HAYES, W. (1957). *J. gen. Microbiol.* **16**, 97-119

The Kinetics of the Mating Process in *Escherichia coli*

By W. HAYES

*Department of Bacteriology, Postgraduate Medical School of London,
London W. 12*

SUMMARY: When broth cultures of donor (HfrH) and recipient (F⁻) strains of *Escherichia coli* K-12 are mixed, zygotes are formed by the transfer of part of the donor chromosome to the recipient cell. The donor parent thus becomes dispensable as soon as transfer is accomplished. The kinetics of zygote formation can therefore be studied by treating samples, removed at intervals from a parental mixture, with virulent bacteriophage to which only the donor parent is susceptible. Only zygotes already formed at the time of treatment can segregate a recombinant cell. A lag of 8-10 min. precedes a linear rise in the number of zygotes when selection is made for inheritance of the donor nutritional markers T⁺ L⁺ only. The formation of zygotes inheriting the marker Lac⁺ as well as T⁺ L⁺ shows a lag of about 18 min. These lag periods represent the times required for the genes T⁺ L⁺ and Lac⁺, respectively, to enter the F⁻ cells and confirm the finding of Wollman & Jacob (1955) that chromosome transfer is an oriented process and that the donor genes penetrate the F⁻ cell in the same order as their arrangement on the chromosome. The process of zygote formation in the equivalent F⁺ × F⁻ cross has also been studied by the phage method. Although the yield of T⁺ L⁺ recombinants is c. 2 × 10⁴ times less than in the Hfr × F⁻ cross under the same conditions, the times of entry of the donor genes T⁺ L⁺ and Lac⁺ are the same in both crosses. In the Hfr × F⁻ cross, significant zygote formation does not occur in unsupplemented buffer but requires the presence of both glucose and sodium aspartate. Zygote formation is a temperature-dependent process which occurs in the absence of multiplication of either parent and is unaffected by the presence of deoxyribonuclease. The number of zygotes (and therefore of recombinants) formed in a given time is a function of two independently variable factors: (i) the frequency and intimacy of chance contacts; (ii) the speed of chromosome transfer which is related to energy production. Decrease of temperature from 37° to 32° about doubles the time required for any given Hfr gene to be transferred to an F⁻ cell. Alteration of the parental population density, or the pH values of the medium (Fisher, 1957*b*), does not affect the times of entry of Hfr genes into the F⁻ cells but does modify the rate of effective contact formation. Segregation of haploid recombinant cells from Hfr × F⁻ zygotes, at 37°, takes place in nutrient broth at about 140 min., and on minimal agar at about 160 min., after mixing the parental suspensions. The phenotypic expression of resistance to sodium azide, inherited from the Hfr parent, commences shortly after the zygotes are formed and becomes complete just before segregation; resistance to phage T₁, however, is not expressed at all until the time of segregation, and requires four generations of the recombinant segregants for completion.

Previous studies of the kinetics of recombination in *Escherichia coli* K-12 were undertaken by Nelson (1951) and by Marguerite Vogt (1952, personal communication) with the aim of obtaining quantitative information about the nature of the cellular contacts which initiated recombination, and of defining

* This work was commenced at the Division of Biology, California Institute of Technology, Pasadena, under a grant from the National Science Foundation, Washington D.C., while the author was on leave of absence from the Postgraduate Medical School of London.

the optimal conditions for mating. Both these studies preceded the discovery that recombination is mediated by contact between cells of different mating type. The cells of one type (F+) act as genetic donors and of the other type (F-) solely as recipients, so that mating involves a one-way transfer of genetic material from F+ to F- cells. Crosses between F- cells are sterile (Hayes, 1952; Lederberg, Cavalli & Lederberg, 1952; Cavalli, Lederberg & Lederberg, 1953; Hayes, 1953 *a, b*).

The maximum yield of recombinants from F+ $\times$ F- crosses is of the order of one per 10⁴ to 10⁵ parental cells. However, mutant donor strains have been isolated which show a very much higher degree of fertility than the F+ strain from which they were derived (Cavalli, 1950; Hayes, 1953*b*). These strains are termed 'Hfr' on account of their high frequency of recombination. One of them (HfrH) regularly yields one recombinant prototroph per 10 F- recipient cells when equal numbers of the two strains are mixed (Hayes; unpublished). Such a high frequency of recombination implies at least as high an efficiency of mating. In fact, conjugation between pairs of cells in mixtures of Hfr and F- cultures is readily observable microscopically (Lederberg, 1956). Hfr $\times$ F- crosses are thus well suited to kinetic analysis.

THEORETICAL BACKGROUND

The mating process begins when an effective contact is established between a donor and a recipient cell, and ends with the appearance of a haploid recombinant cell in which those characters inherited from the donor parent are functionally expressed. Several intervening steps can arbitrarily be distinguished. Once effective contact is made, the chromosomal contribution of the donor cells is transferred to the recipient cell to form a zygote. The function of the donor cell is then fulfilled and its further participation is not required. Within the zygote, a process formally analogous to crossing-over then occurs which leads to the creation of a recombinant chromosome.

Certain important assumptions may be made as to the constitution and behaviour of the zygote. First, genetic analysis of F+ $\times$ F- crosses reveals a continuous system of linkage uniting all of the large number of genetic markers of *Escherichia coli* K-12 (Clowes & Rowley, 1954; Cavalli-Sforza & Jinks, 1956) so that the haploid nucleus of this organism may be assumed to comprise a single chromosome. Secondly, the majority of the characters inherited by recombinants are derived from the recipient parent, suggesting that the donor cell usually contributes only part of its chromosome to the zygote (Hayes, 1953*a, b*). This theory of partial transfer has recently been substantiated (see Wollman, Jacob & Hayes, 1956). The zygote therefore contains the complete chromosome of the recipient cell, but only part of the chromosome of the donor cell, and consequently cannot yield more than one viable recombinant cell, unless replication of the parental chromosomal contributions occurs before recombination. Thirdly, it may be concluded that the zygote does not usually divide before recombination since each recombinant colony, representing the selected progeny of a single zygote, tends to contain only cells of one genotype (Leder-

berg, 1947); if the zygote divided before recombination to produce two or more daughter zygote cells, each of these would often yield a different type of recombinant prototroph so that prototroph colonies stemming from single, fertilized recipient cells would tend not to be homogeneous.

Following recombination of genes within the zygote, segregation occurs so that a haploid cell possessing the recombinant chromosome is produced. Since this cell alone is able to multiply on the selective medium designed to demonstrate its occurrence, it will proceed to divide to form a colony of recombinant cells. But the cytoplasm and cell wall of the zygote are those of the F-recipient cell so that not all of the genes inherited from the donor parent may be capable of functional expression immediately after segregation. The presence or absence of delay in the phenotypic expression of any character will depend upon the particular way in which the controlling gene determines the character. For example, a character which is directly expressed through synthesis of an enzyme may be expected to become manifest more rapidly than one, such as resistance to bacteriophage, which requires a radical reorganization of cell-wall structure.

In the work to be described, the stages of zygote formation, segregation and phenotypic expression in an Hfr $\times$ F- cross are experimentally defined and analysed.

DEVELOPMENT OF METHODS

The kinetics of zygote formation

In previous kinetic studies, such as that of Nelson (1951), suspensions of the two parental strains were mixed in a fluid medium and, at intervals thereafter, samples were withdrawn and plated on minimal agar which permitted the growth of prototrophic recombinants while suppressing that of the auxotrophic parents. When, in an experiment of this kind, the number of prototrophic colonies is plotted as a function of time, a curve is obtained which rises linearly from the origin. This curve must describe the rate at which irreversible unions are formed between donor and recipient cells in the mixed population, since the sole effect of diluting and plating the samples is to separate those cells which have not united firmly, and to prevent further contacts. The pairs of cells thus transferred to the minimal agar can continue to mate on the plate and may produce prototrophic recombinants. These unions must be established very rapidly since the curve expressing the rate of their formation begins to rise from zero time.

It is clear that this method can yield no information about the kinetics of zygote formation since it only scores the total number of mating pairs present in the population at any given time, which yield a prototrophic recombinant. It cannot distinguish between freshly formed unions and those which may already have mediated genetic transfer.

The basic function of the donor cell in recombination is to transfer part of its chromosome to the recipient cell to form the zygote. As soon as the zygote has been formed, but not before, the donor cell plays no further role in mating and is dispensable. If, therefore, it were possible to kill the donor cells rapidly

and selectively, in samples removed at intervals from a mating mixture, so that chromosomal transfer between mating pairs was prevented or arrested, recombinants arising from the treated mixture should be derived only from those zygotes which had already been formed at the time of treatment. One of the virulent bacteriophages of the 'T' series seemed to be an ideal agent for this purpose. These phages irreversibly inhibit the metabolic activities of the sensitive host cell almost immediately after they are adsorbed (Cohen, 1947), while bacterial strains resistant to them can easily be obtained by mutant selection.

One of the characteristic features of the Hfr donor strain used in this study is that only a limited number of Hfr genetic markers is inherited with significant frequency among prototrophic recombinants (Hayes, 1953*b*; Wollman & Jacob, 1954; see Fig. 1). Among the Hfr markers which are not inherited is sensitivity (or resistance) to phage T_3 which therefore seemed to be the ideal choice since, if the Hfr parent were sensitive and the F— parent resistant, all the zygotes (i.e. fertilized F— cells) and their prototrophic progeny would be resistant so that no distortion of segregation ratios would follow phage treatment. Preliminary experiments, however, revealed two disadvantages in the use of phage T_3 :

(1) Since the original auxotrophic Hfr donor strain was inherently resistant to phage T_3 , a sensitive Hfr derivative of this strain, obtained by recombination, had to be used instead. This strain had fewer differential genetic markers than the original strain so that genetic analysis of recombinants was limited.

(2) The high-titre phage T_3 preparations, necessary to ensure high multiplicity of infection, frequently contained sufficient host-range mutants to initiate visible lysis of the resistant F— population under the experimental conditions then observed. For these reasons phage T_6 , to which the original Hfr strain (as well as the analogous F+ strain, 58-161) is sensitive and in which host-range mutants have not been found (Doermann, 1953), was substituted for phage T_3 and was found satisfactory. Unlike phage T_3 sensitivity, the sensitivity to phage T_6 of the Hfr (or F+) parent is normally inherited by c. 20 % of prototrophs (Fig. 1). This proportion was found to remain unchanged despite treatment of the parental mixture with phage T_6 since the subsequent dilution (or washing) of the treated mixture reduces to insignificance the probability of residual phage particles contacting sensitive recombinant segregants on the surface of the selective medium on which they are spread. The use of phage T_6 antiserum to neutralize surplus phage after treatment is, therefore, unnecessary.

Before studying the kinetics of zygote formation by means of the phage method, it is necessary to define the word 'zygote' in operational terms. Fig. 1 is a schematic representation of the chromosomes of the donor (Hfr or F+) and recipient (F—) strains employed, showing the relative positions of the genes controlling various characters to which reference will be made (see Cavalli-Sforza & Jinks, 1956). That part of the donor chromosome represented by an interrupted line is not transferred to the zygote by the Hfr strain. If the interrupted line is ignored, therefore, Fig. 1 portrays the constitution of an

average zygote from the Hfr $\times$ F $-$ cross. To demonstrate recombinants of any given class in such a cross, selection is made, in effect, for inheritance by the F $-$ cell of the Hfr gene or genes which characterize this class (Hayes, 1953*b*). Thus on minimal agar supplemented with vitamin B₁ (thiamine) the F $-$ cell cannot grow because it lacks the ability to synthesize threonine and leucine (T $-$ L $-$). When zygotes are plated on this medium, therefore, selection is, in effect, made for recombinants possessing an F $-$ chromosome which has inherited that part of the donor chromosome carrying the closely linked genes T $+$ and L $+$, so that a prototrophic (T $+$ L $+$) recombinant cell is produced. When recombinants are selected in this way, on the basis of inheritance of T $+$ L $+$ alone, it is found that the frequency with which the other, unselected,

Fig. 1. The order of arrangement of various loci on the *Escherichia coli* K-12 chromosome (after Cavalli-Sforza & Jinks, 1956). The probable positions of the loci are only approximately represented since the map is only intended to clarify some concepts explained in the text. That part of the HfrH chromosome which is not transferred to the F $-$ cell during mating is shown as an interrupted line. The symbols represent:

T = threonine	} synthesis	Az = sodium azide	} resistance or sensitivity	Lac ₁ = lactose	} fermentation
L = leucine		S = streptomycin		Mal ₁ = maltose	
M = methionine		T ₁ = phage T ₁		Xyl ₁ = xylose	
B ₁ = vitamin B ₁		T ₆ = phage T ₆			

'O' represents the extremity of the HfrH chromosome which first penetrates the F $-$ cell (Wollman & Jacob, 1955). The phage T₃ locus is not marked since its position has not yet been mapped; but it is known to be situated on that part of the HfrH chromosome shown as an interrupted line.

markers are inherited among them varies. Thus about 90% inherit the Hfr marker Az^r (resistance to sodium azide), about 75% the marker T₁^r (resistance to phage T₁) and about 40% the marker Lac⁺ (ability to ferment lactose). Since the more closely two markers are linked together on the chromosome the greater is the likelihood that they will be inherited together, these frequencies are inversely related to the distance each marker is situated on the chromosome from the selective markers T⁺ L⁺, as Fig. 1 shows. Similarly, the F $-$ cell does not ferment lactose (Lac⁻) so that if glucose is replaced by lactose in the minimal agar, selection is now made for another type of recombinant in which the F $-$ chromosome acquires, by crossing-over, both T⁺ L⁺ and Lac⁺ from the donor parent. In such a case it is usually found that the whole donor chromosomal segment, TL—Lac, is inherited since the great majority of T⁺ L⁺ Lac⁺ prototrophic recombinants also possess the intervening unselected Hfr markers (Az^r and T₁^r).

In all early experiments defining the kinetics of zygote formation, selection was made for T⁺ L⁺ recombinants only. Thus, in these experiments, the term 'zygote' means, operationally, an F $-$ cell to which the genes T⁺ L⁺

have been transferred and which is therefore potentially able to segregate a T+ L+ recombinant. It is unlikely, from the genetic data, that every zygote which receives the T+ L+ genes in fact produces a T+ L+ recombinant, so that the actual number of recombinants observed should be regarded as proportional, but not equivalent, to the total number of zygotes formed.

The kinetics of segregation

Segregation is the process whereby the T+ L+ recombinant chromosome, which has already been formed within the diploid zygote, attains an independent position in a haploid cell. The situation is complicated by the fact that what is called a bacterial cell normally possesses two to four chromatinic bodies which are assumed, with reason, to be identical nuclear analogues (see Robinow, 1946). Hence when we refer to a zygote (i.e. a fertilized F- cell) we really connote a structure at the nuclear rather than at the cellular level, for if a part of only one Hfr chromosome enters the F- cell, as the usual clonal nature of recombinant colonies suggests, it presumably pairs with only one of a possible four of its F- homologues with the result that three of the nuclei of the fertilized F- cell will be haploid F- while the fourth will be diploid Hfr/F-. It is thus to be expected that the zygote cell, after recombination and segregation, will produce one or more F- cells in addition to whatever segregants the diploid nucleus may yield. This has been demonstrated experimentally by allowing freshly formed zygotes to segregate without selection on nutrient agar; every colony which was found by replica plating (Lederberg & Lederberg, 1952) to contain a prototrophic recombinant was also found to contain F- cells (Hayes, unpublished). With regard to the process of recombination, the main point of interest is the time at which that component of the cell containing the diploid nucleus divides to liberate a haploid recombinant. The interval between the formation of the zygote and its segregation defines, for future analysis, the vital period during which the process of recombination proper takes place.

The time at which the recombinant haploid cells issuing from the zygotes undergo their first and subsequent divisions, either on minimal agar or in broth, is easily ascertained. Young nutrient broth cultures of Hfr and F- cells are mixed and aerated at 37° for 30 min. to allow zygotes to form. The Hfr parental cells are destroyed by adding a high multiplicity of phage T₆ and aerating for 10 min. so that only zygotes and F- cells remain. Such a preparation is called a 'zygote suspension'. To assess the time of segregation on minimal agar, a series of minimal agar (+ vitamin B₁) plates, at 37°, is inoculated with diluted zygote suspension so as to yield, after incubation, about twenty to thirty prototroph colonies/plate, each colony being composed of the progeny of a T+ L+ recombinant segregant issuing from a single zygote. At intervals after inoculation and incubation, the surfaces of the plates, in turn, are vigorously rubbed with distilled water by means of a glass spreader. This separates the progeny of any T+ L+ recombinants that have already divided at the time of rubbing so that the subsequent colony count is doubled for each generation. Prior to division, of course, the colony count remains constant

since the effect of rubbing is simply to alter the position of the zygotes or segregants on the plate.

To study segregation in nutrient broth, the zygote suspension is diluted 1/50 into fresh broth at the desired temperature. Samples are removed as a function of time, appropriately diluted and plated on minimal agar to select for T + L + recombinants. As before, every division is reflected in a doubling of the count of prototroph colonies.

The number of colonies obtained in such experiments remains static for a period and then, usually abruptly, commences to increase exponentially. The time at which this increase begins represents the time when the first division of the recombinant cells is initiated and is assumed to coincide with their first appearance as independent units (see Fig. 5, solid curves). Although we cannot be sure that this assumption is correct, it is at least certain that segregation has occurred at this time.

The kinetics of phenotypic expression

The Hfr genes determining resistance to sodium azide and to phage T₁ are closely linked to the selective markers T + L + and are inherited respectively by about 90 and 75 % of T + L + recombinants (see Fig. 1). For this reason, and because they manifest their effects in very different ways, these markers are well suited to the study of phenotypic expression. The experiments are run in parallel with those to determine the kinetics of segregation, either on minimal agar or in nutrient broth, but selection is made for the inheritance and expression of resistance, as well as for inheritance of prototrophy from the Hfr parent, by exposure to either sodium azide or phage T₁ at the time of sampling. Since, under the conditions employed, these agents prevent any further division of sensitive organisms, only those cells, whether zygotes or T + L + segregants, which have inherited the gene controlling resistance, and in which the character of resistance has become expressed, can produce colonies. When the proportion of T + L + recombinant colonies obtained in the presence of the drug reaches the proportion of T + L + recombinants which have inherited the gene for resistance (i.e. c. 90 % for resistance to sodium azide and c. 75 % for phage T₁ resistance), expression is said to be complete.

Additional details of the experimental techniques used to demonstrate these methods will be found at the end of the next section.

MATERIALS AND TECHNIQUES

Bacterial strains

The great majority of the experiments to be described employed variants of two strains of *Escherichia coli* K-12: (1) The methionine-requiring (M-) strain, HfrH, isolated and described by Hayes (1953*b*); Hfr derivatives from this strain were occasionally used when alternative unselected markers were needed in the Hfr parent. Such derivatives were obtained either by recombination in crosses with a suitable F⁻ parent (Hayes, 1953*b*) or by simple selection of

spontaneous mutants, and showed no variation from the parent strain with respect to the properties under investigation.

(2) An auxotrophic K-12F- strain called W-1 (Lederberg & Lederberg, 1952) which requires threonine, leucine and vitamin B₁ for growth (TLB₁-) and does not ferment lactose (Lac₁-) or maltose (Mal₁-). This strain was preferred to the classical TLB₁-F- strain, W-677, which was derived from it and which it closely resembles in its genetic markers, since some experiments demanded an F- parent sensitive to phage T₁ to which strain W-677 is resistant. The derivative of strain W-1 actually used was resistant to streptomycin (S^r) and to phages T₃ and T₆ (T₃.T₆), and was obtained from the parent strain in three steps by simple selection of spontaneous mutants.

Other strains occasionally employed will be specified in the text. All strains were lysogenic for λ phage.

Media

Minimal agar. The basic medium was that of Tatum & Lederberg (1947), solidified with 2.0 % (w/v) powdered New Zealand agar which had been washed, by suspension in a muslin bag, in several changes of distilled water during 48 hr. The medium was supplemented before use with either glucose or lactose (0.25 %, w/v) depending on the selective requirements, and, unless otherwise stated, with vitamin B₁ (thiamine) (5.0 μ g./ml.). Sodium aspartate was usually added to a final concentration of 0.1 % (w/v) for reasons to be discussed later.

Nutrient broth. This was, in general, the tryptic digest of beef routinely employed by this Department. Latterly, however, a yeast extract + casein hydrolysate medium (0.5 % Difco dehydrated 'Bacto' Yeast Extract + 2.0 % Difco 'Bacto' Casamino Acids, Technical, in distilled water; pH adjusted to 7.4) was used.

Nutrient agar. This was made from the nutrient broth described above.

Buffer. An aqueous solution containing (% w/v): NaCl, 0.4; MgSO₄.7H₂O, 0.02; Na₂HPO₄, 0.7; KH₂PO₄, 0.3. The required pH value (usually 7.2) was obtained by addition of NaOH or HCl solution.

Basic technical procedures

Maintenance of stock cultures. Stock cultures were maintained at 4° on Dorset's egg medium in screw-capped bottles. Every 4-6 weeks, overnight broth cultures from the stock cultures were diluted and spread on blood agar plates so as to yield *c.* 50-100 colonies/plate. The plates were incubated overnight at 30° so that the colonies developed to only a small size, and were then stored at 4°.

Preparation of working cultures. The starting-point of most experiments was logarithmic phase nutrient broth cultures of the Hfr and F- parental strains. The evening before an experiment a single colony of each of the required strains was subcultured, from the blood agar plates maintained at 4°, to nutrient broth and incubated overnight at 37°. Cultures for use in the experiment were prepared by inoculating the desired volume of nutrient broth, warmed to 37°, with 1/10 vol. of the overnight broth culture and then aerating

at 37° for 1½ hr. Control experiments showed that these cultures were at about the middle point of the logarithmic phase of growth and had a viable count of $c. 2.5 \times 10^8$ organisms/ml.

Preparation of washed suspensions and mixtures. Unless otherwise stated, cultures were washed in three changes of buffer (pH 7.2) at room temperature. In most experiments involving comparison of the rates of zygote formation in different media or under different environmental conditions, washed suspensions of each parent were resuspended to 1/10 their original volume in buffer and 0.5 vol. of each were then added separately to 9.0 vol. of the test medium, so that the final population density was equivalent to that of a mixture of the original cultures ($c. 1.25 \times 10^8$ organisms/ml. of each parent).

Aeration. Cultures or suspensions were aerated in screw-capped bottles clipped flat to the periphery of a gramophone turntable, inclined at 45° to the horizontal and rotating at 33 r.p.m. in an incubator. The bottles were usually less than half full.

Viable counts. Appropriate dilutions of the suspension were made in buffer. Standard loopfuls were then transferred, by means of a welded platinum loop, to the surface of the agar medium and spread over areas of $c. 3$ cm. diam. Seven such areas can be accommodated on a plate of 9 cm. diam. The standard loop was calibrated by weighing and contained $c. 0.0125$ ml. The counts recorded here are, in general, the average of triplicates. When a higher degree of precision was required, counts were made in sextuplicate. Colonies were enumerated after overnight incubation, at 37° in the case of recombinant colonies on minimal agar, and at 30° for total viable counts on nutrient agar. Under these conditions the colonies, although easily visible without magnification, are small and as many as $c. 250/3$ cm. diam. area can be counted with accuracy.

Preparation of bacteriophage (T_6) suspensions. A suspension of the phage was added, at low multiplicity, to an exponentially growing nutrient broth culture ($c. 10^8$ viable organisms/ml.) of *Escherichia coli* strain B and aerated at 37° until clearing occurred. Residual viable bacteria were killed by heating at 58–60° for 20 min. in a water bath; further clarification by centrifugation was rarely required. Phage titres were usually 10^{10} – 10^{11} plaque-forming particles/ml. Phage T_6 , which was routinely used throughout this work, was the wild-type r^+ strain and had approximately the same efficiency of plating on *E. coli* K-12 strains as on *E. coli* strain B.

The techniques of kinetic analysis

Zygote formation. Suspensions of the two parents were mixed, usually in equal proportions, in a fluid medium warmed to the temperature at which the experiment was to be conducted. The mixture was aerated at that temperature. At intervals after mixing, 1.0 ml. samples were rapidly transferred to 1.0 ml. of phage T_6 suspension in nutrient broth, contained in screw-capped bottles of $c. 4.0$ ml. capacity. The mixture was shaken by hand for a few seconds and then aerated for 10–20 min. at 37°. In most experiments the multiplicity of phage T_6 infection was of the order of 50–100/Hfr (or F+) cell, giving a very

rapid rate of adsorption. The viable count of Hfr cells was reduced at least 1000-fold by the treatment. In $Hfr \times F^-$ crosses, the treated mixture was diluted in buffer and plated, without washing, on the selective medium. The extent of dilution required obviously depends on the experimental conditions: in media allowing optimal zygote formation, a final dilution of 10^{-3} to 10^{-4} was usually used. The amount of nutrient broth carried over to minimal medium in inocula from 10^{-2} dilutions was insufficient to allow visible growth of auxotrophic strains. In the case of $F^+ \times F^-$ crosses the treated mixture was washed 2-3 times, resuspended to (usually) $\frac{1}{4}$ - $\frac{1}{2}$ vol. in buffer and plated without dilution. Three washings are required when selection is made for inheritance from the F^+ parent of the ability to synthesize vitamin B_1 . Using this technique, samples taken immediately after mixing never yielded any recombinant colonies.

Segregation. All the relevant information has already been given under 'Development of Methods'.

Phenotypic expression. To study expression on minimal agar, separate 3 cm. Petri dishes, containing 2.0 ml. minimal agar (+ vitamin B_1 + 0.1 % sodium aspartate), were used for each plating. When the expression of sodium azide resistance was being assessed, the amount of sodium azide rubbed over the surface, in place of distilled water, was 0.025 ml. of an M/20 solution, giving a final concentration in the agar, after diffusion, of M/1600. This concentration was found to be optimally differential. In nutrient agar M/500 sodium azide is recommended for distinguishing resistant from sensitive organisms (Lederberg, 1950), but this concentration in minimal agar inhibits the growth of resistant prototrophs. In the phage T_1 experiments, 0.025 ml. of a washed phage suspension, containing 10^{12} plaque-forming particles/ml., was used.

To study expression in nutrient broth, the samples were spread on each of two series of minimal agar (+ vitamin B_1 + 0.1 % sodium aspartate) plates, one of which was not supplemented further and was used to assess the kinetics of segregation; plates of the other series either contained M/1600 sodium azide, or had been preseeded with 0.1 ml. of the high-titre, washed, phage T_1 suspension.

RESULTS

Analysis of zygote formation in $Hfr \times F^-$ crosses

Young broth cultures of T_6^+ Hfr and T_6^- F^- strains, containing approximately equal numbers of cells, were mixed and aerated at 37°. Samples were withdrawn at intervals, treated with phage T_6 and plated for $T^+ L^+$ recombinants. A typical curve relating the number of recombinants to the interval of time after mixing is given in Fig. 2. Unlike the curves obtained by Nelson (1951) with $F^+ \times F^-$ crosses, which arose from the origin, a lag of 8-10 min. regularly preceded the linear rise in the number of recombinants. Assuming a similar basic mechanism of mating in the two varieties of cross, this lag must result from the killing of the Hfr cells by phage T_6 and therefore represents the time required for transfer of the genes $T^+ L^+$ from the Hfr to the F^- cell; i.e. the time required for zygote formation. Between 30 and 40 min.

after mixing the slope of the curve begins to flatten, presumably due to the decreasing probability of fresh contacts arising between unpaired Hfr and F⁻ cells. Analysis of T⁺L⁺ recombinants derived from samples treated with phage about 45 min. after mixing showed the same percentage inheritance of unselected markers from the Hfr parent as is usual in crosses made *ab initio* on minimal agar. At 60 min. after mixing, the number of recombinants formed

Fig. 2. The kinetics of zygote formation. Logarithmic phase broth cultures of HfrH.M-T₆⁺ and F⁻.TLB₁-T₆⁺ were mixed and aerated at 37°. Samples removed at intervals were treated with phage T₆, diluted, and plated to select the growth of T⁺L⁺ recombinants. Thus the term 'zygote' here connotes an F⁻ cell which, at the time of treatment, had already received the genes T⁺L⁺ from an Hfr cell.

was usually about 10% the number of viable F⁻ cells initially present. Exceptionally the ratio of T⁺L⁺ recombinants to F⁻ cells was as high as 1:3 to 1:5. Decreasing 20-fold the number of F⁻ cells in the mixture, so that the Hfr cells were in gross excess, did not significantly raise the ratio nor alter the slope of the curve.

Physiological and environmental requirements for zygote formation

Nutritional requirements. About the time that the phage method for studying zygote formation was being evolved, it was observed that when thoroughly washed Hfr (or F⁺) and F⁻ cells were plated together on minimal agar prepared with highly purified agar (see Fisher, 1957a) virtually no recombinants developed. When, however, the agar was supplemented with

asparagine (Gray & Tatum, 1944) or, preferably, with sodium aspartate, the expected number of recombinants arose. The minimal effective concentration of sodium aspartate was about 0.01 % (w/v). Sodium pyruvate, succinate, malate or fumarate replaced aspartate, though much less efficiently, but the addition of amino acids other than those required for the growth of the parental strains was ineffective. Further investigation revealed:

(1) Prototrophic recombinants derived from a cross on highly purified minimal agar, supplemented with sodium aspartate, grew well, though rather more slowly, when subcultured to minimal agar without aspartate.

(2) Parental mixtures aerated together for 30 min. in broth, treated with phage to kill the donor cells and thoroughly washed as before (i.e. washed zygote suspensions), yielded the same number of recombinant colonies whether sodium aspartate was present or not.

These facts suggested that the stage of mating which was suppressed on purified minimal agar was that of zygote formation, and that the effect of aspartate might be associated with the operation of the Krebs cycle in the mating cells. The phenomenon was therefore investigated quantitatively by means of the phage method. When the parental cultures were washed and mixed in unsupplemented buffer, the number of zygotes formed at 30–45 min. after mixing was usually less than 1 % of the number arising from the same mixture in nutrient broth. Addition to the buffer of either glucose (0.25 %) or sodium aspartate (0.1 %) alone did not appreciably increase the yield of zygotes. When both glucose and sodium aspartate were added, however, the number of zygotes rose to about the same level as that found in broth. When the kinetics of zygote formation was studied in this medium, curves started to rise at 8–10 min. after mixing and began to flatten 20–30 min. later. The recent work of Fisher (1957*a*) has confirmed and greatly extended these findings.

A defined fluid medium which permits optimal zygote formation while restricting the multiplication of both parents is more suitable for the precise analysis of the requirements of this stage of mating than is nutrient broth. The following experiments were therefore carried out in buffer, usually at pH 7.2, supplemented with glucose (0.25 %) and sodium aspartate (0.02 %).

Cellular multiplication and zygote formation. In order to determine whether zygote formation could take place in the complete absence of cellular multiplication, washed parental suspensions were aerated for 30–45 min. in buffer + glucose + aspartate, both separately and after mixing. The number of F-cells (TLB₁–) usually remained constant during this period, but the count of Hfr cells (M–) increased by as much as 25 %. It was suggested by Mr K. W. Fisher that this multiplication of Hfr cells might be due to their endogenous food reserves. The experiments were therefore repeated using washed parental suspensions which were starved, by aeration in unsupplemented buffer at 37° for 30 min., before being mixed in buffer + glucose + aspartate. Under these conditions the rate of zygote formation was only slightly decreased while no increase in the numbers of either parent could be detected by viable counts or nephelometry.

Washed suspensions mixed in unsupplemented buffer usually yield less than 1 % the number of zygotes formed by the same mixtures in buffer + glucose + aspartate. When the suspensions were starved to exhaust their endogenous reserves before mixing in unsupplemented buffer, the yield of zygotes became negligible. Zygote formation, therefore, does not require the multiplication of either parent but is strictly dependent on the presence of glucose and sodium aspartate when other nutrients are absent from the medium (see Fisher, 1957 *a, b*).

Fig. 3. The effect of parental population density on the number of zygotes formed. Cross = $HfrH.M - T_6^+ \times F - .TLB_1 - T_6^+$ in buffer + glucose + sodium aspartate (pH 6.5) at 37°. A series of dilutions of each parental suspension, differing by 25 %, were made in the test medium. Equal vols. of equivalent dilutions of each parental suspension were mixed and aerated at 37°; 35 min. later each mixture was treated with phage T_6 , appropriately diluted, and plated to select the growth of T + L + recombinants. The arbitrary unit was defined as the product of the no. cells/ml. of each parent in the most dilute mixture yielding a significant no. of recombinants (i.e. $Hfr:2.2 \times 10^7 \times F - : 2.0 \times 10^7$).

Effect of parental population densities. Washed parental cultures were suspended separately in buffer + glucose + aspartate (pH 6.5) at 37° to give *c.* 10^8 organisms/ml. A series of dilutions of each suspension was then made in the same medium in such a way that each successive dilution contained 25 % fewer cells than the previous one. Equivalent dilutions of each parent were mixed in equal parts and aerated at 37° for 35 min. Pilot experiments had shown that at this time the number of zygotes in the undiluted mixture was approaching a maximum. The mixtures at every dilution were then treated with phage T_6 , suitably diluted and plated for T + L + recombinants. The yield of recombinants from each dilution was found to be proportional to the product of the population densities of each parent (cells/ml.) as shown in Fig. 3. In conformity with the conclusions of Nelson (1951) in the case of

$F+ \times F-$ crosses, this finding, together with the initial linearity of the rate of zygote formation (Fig. 2), shows that the frequency with which zygotes are formed in $Hfr \times F-$ systems is a function of the probability of collision between two parental cells.

Effect of temperature on zygote formation. Equal parts of washed parental suspensions were mixed in buffer + glucose + aspartate at 44°, 37°, 32°, 25° and 4°. After incubation at these temperatures for 45 min., each mixture was treated with phage T_6 and plated for $T+L+$ recombinants as before. The numbers of recombinants formed, expressed as a percentage of those formed at 37°, was: 25 % at 44°, 80 % at 32°, 6 % at 25° and less than 1 % at 4°. Zygote formation is thus a temperature-dependent process and its efficiency falls rapidly as the temperature diverges from the optimal at 37°.

Effect of deoxyribonuclease (DNA-ase) on zygote formation. Equal portions of washed parental suspensions were mixed in buffer + glucose + aspartate + $MgCl_2$ (m/200), with and without DNA-ase (200 $\mu g./ml.$), at 37°. The mixtures were aerated at 37° for 30 min. and then treated with phage T_6 and plated as before. The presence of DNA-ase had no effect on the efficiency of zygote formation (cf. Lederberg, 1947).

The kinetics of chromosomal transfer

Wollman & Jacob (1955), in their study of the kinetics of zygote formation in the $HfrH \times F-$ cross, treated samples from the mating mixture in a Waring blender, instead of with virulent phage, in order to separate the mating cells. When untreated samples of the mixture were plated and the number of $T+L+$ recombinants scored as a function of time, a curve was obtained which commenced to rise linearly from the time of mixing, began to flatten about 30 min. later and reached a plateau at about 50 min. This curve is similar to those obtained by Nelson (1951) from $F+ \times F-$ crosses and describes the kinetics of the formation of effective unions between Hfr and $F-$ cells. On the other hand, curves obtained from the same samples after treatment in the blender showed a lag of 8–10 min. as in the kinetic experiments using phage, and thereafter rose to join the first curve at about 50 min. after mixing. Wollman & Jacob then scored the percentage inheritance of various unselected Hfr markers among the $T+L+$ recombinants obtained at intervals after mixing (see Fig. 1). In the case of the untreated samples the unselected markers appeared in their normal frequencies irrespective of the time of sampling. In the treated series, however, the proportion of unselected Hfr markers steadily increased among the $T+L+$ recombinants from successive samples, appearing in a definite sequence which corresponded to the order in which the genes are arranged on the chromosome as determined by genetic analysis. Thus the Hfr character T_1 was first found in $T+L+$ recombinants within a few minutes of their appearance; but the first appearance of $Lac+$ was delayed until 18 min., and of $Gal+$ until about 28 min. after the parental suspensions were mixed. These results indicated (Wollman & Jacob, 1955) that the chromosome of the donor strain, $HfrH$, always enters the $F-$ cell by an extremity, O , which is followed by the genes $T+L+$ 8 min. later and then by the other loci at

intervals of time proportional to their distance from O on the chromosome. The transfer of the entire chromosomal segment O—Gal occupies roughly 30 min. The effect of treatment in the blender is to separate the mating cells and thus to break the chromosome during its transfer so that only that fragment which has already entered the F— cell at the time of treatment can participate in subsequent recombination.

These important findings of Wollman & Jacob have been fully confirmed by using the phage method to kill the Hfr parental cells, instead of separating the mating pairs by agitation. For example, at 37°, the times of entry into the F— cell of the Hfr loci T + L + and Lac + were found to be 8–10 and 17–20 min. respectively after mixing, whether the mixture was made in nutrient broth or in buffer + glucose + aspartate (see Fig. 4A).

It thus turns out that the kinetics of zygote formation only become meaningful when expressed in terms of the particular marker or markers transferred to the F— cell. For example, at about 8 min. after mixing an Hfr and an F— culture, zygotes which can segregate a T + L + recombinant begin to be formed; if, however, selection is made for zygotes which can segregate T + L + Lac + recombinants, it is found that such zygotes do not appear until 18 min. after mixing, since the Lac + gene does not begin to enter the F— cells, which have already received T + L +, until this time.

*The influence of environmental factors on the kinetics of
chromosomal transfer*

Fisher (1957*a, b*) has shown that energy is required for zygote formation to enable the Hfr cell to convert a chance collision with an F— cell into an effective union, and thereafter to inject its chromosomal contribution. The dependence of zygote formation upon temperature is due to this requirement for energy. There are also factors influencing zygote formation which do not involve energy-dependent processes. Among these is population density which determines the frequency of chance collisions, and the differences in surface structure, described by Maccacaro (1955) and Maccacaro & Comolli (1956) as distinguishing F + from F— cells, which may well be decisive in permitting chance collisions to become effective unions.

Experiments were devised to analyse the effect of these various factors on the form of the curves describing the kinetics of zygote formation and chromosome transfer in Hfr × F— crosses.

Effect of energy restriction. Diminution of temperature was used to lower the overall capacity of the Hfr cells to produce energy by oxidation of carbohydrate. Two equivalent mixtures of washed Hfr and F— cells were made in buffer + glucose + aspartate (pH 7.2), one at 37° and the other at 32°; these mixtures were then aerated under standard conditions at 37° and 32°, respectively. Samples were removed at intervals, treated with phage T₆ and plated on each of two minimal media, one selecting for all recombinants inheriting the Hfr genes T + L +, the other for recombinants inheriting both T + L + and Lac +. Results are shown in Fig. 4A. At 37°, the genes T + L + (continuous line) begin to enter the F— cells at 8 min. and the gene Lac +

(interrupted line) at 17 min. after mixing, so that transfer of the piece of chromosome from TL to Lac takes 9 min. at this temperature. At 32°, however, T + L + enters at 18 min. and Lac + at 38 min., so that transfer of the same piece of chromosome takes just twice as long at the lower temperature. Since effective unions between Hfr and F- cells are formed very rapidly (see above), it may be assumed that transfer of the O—TL segment of chromosome occupies virtually the whole of the 8 min. lag period which precedes entry of the T + L + genes into the F- cells. At 37°, therefore, transfer of the piece

Fig. 4. The effect of environmental factors on the kinetics of zygote formation and chromosomal transfer. Cross = HfrH.M - T₆ × F- .TLB₁ - T₆ in buffer + glucose + sodium aspartate. At intervals after mixing, samples were treated with phage T₆, diluted and plated on:

- Minimal agar + vitamin B₁ + glucose (selection for inheritance of T + L + only from the Hfr parent).
- Minimal agar + vitamin B₁ + lactose (selection for inheritance of T + L + and Lac +).

(A) Shows the effect of varying the temperature at which zygote formation occurs.
 (B) Shows the effect of varying the concentration of the parental cells, or the pH value of the medium.

of chromosome O—TL takes 8 min. and of the piece O—TL—Lac 17 min.; at 32°, transfer of these same pieces takes 18 and 38 min. respectively. The proportionality between these times strongly suggests that that part of the chromosome between O and Lac, at least, enters the F- cell at a uniform rate which depends exclusively upon the available energy. The fact that the comparable curves of Fig. 4A are parallel, although displaced on the time axis, indicates that the only effect of this degree of limitation of energy is to slow the rate of chromosome transfer.

Effect of varying the frequency of chance collisions. Washed cultures of each parent were suspended separately in buffer + glucose + aspartate (pH 7.2) at 37°. A portion of each suspension was then diluted 1/1.5 in the same medium. Equal volumes of the two parental suspensions at each concentration were

mixed, aerated at 37° and sampled at intervals as before. Results are shown in Fig. 4B. The times of entry of the genes T+L+ and Lac+ into the F- cells remain unaltered when the population density of the parental cells is reduced. In each case the curves arise from the same point on the time axis but their slopes differ.

Fisher (1957*b*) has shown that lowering the pH value of the medium from 7.2 to 6.2 more than doubles the number of zygotes formed. When the kinetics of chromosomal transfer were compared for identical Hfr × F- crosses in buffer + glucose + aspartate at these two pH's, the same kind of result was obtained as when the population density was varied (see Fig. 4B). Since the population density was the same in both crosses, lowering the pH value presumably exercises a surface effect, probably ascribable to an alteration of charge, which increases the proportion of random collisions which can subsequently become effective unions (Maccacaro & Comolli, 1956).

The kinetics of zygote formation in F+ × F- crosses

The blender technique is not well adapted to study of the kinetics of F+ × F- crosses since the high concentrations of parental mixtures which must be plated, after treatment, to give significant prototroph counts will lead to secondary contacts, followed by the occurrence of recombination, on the plate. On the other hand, the phage method, in which the donor parental population is reduced to an insignificant level before plating, is well suited to such an investigation.

Young nutrient broth cultures of the M- F+ strain 58-161 (genotypically identical with strain HfrH) and the TLB₁- F- strain previously used (or the similar strain, W677.T₆) were mixed and aerated at 37°. Samples were removed at intervals, treated with phage T₆, washed, resuspended in the appropriate volume of buffer and finally plated on minimal agar supplemented with:

- (1) Glucose + vitamin B₁ (i.e. selection for inheritance of T+L+ only).
- (2) Lactose + vitamin B₁ (i.e. selection for inheritance of T+L+ and Lac+ from the F+ parent).
- (3) Glucose only (i.e. selection for inheritance of T+L+ and B₁+ from the F+ parent).

The times at which the F+ markers T+L+ and Lac+ began to appear were the same as in the equivalent Hfr cross (i.e. *c.* 8 and 18 min. after mixing respectively). The F+ marker B₁+ began to appear among recombinants about 45 min. after mixing and rose to its usual level of inheritance (*c.* 5.0 %) at about 60 min. When selection was made for the F+ markers Xyl+, Mal+ or S^r (streptomycin resistance) in addition to T+L+ (see Fig. 1) no recombinants were found (i.e. less than 0.5 % of those formed when only T+L+ was selected) up to 90 min. after mixing.

The inheritance among T+L+ recombinants of markers from the donor parent is therefore very much the same whether the cross is made by the classical method of mixing the parental suspensions *ab initio* on minimal agar, or by the method here described of permitting the zygotes to form under

controlled conditions in fluid medium before plating, provided that in the latter method adequate time is allowed for chromosomal transfer before the mating process is arrested with phage (or agitation). The two methods have, however, been found to yield significant quantitative differences in two respects:

(1) When direct plating is used, the frequency with which $T+L+$ recombinants appear in the $Hfr \times F-$ cross is $c. 10^3$ times higher than in the equivalent $F+ \times F-$ cross (Hayes, 1953*b*). Yet when the two crosses are made in aerated broth under the same conditions of population density, and sampled when the number of zygotes has reached its peak, the Hfr cross yields $c. 2 \times 10^4$ times as many prototrophs as the $F+$ cross.

(2) When an $M-Hfr \times TLB_1-F-$ cross is made by direct plating on minimal agar devoid of vitamin B_1 so that the gene B_1+ as well as $T+L+$ must be inherited from the Hfr parent to form a prototroph, the recombination rate is $c. 10^3$ times less than when the same cross is made on minimal agar supplemented with vitamin B_1 . This means that B_1+ is only inherited by $c. 0.1\%$ of the $T+L+$ recombinants formed at high frequency as compared with $c. 5.0\%$ in the equivalent $F+ \times F-$ cross (see Hayes, 1953*b*). Since, however, the recombination rate of the $F+ \times F-$ cross is $c. 10^3$ times lower than that of the $Hfr \times F-$ cross when selection is made for $T+L+$ only, a simple calculation will show that, cell for cell, the Hfr cross yields about 20 times more B_1+ prototrophs than does the $F+$ cross. Nevertheless, when $Hfr \times F-$ zygotes are formed in aerated broth, no $T+L+B_1+$ recombinants have ever been isolated from samples taken up to 90 min. after mixing, although the technique is sensitive enough to detect one B_1+ prototroph in 5×10^5 .

The kinetics of segregation

Experiments to determine the times of segregation and of phenotypic expression in $Hfr \times F-$ crosses, both on minimal agar and in nutrient broth, have given very constant results. The kinetics of segregation is exemplified by the curves (continuous line) shown in Fig. 5. On minimal agar at 37° (Fig. 5A), $T+L+$ recombinants begin to divide about 120 min. after plating (i.e. 160 min. after mixing the parental cultures) and thereafter multiply with a generation time of about 60 min. In aerated broth at 37° (Fig. 5B), division of the recombinant segregants commences at about 100 min. after dilution of the zygote suspension into fresh broth (i.e. about 140 min. after mixing the parental cultures), and the generation time is 20 min.

Two difficulties arise when one tries to infer the actual time of segregation from these findings. First, when zygotes are made in broth (as described above) and aerated without dilution, it is found that no increase in the number of $T+L+$ recombinants occurs for at least 6 hr., whereas division begins 100 min. after dilution into fresh broth. Furthermore, there appears to be a tendency for the early divisions of the segregants to occur in bursts. This suggests that events in the zygotes achieve a measure of synchrony through inhibition of some step preceding segregation, which is released by dilution. The mechanism of this inhibition has not yet been investigated. It poses the

question of whether the timing of segregation should start when the parental cultures are mixed, or when the zygote suspension is diluted. This dilemma may assume importance when it becomes possible to attempt to correlate segregation with events occurring within the zygote during recombination.

The second difficulty is that although the time can be fixed at which T + L + segregants first begin to divide, we cannot be sure that this coincides with the first appearance of the recombinant cells as independent units. For example, physiological delay in the expression of the selective markers used could introduce a lag between segregation and initiation of the first division; the relatively similar periods required for the first division in broth and on minimal agar, in contrast to the threefold increase in generation time on the latter medium (see Fig. 5A, B), make such a lag improbable when T + L + is selected.

The absence of non-recombinant donor cells among zygote progeny

Since the discovery of one-way genetic transfer in *Escherichia coli* there has been controversy whether the absence among recombinants of certain markers of the donor parent is due to transfer of only part of the donor chromosome to the F⁻ cell to form an incomplete zygote (pre-zygotic elimination; Hayes, 1953*a, b*) or to elimination from a complete zygote of part of the donor contribution, either during or after recombination (post-zygotic elimination). The latter hypothesis is supported by diploid analysis (Nelson & Lederberg, 1954). If zygotes from the HfrH × F⁻ cross really receive the complete Hfr chromosome, then a proportion of them should segregate an Hfr parental cell, especially if recombination occurs at the 4-strand stage. The HfrH and F⁻ strains used in the present work yield colonies of such different appearance, when examined by oblique illumination, that Hfr sectors can clearly be observed in F⁻ colonies when the two strains are mixed together and plated on nutrient agar without phage treatment. Thus segregation of an Hfr cell from a zygote should reveal itself as a sector, since colonies arising from zygotes are nearly always similar in appearance to F⁻ colonies. In the course of performing viable counts on parental mixtures, after zygote formation and destruction of the Hfr parent by phage, many thousands of colonies on nutrient agar have been examined, including many hundreds derived from zygote segregation. Only once was an Hfr sector observed, and since a recombinant could not be isolated from this colony there is no evidence that it was indeed derived from a zygote and that the sector did not arise accidentally from an Hfr contaminant which had survived the phage treatment.

The kinetics of phenotypic expression

When the kinetics of phenotypic expression of either sodium azide or phage T₁ resistance was plotted in terms of the generation time of the recombinants, closely similar results were obtained on minimal agar, nutrient agar and in nutrient broth. The patterns of expression of these two markers are quite different. Expression of the character Az^r begins at the time of dilution (or of plating) of the zygotes and then rises exponentially to become complete

just before the recombinants which inherit it start to divide (Fig. 5B: interrupted line). In contrast, the character T_1^+ does not begin to be expressed until after segregation while full expression (i.e. in 75 % of all T + L + recombinants) is delayed until the 4th recombinant generation (Fig. 5A: interrupted line). Work on *Escherichia coli* K-12 diploid strains has shown that the gene T_1^+ is recessive to T_1^- (Lederberg, 1949) so that delay in its expression until after segregation has occurred is to be expected; the fact

Fig. 5. The kinetics of segregation and phenotypic expression.

Cross = $HfrH.M - Az^rT_1^+T_6^+ \times F^- .TLB_1 - Az^sT_1^+T_6^+$.

The methods and techniques employed are described in the text.

that four generations are required before expression is manifest in all the cells which have inherited the gene, is probably a consequence of the dependence of phage resistance on structural changes in the cell wall. Since expression of the character Az^r increases rapidly during a period when the genes Az^r from the donor parent and Az^s from the recipient parent must be present together in the diploid zygote, it follows that Az^r is dominant to Az^s .

DISCUSSION

This study, which has been so notably extended by the recent work of Wollman & Jacob, and of Fisher, has attempted to define and analyse experimentally some of the presumptive stages of mating in *Escherichia coli* K-12. Since a comprehensive account of present knowledge about the mating process will be published elsewhere (Wollman *et al.* 1956), only a few points specifically raised by this paper will be discussed. The conclusion of Wollman & Jacob (1955), that the observed effects of treating mating cells in a blender are due to breakage of the Hfr chromosome during its transit into the F^- cell, has been criticized on the ground that similar effects might result from violent agitation of fully formed zygotes at different stages of pairing or recombination (Lederberg,

1955). This objection also applies to the use of 2, 4-dinitrophenol to arrest genetic transfer (Fisher, 1957*b*) since this agent interferes with the energy utilization of the recipient as well as of the donor cells. Since precisely similar effects are observed to follow selective killing of the donor parent by the virulent phage T_6 , the validity of this objection is greatly weakened. The recipient cells are resistant and do not adsorb the phage which, therefore, could only influence the course of pairing or recombination if its DNA entered the zygote by the same route as the donor genetic material. Although the rare occurrence of such an event has not been excluded it is likely that its outcome, if it did occur, would be death of the zygote from the multiplication of phage within it. The number of recombinants found, and thus the number of zygotes formed, is not decreased by the use of phage. Moreover, Jacob & Wollman (1954) showed that the virulent phage, λ_2 , is not transferred in this way when sensitive HfrH cells mating with resistant F⁻ cells are superinfected with it.

Repeated attempts to obtain kinetic curves, with untreated mixtures, which did not show a lag were unsuccessful until the rather vigorous dilution technique used was replaced by a very gentle one. In fact, the only really reliable method is initially to dilute the samples very gently to 10^{-2} in buffer + glucose + sodium aspartate at 37°, in order to prevent further contacts, and then to incubate the diluted mixture for 10 min. before making the final dilution for plating. The short period of incubation allows the Hfr selective markers, T + L +, to enter the F⁻ partner of all the pairs which had been formed at the time of the initial dilution, so that subsequent separation of these pairs by further dilution and plating does not lower the recombinant count. An intensity of agitation very much milder than that imposed by a blender is thus adequate to produce the Wollman-Jacob effect. It should be stressed that the sensitivity of the rate of zygote formation to small changes in temperature, population density and pH value makes the strict control of these factors vital in comparative studies.

Jacob & Wollman (1956) have produced evidence that the fertility of F⁺ × F⁻ crosses is due solely to the occurrence of Hfr mutants in the F⁺ population. When they analysed crosses involving some of these Hfr mutant strains by the blender method, they found not only that different Hfr strains might transfer different segments of the donor chromosome to the F⁻ cells at high frequency, but that the order in which the same genes were transferred might differ from strain to strain. There is thus evidence that mutation to the Hfr state may be associated with chromosomal rearrangements. Application of the phage method to the kinetics of chromosomal transfer in an F⁺ × F⁻ cross, analogous in genotype and polarity of transfer to the standard HfrH × F⁻ cross, has revealed an identity of the two systems with respect to the timing and the order of entry of the genes T +, L + and Lac +. This suggests that the great majority of Hfr mutants (of the F⁺ strain used) which can transfer these markers are similar to the standard HfrH strain. However, the inheritance of the F⁺ marker B₁ + among T + L + recombinants with a frequency of 5 %, and its entry into the F⁻ cell at 45 min., implies that at least 5 % of Hfr mutants which transfer T + L + also transfer B₁ + at high

frequency, and that a greater length of chromosome than usual is involved. The marker $B_1 +$ is not transferred to the zygote by strain HfrH.

The difference between the kinetics of phenotypic expression of sodium azide and phage T_1 resistance is striking. The fact that resistance to sodium azide is expressed in zygotes which are heterozygous for this character shows that resistance must be dominant to sensitivity to this drug. This is an exception to the rule that wild-type alleles are dominant to their mutant alleles in *Escherichia coli*. On the other hand, the dominance of sensitivity to phage T_1 is confirmed by the fact that resistance to this agent is not expressed until the recombinants begin to divide. This coincidence in timing also suggests that there is no lag between segregation and the initiation of division of the haploid segregants. The exponential nature of the rise in phenotypic expression of both the characters studied may reflect the fact that both are, basically, the result of enzyme synthesis.

Knowledge of the kinetics of phenotypic expression following recombination in bacteria is important for two reasons. First, it serves as a control against which the results of mutation kinetics can be studied. Secondly, it is necessary for the rational design of recombination experiments, whether in *Escherichia coli* or in other bacterial species. For example, the choice of selective markers which are recessive, or whose expression is manifested slowly, is unlikely to yield recombinants from an otherwise fertile cross unless application of the selective agent is withheld until expression has occurred. This is clearly demonstrated by the fact that virtually no recombinants appear if recently formed zygotes, which have inherited from the donor parent the gene determining resistance to sodium azide, are plated directly on to media containing the drug, although resistance is dominant to sensitivity and becomes rapidly expressed.

I wish to express my appreciation of the hospitality and kindness of Professor Max Delbrück, and of the many helpful discussions I enjoyed with him and his colleagues at the California Institute of Technology. I am also much indebted to Professor Boris Ephrussi, in whose laboratory part of the work was done, for his interest in this research, and to the British Empire Cancer Campaign for a grant in support of it. My thanks are also due to Dr J. C. White for a gift of deoxyribonuclease and to Dr G. Popjak for the use of a Spinco ultracentrifuge.

REFERENCES

- CAVALLI, L. L. (1950). La sessualità nei batteri. *Boll. Ist. sieroter. Milano*, **29**, 1.
CAVALLI, L. L., LEDERBERG, J. & LEDERBERG, E. M. (1953). An infective factor controlling sex compatibility in *Bact. coli*. *J. gen. Microbiol.* **8**, 89.
CAVALLI-SFORZA, L. L. & JINKS, J. L. (1956). Studies on the genetic system of *Escherichia coli* K-12. *J. Genet.* **54**, 87.
CLOWES, R. & ROWLEY, D. (1954). Some observations on linkage effects in genetic recombination in *Escherichia coli* K-12. *J. gen. Microbiol.* **11**, 250.
COHEN, S. S. (1947). The synthesis of bacterial viruses in infected cells. *Cold Spr. Harb. Symp. quant. Biol.* **12**, 35.
DOERMANN, A. H. (1953). The vegetative state in the life cycle of bacteriophage: evidence for its occurrence, and its genetic characterization. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 3.

- FISHER, K. W. (1957*a*). The role of the Krebs cycle in conjugation in *Escherichia coli* K-12. *J. gen. Microbiol.* **16**, 120.
- FISHER, K. W. (1957*b*). The nature of the endergonic processes in conjugation in *Escherichia coli* K-12. *J. gen. Microbiol.* **16**, 136.
- GRAY, C. H. & TATUM, E. L. (1944). X-ray induced growth factor requirements in bacteria. *Proc. nat. Acad. Sci., Wash.* **30**, 404.
- HAYES, W. (1952). Recombination in *Bact. coli* K-12: unidirectional transfer of genetic material. *Nature, Lond.* **169**, 118.
- HAYES, W. (1953*a*). Observations on a transmissible agent determining sexual differentiation in *Bacterium coli*. *J. gen. Microbiol.* **8**, 72.
- HAYES, W. (1953*b*). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 75.
- JACOB, F. & WOLLMAN, E. (1954). Induction spontanée du développement du bactériophage λ au cours de la recombinaison génétique chez *Escherichia coli* K-12. *C.R. Acad. Sci., Paris*, **239**, 317.
- JACOB, F. & WOLLMAN, E. (1956). Recombinaison génétique et mutants de fertilité chez *Escherichia coli*. *C.R. Acad. Sci., Paris*, **242**, 808.
- LEDERBERG, J. (1947). Genetic recombination and linked segregations in *E. coli*. *Genetics*, **32**, 505.
- LEDERBERG, J. (1949). Aberrant heterozygotes in *Escherichia coli*. *Proc. nat. Acad. Sci., Wash.*, **35**, 178.
- LEDERBERG, J. (1950). The selection of genetic recombinations with bacterial growth inhibitors. *J. Bact.* **59**, 211.
- LEDERBERG, J. (1955). Genetic recombination in bacteria. *Science*, **122**, 920.
- LEDERBERG, J. (1956). Conjugal pairing in *Escherichia coli*. *J. Bact.* **71**, 497.
- LEDERBERG, J., CAVALLI, L. L. & LEDERBERG, E. M. (1952). Sex compatibility in *Escherichia coli*. *Genetics*, **37**, 720.
- LEDERBERG, J. & LEDERBERG, E. M. (1952). Replica plating and indirect selection of bacterial mutants. *J. Bact.* **63**, 399.
- MACCAGARO, G. A. (1955). Cell surface and fertility in *E. coli*. *Nature, Lond.* **176**, 125.
- MACCAGARO, G. A. & COMOLLI, R. (1956). Surface properties correlated with sex compatibility in *Escherichia coli*. *J. gen. Microbiol.* **15**, 121.
- NELSON, T. C. (1951). Kinetics of genetic recombination in *Escherichia coli*. *Genetics*, **36**, 162.
- NELSON, T. C. & LEDERBERG, J. (1954). Post-zygotic elimination of genetic factors in *Escherichia coli*. *Proc. nat. Acad. Sci., Wash.* **40**, 415.
- ROBINOW, C. F. (1946). Addendum to *The Bacterial Cell* by R. J. Dubos. Cambridge, Massachusetts: Harvard University Press.
- TATUM, E. L. & LEDERBERG, J. (1947). Gene recombination in the bacterium *Escherichia coli*. *J. Bact.* **53**, 673.
- WOLLMAN, E. & JACOB, F. (1954). Lysogénie et recombinaison génétique chez *Escherichia coli* K-12. *C.R. Acad. Sci., Paris*, **239**, 455.
- WOLLMAN, E. & JACOB, F. (1955). Sur le mécanisme du transfert de matériel génétique au cours de la recombinaison chez *Escherichia coli* K-12. *C.R. Acad. Sci., Paris*, **240**, 2449.
- WOLLMAN, E., JACOB, F. & HAYES, W. (1956). Conjugation and genetic recombination in *Escherichia coli* K-12. *Cold Spr. Harb. Symp. quant. Biol.* **21** (in the Press).

(Received 11 July 1956)