 37th minute to neutralize free phage and diluted at the 40th minute. Aliquots were spread at intervals on EMB lactose medium. The entrance of *Lac*⁺ genes into *F*⁻ cells was measured by the blender technique¹¹ using a separate culture.

is, clones which contain any *Lac*⁺ cells. The following experiment indicates that the efficiency of detection is high. The *Lac*⁻ character of W1 and some of its derivatives is relatively unstable, reverting to *Lac*⁺ with a frequency of about 1 in 10⁷ divisions. Colonies of an unstable strain on EMB lactose medium observed after incubation for 20 hours were typical *Lac*⁻. On further incubation for two more days, at least five *Lac*⁺ papillae appeared on every colony. This indicates that *Lac*⁺ cells arising as a small minority in the colony can be detected efficiently after incubation for three days. By contrast, a strain with a more stable *Lac*⁻ character, for example W1-4, almost invariably produced colonies without papillae after incubation for three days. Both unstable and stable *F*⁻ cells were mated with *Hfr* CS 101. After 20 hours incubation, the recombinant cells of both strains produced colonies of similar morphology on EMB lactose medium, suggesting that the metabolic advantage of the *Lac*⁺ cells over *Lac*⁻ cells is similar for both strains. On further incubation, however, papillae appeared on all the colonies of the unstable strain but rarely on colonies of the stable strain. This result indicates that

virtually all the recombinant cells produced in consequence of the mating arise early enough during the development of the clones to be detected as variegated colonies.

Finally, in order to assure that the results were not biased by selection on the plates, an experiment was carried out in which aliquots of the mating culture of CS 101 and W1-4 were first spread on agar medium containing glucose, proline, vitamin B₁, and streptomycin, but no lactose. After incubation of the plates for 20 hours, the colonies were replicated on EMB lactose plates. The results were not significantly different from those obtained by direct plating on EMB lactose medium.

Genetic purity of recombinants: In the cross between CS 101 (*Lac*⁺ *Pro*⁺ *T5*^r *T6*^s *Strep*^s) and W1-4 (*Lac*⁻ *Pro*⁻ *T5*^s *T6*^r *Strep*^r), the *Lac*⁺ colonies formed after plating on EMB lactose medium at the 100th minute were restreaked to determine their purity with respect to the markers *Pro*, *T5*, and *T6*. The *Pro* character was tested on glucose medium supplemented with vitamin B₁ and 0.2 µg per ml of proline. Phage sensitivity was tested on plates seeded with the appropriate phage. Of 250 *Lac*⁺ colonies tested, 145 were pure *Pro*⁺, 100 were pure *Pro*⁻, 5 were mixtures of *Pro*⁺ and *Pro*⁻, 220 were pure *T5*^s, and 218 were pure *T6*^s. The fact that most of the recently segregated *Lac*⁺ recombinants were pure with respect to the other markers tested suggests that all these genes are incorporated into an *F*⁻ chromosome at the same generation in most of the zygotes.

Microscopic observation of F⁻ cells: To obtain information about the number of nuclei (chromatin bodies) present in *F*⁻ cells under the conditions used here, bacteria were examined microscopically. The parental cultures, CS 101 and W1-3, were mixed at time zero and 30 minutes later ultraviolet-irradiated phage T6 was added to the mating tube at a multiplicity of infection of about 1000 to insure prompt lysis of the *Hfr* cells. In this culture the number of *Lac*⁺ recombinants was 15 per cent of that of input *F*⁻ cells. Observations were made on randomly chosen microscopic fields of samples taken at 40, 80, and 130 minutes after mixing. A sample was taken from the *F*⁻ culture just before mixing. As a control, aliquots were also taken from a culture in which the *T6*^s *F*⁻ strain W1, instead of the *Hfr*, was mixed with the *F*⁻ strain W1-3. Between 600 to 1000 cells were observed in each sample. The samples were stained by the hydrochloric acid-Giemsa method.¹⁸ Special attention was given to cells with uneven numbers of nuclei.

The results showed that the overwhelming majority of cells contained either two or four nuclei; binucleated cells constitute about two thirds (e.g., 71 per cent at the 80th minute), and tetranucleated ones about one third (e.g., 27 per cent at the 80th minute), of the total cells. The proportion of uninucleated cells was less than two per cent before the 40th minute and less than 0.5 per cent after the 80th minute. Trinucleated cells, which generally showed asymmetric nuclear figures, were less than one per cent of the total cells throughout the experiment. The only difference between the mating suspension and the control culture was that the former contained slightly more cells with more than four nuclei. The proportion of such cells never exceeded one per cent. These observations indicate that the increase in the number of *F*⁻ cells results mainly from a division of tetranucleated cells into binucleated cells.

Lederberg¹⁹ and Anderson²⁰ reported frequent death of the descendants of zy-

gotes. The following results show that in our mating system death of early descendants of zygotes must be infrequent. The same test and control cultures used for the staining experiment were examined. At 100 minutes after mixing the parental cells, aliquots were diluted into broth containing melted one per cent agar at 45°C. The suspension was immediately mounted in a counting chamber, warmed for one hour at 37°C, and examined by phase contrast microscopy to determine the fraction of nonviable cells. The cells which produced microcolonies containing several cells were classified as dividing cells and the cells which remained single or double were classified as nondividing cells. Some nondividing cells may divide after the observation time. In the test culture, 41 (3.1 per cent) out of 1303 cells were nondividing; 21 (1.6 per cent) of these had normal shape, and 20 (1.5 per cent) showed abnormal morphologies. Of the abnormal cells, 12 were abnormal soon after addition to the agar. In the control culture, 13 (2.3 per cent) out of 553 cells were nondividing, 9 (1.6 per cent) having normal shapes and 4 (0.7 per cent) abnormal shapes. Thus the test culture contained only about one per cent more nondividing cells than its control. Since about 10 per cent of the cells in the test culture were derived from zygotes, nondividing cells cannot represent a large fraction of them.

Discussion.—The experimental results can be summarized as follows. (1) *Lac*^o cells originating in zygotes do not multiply as such, but segregate into *Lac*⁺ and *Lac*[−] cells. (2) Most of the segregations that produce genetically pure cells takes place between two and 3.5 generations after the entrance of the first *Lac*⁺ marker into a zygote. (3) Most of the *F*[−] cells are viable and have either two or four nuclei. (4) In most of the zygotes incorporation of *Hfr* markers into an *F*[−] chromosome takes place in a single generation, linked markers being incorporated by a single act.

We shall now discuss the assumptions indicated in the introduction. First of all we assumed that a bacterial chromosome is composed of two units with equivalent genetic information. Although this is nowhere proved, information on DNA as genetic material,^{4, 5} on its two-stranded structure,⁹ and on its duplication in various organisms^{6–8} makes this acceptable as a working hypothesis.

According to assumption (a), each nucleus contains one complete chromosome, its duplication resulting in nuclear division. A chromosome is most likely to be located in a nucleus but we are not certain of the ploidy of each nucleus. The present results showing early segregation, however, make it improbable that a nucleus contains two or more copies of *Lac* genes which multiply independently. Experiments on the sensitivity of a bacterium to inactivation by the decay of incorporated radiophosphorus suggest that a nucleus contains a single unit of DNA, and that the survival of the cell requires the integrity of this unit in at least one nucleus.²¹ These results suggest that a nucleus is haploid.

Assumption (b) states that the increase in the number of *F*[−] cells results from division of tetranucleated cells into binucleated cells and that at cell division the nuclei assort in a specific and regular way, so that two sister nuclei pass into the same daughter cell. The staining experiment supports the assumption as to the number of nuclei. If at cell division the nuclei assort randomly instead of in a regular manner, the number of *Lac*^o cells would never decrease. The results also exclude any specific way of assortment that would predict increase or persistence of

Lac^o cells. Thus, sister nuclei must be transmitted to the same daughter cell, as suggested by serial photographic observations on dividing cells.²² In addition, the fact that the formation of a zygote does not result in the formation of a cell with an uneven number of nuclei suggests that an *F*⁻ chromosome doubles whether or not it engages in recombination; that is, a recombinant chromosome does not exist as an "extra" chromosome in the cell.

Assumption (c) states that the chromosome fragment derived from the *Hfr* parent does not engage in recombination more than once. If repeated recombination occurred between the *Hfr* fragment or its replicas and the *F*⁻ nonrecombinant or heterozygous recombinant chromosome, the cells containing such fragments would be scored as *Lac*^o cells as long as they persisted. The fact that *Lac*^o cells do not increase in number but segregate to pure *Lac*⁺ and *Lac*⁻ cells within a few generations indicates that repeated recombination, if it occurs, must be infrequent.

Assumption (d) states that recombination does not take place between the established chromosomes of an *F*⁻ cell. Early segregation excludes the possibility of repeated recombination between established recombinant and nonrecombinant chromosomes.

Assumption (e) states that all the descendants of a zygote are viable. We have described results showing that nondividing cells cannot represent a majority of cells derived from zygotes. However, it is of critical importance for the present discussion to know whether or not the cells formed immediately after segregation are viable. If a cell which has both *Lac*⁺ and *Lac*⁻ chromosomes segregates into one cell with *Lac*⁺ chromosomes and another with *Lac*⁻ ones and if the latter fails to produce viable progeny, then that cell would form a pure *Lac*⁺ colony. The appearance of pure *Lac*⁺ recombinant colonies might, therefore, result from selective death of *Lac*⁻ cells. If this were the case, the production of a cell with a pair of recombinant chromosomes would require one generation beyond the formation of an apparently pure *Lac*⁺ cell, and two generations would be needed for doubling the number of recombinant cells. The rapid increase in *Lac*⁺ recombinants observed excludes this possibility. Most of the *Lac*⁺ recombinants must, therefore, have been produced when *Lac*^o recombinants segregated into viable *Lac*⁺ recombinants and nonrecombinants.

Having justified our assumptions, we may now turn to the models between which we wish to discriminate (Fig. 1). Model C, which predicts the multiplication of *Lac*^o cells, is incompatible with the experimental results. Models A and B, as far as our experiments are concerned, differ only in their prediction of the time at which pure recombinants appear. Model A predicts that segregation should occur at the second division after genetic recombination, while model B predicts that it should occur at the third division. Our measurements show that most segregations occur between 2 and 3.5 generations after *Lac*⁺ markers first start to enter the *F*⁻ cells. To compare the experimental finding with the predictions, it is necessary to subtract the time required for recombination to occur from the measured interval. How large should this correction be?

First, the distribution of entry times of *Lac*⁺ markers into mating cells covers half a generation time (Fig. 2), which can be subtracted from the upper limit of the measurement. Second, if a *Lac*⁺ marker enters an *F*⁻ cell at a time when the *Lac* region has already duplicated, recombination might not be possible until the next

generation. If so, an additional generation should be subtracted from the upper limit to the measured segregation time. Finally, the interval between the formation of a recombinant chromosome and the subsequent cell division must itself occupy some part of a generation time. These three considerations lead to the conclusion that the minimal observed segregation time, namely two generations, is an appropriate measure of the interval between recombination and segregation, in good agreement with model A, but not with model B or C. We conclude that recombinant chromosomes are usually already homozygous at the time of formation.

We shall now discuss some results obtained with other mating systems which are pertinent to the present problem. Hayes¹⁷ has described the kinetics of formation of $T^+ L^+$ recombinants in zygotes formed in a cross between *Hfr* H ($T^+ L^+ T1^+ T6^s$ *Strep*^s) and P678 ($F^- T^- L^- T1^s T6^r$ *Strep*^r). The number of recombinants stayed constant for the first few generations after mixing the parental cultures and then increased exponentially. The essential features of his result were confirmed in our studies. Under our standard procedure, $T^+ L^+$ recombinants begin to increase at about 100 minutes after mixing, which is also the time at which the number of *Lac*⁺ plus *Lac*^s cells begins to increase in the system described above. The kinetics of segregation of $T^+ L^+$ recombinants is unknown, but the following result of Hayes¹⁷ is suggestive. In his cross about 75 per cent of $T^+ L^+$ recombinants are $T1'$. The number of genetically $T1'$ recombinants begins to increase a few generations after mixing, but phenotypic expression of phage resistance is delayed. The resistance begins to be expressed when the number of the recombinants starts to increase and is fully expressed during the subsequent three or four generations. Hayes interprets his result in the following way. The gene $T1'$ is recessive to $T1^s$ and, even after segregation, the genetically pure $T1'$ cells do not express the resistant phenotype immediately. The fact that some cells express the resistant phenotype at the time when the number of recombinants begins to increase indicates that by the time there is an increase in the number of recombinants, some recombinants are genetically pure and have passed the phenotypic lag period. This result again suggests that genetically mixed recombinant cells do not multiply as such.

Lederberg¹⁹ described pedigree analyses of zygotes in a mating system similar to the present one. He found that, usually at the second division, three out of four cells in a clone were pure nonrecombinant type, while the fourth was still generating both parental and recombinant types. These became pure at the third or fourth division. Lederberg also mentions that repeated recombinations were rare events. His results do not generally conflict with the present ones. The results reported by Anderson²⁰ for a mating system similar to that used by Hayes¹⁷ are quite different, however. Segregation of pure recombinant clones occurred only after three to nine divisions, repeated recombinations were rather frequent, and many cells were nonviable. Also, delayed segregation was described by Lederberg²³ in crosses between *E. coli* strains and by Luria and Burrous²⁴ in a cross between *E. coli* and *Shigella*. Differences in mating strains, cultural conditions, and technique may underlie these discrepancies. We found slight differences in the kinetics of segregation by different substrains of W1. We also observed considerable differences under different cultural conditions, especially in different media. Differences in techniques may also be im-

portant. In the mass culture method we used, the F^- cells are separated from Hfr cells after relatively short contacts, while in pedigree analyses those pairs that remain in contact for a relatively long time tend to be selected. The selection might be nonrandom in other respects if a choice is made on morphological differences. Prolonged contact between mating cells may cause metabolic and divisional disturbances in the zygote.

Results of bacterial cross furnish information about the consequences of recombinational acts but do not tell much about the mechanism. A recombinant chromosome could arise by direct incorporation of an Hfr fragment into an F^- chromosome. In this case the present results suggest that both units of the fragment are incorporated simultaneously. Alternatively, recombination may occur by a "copy choice" mechanism²⁵ during replication. In that case the present results show that the assortment is different depending on whether or not recombination has occurred. In ordinary replication, each daughter molecule of DNA contains an old and a new unit.⁷ A recombinant chromosome, however, according to our results contains only new units.

Levinthal²⁶ proposed a mechanism of recombination to account for the findings in phage genetics. It is consistent with the basic features of the DNA duplication scheme of Watson and Crick⁹ and with our results. According to this mechanism, a recombinant strand of DNA is produced that does not remain associated with either of the parental units from which it is copied, but emerges from a mating in a homozygous, or only locally heterozygous, condition.

Summary.—The kinetics of segregation of several genetic markers in *E. coli* K12 are analyzed. The results suggest that the first recombinant chromosome formed from a zygote is homozygous for most of the genetic markers and that generally only one such recombinant chromosome is formed from each zygote. The implication of this finding for the mechanism of genetic recombination is discussed.

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¹ Demerec, M., I. Blomstrand, and Z. E. Demerec, these PROCEEDINGS, **41**, 359 (1955).

² Jacob, F., and E. L. Wollman, in *Symposia of the Society for Experimental Biology*, **12** (New York: Academic Press, 1957), p. 75.

³ Benzer, S., in *The Chemical Basis of Heredity*, ed. W. D. McElroy and B. Glass (Baltimore: Johns Hopkins Press, 1957), p. 70.

⁴ Avery, O. T., C. M. MacLeod, and M. McCarty, *J. Exp. Med.*, **79**, 137 (1944).

⁵ Hershey, A. D., and M. Chase, *J. Gen. Physiol.*, **36**, 39 (1952).

⁶ Levinthal, C., these PROCEEDINGS, **42**, 394 (1956).

⁷ Meselson, M., and F. Stahl, these PROCEEDINGS, **44**, 971 (1958).

⁸ Taylor, J. H., P. S. Woods, and W. L. Hughes, these PROCEEDINGS, **43**, 122 (1957).

⁹ Watson, J. D., and F. H. C. Crick, *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 123 (1953).

¹⁰ Lederberg, J., and E. L. Tatum, *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 113 (1946).

¹¹ Wollman, E. L., F. Jacob, and W. Hayes, *Cold Spring Harbor Symposia Quant. Biol.*, **21**, 141 (1956).

¹² Lederberg, J., in *Methods in Medical Research* (Chicago: Year Book Publishers, 1950), vol. 3, p. 5.

¹³ Skaar, P. D., and A. Garen, these PROCEEDINGS, **42**, 519 (1956).

¹⁴ Cavalli, L. L., *Boll. 1st. Seroter. Milano*, **29**, 281 (1950).

¹⁵ Strains CS 101 and W1 were kindly furnished by Dr. N. D. Zinder.

¹⁶ The designations *Lac*, *T*, *L*, *Pro*, *T1*, *T5*, *T6*, and *Strep* refer to lactose, threonine, leucine, proline, phage T1, phage T5, phage T6 and streptomycin, respectively. The + and - superscripts indicate ability or inability to synthesize an amino acid or ferment lactose. The superscripts *s* and *r* indicate sensitivity or resistance to the agent in question. All strains require vitamin B₁ for growth.

¹⁷ Hayes, W., *J. Gen. Microbiol.*, **16**, 97 (1957).

¹⁸ Robinow, C. F., *J. Hyg.*, **43**, 413 (1944).

¹⁹ Lederberg, J., these PROCEEDINGS, **43**, 1060 (1957).

²⁰ Anderson, T. F., *Cold Spring Harbor Symposia Quant. Biol.*, **23**, 47 (1958).

²¹ McFall, E., A. B. Pardee, and G. S. Stent, *Biochim. Biophys. Acta*, **27**, 282 (1958).

²² Mason, D. J., and D. M. Powelson, *J. Bacteriol.*, **71**, 474 (1956).

²³ Lederberg, J., these PROCEEDINGS, **35**, 178 (1949).

²⁴ Luria, S. E., and J. W. Burrous, *J. Bacteriol.*, **74**, 461 (1957).

²⁵ Lederberg, J., *J. Cell. Comp. Physiol.*, **45 suppl.**, **2**, 75 (1955).

²⁶ Levinthal, C., in *The Viruses*, ed. F. M. Burnet and W. M. Stanley (New York: Academic Press Inc., 1959), vol. 2, p. 281.

EFFECTS OF HEATING DRY BACTERIA AND SPORES ON THEIR PHENOTYPE AND GENOTYPE*

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It has recently been reported that if the cells of *Escherichia coli*, suspended in sucrose solution or dried in air, are heated to 60°C, a high frequency of mutations is produced.¹ The mechanism of the mutagenic action of heat is being studied.² The conditions used above for heating offer some protection to the cells; nevertheless viability is lost at temperatures lower than those known to inactivate the deoxyribonucleic acids (DNA).³ Other methods must be sought if one intends to inflict more frequent genetic damage to DNA without excessive lethality to the cell; and the study of such methods is the object of this paper. It was found that survival at higher temperatures and higher mutation frequencies can be obtained by subjecting vegetative cells (*Escherichia coli*) or spores (*Bacillus subtilis*) to heat in the *dried state in vacuum*. Sufficient survival for determination of mutants was obtained after heating to 135°C to 155°C for 16 min. The corresponding mutant frequencies were 1 to 10 per cent. In the course of this study it was found that in such conditions the vegetative cells of *E. coli* are almost as resistant to heat as the spores of *B. subtilis*; however, the former differ from the latter by exhibiting the phenomenon of pseudoauxotrophy.¹

Methods.—In series 1C, 24-hr-old (37°C) cultures of *E. coli* (a mutant of strain 15, A.T.C.C. 9723, which is somewhat more resistant to heat) were centrifuged, the sediment transferred to 5-ml glass drying bulbs and dried over P₂O₅ in vacuum. The bulbs were evacuated (2×10^{-2} mm Hg), sealed, totally submerged in boiling water for 3 to 6 hrs (Table 1) and opened. The contents were suspended in 1 ml