

ANTIGENIC AND SURFACE PROPERTIES OF FERTILE
STRAINS OF ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM

by

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	3
Bacterial Recombination	3
Bacterial Conjugation in Species Other Than <u>E. coli</u>	14
Episomes	19
Sex-specific Bacteriophage	22
Surface Properties and Conjugation	24
Antigens and Conjugation	35
MATERIALS AND METHODS	39
Bacterial Strains	39
Media	39
Antigen Preparation	41
Antisera	42
Tube Agglutination Tests	43
Agglutinin Adsorption	44
Antigen Typing	45
Gel Diffusion Precipitation System	45
Electron Microscopy	47
RESULTS	48
Agglutinin Adsorption	48
O and H Antigen Typing	51
Microscopic Gel Diffusion Test	52
Electron Microscopic Examination	55

	PAGE
DISCUSSION	59
CONCLUSION	64
LITERATURE CITED	66

INTRODUCTION

The discovery of a unique type of bacterial genetic recombination in Escherichia coli by Tatum and Lederberg in 1947 led to the discovery of a means of sexual reproduction in bacteria. The mechanism of bacterial conjugation is not only a subject of intensive study in itself, but also leads to basic knowledge of the genetics and cellular organization and regulation of a bacterial cell.

Bacterial conjugation proceeds through a series of steps, beginning with the pairing of sexually compatible cells, followed by genetic transfer from donor to recipient, and terminating with the integration and segregation of the genetic material to produce the offspring recombinants. The formation of stable conjugating pairs is assumed to require a reaction between complementary male and female cell surfaces. It is this stage in bacterial conjugation with which this thesis is concerned.

Although most of the information gathered on bacterial conjugation is based upon the phenomenon as it occurs in E. coli, it is found in other species as well. The existence of mating types in S. typhimurium is now well established. The system originated by crossing an E. coli male with a wild-type S. typhimurium, which served as a recipient. It would be of interest to determine if the surface factors responsible for conjugal pairing in Salmonella are identical with those of E. coli.

This thesis presents the results of antigenic analyses of S. typhimurium and E. coli donor (Hfr)¹ and recipient (F-) cells, as well as E. coli-S. typhimurium hybrids. Immunological interaction between the two species was also sought. Electron microscope photographs and bacteriophage susceptibility tests supplemented the immunological tests.

Following is a list of all symbols and abbreviations in alphabetical order used throughout this thesis: col (colicin); CT (E. coli F- x S. typhimurium Hfr); fer (fertility); F- (female; recipient); F+ (male; donor); Hfr (high frequency donor); infer (infertility); leu (leucine requiring); mut (mutator gene); TC (S. typhimurium F- x E. coli Hfr).

REVIEW OF LITERATURE

Bacterial Recombination

The first evidence of sexual recombination in E. coli was presented by Lederberg and Tatum in 1946, when they found prototrophic recombinants in a mixture of mutants which lacked the ability to synthesize certain growth factors. They also found recombinant characters of resistance to certain bacteriophage (Tatum and Lederberg, 1947). The frequency of recombinants observed, however, was only approximately one in a million of each parental type.

Not only nutritional markers, sugar fermentation, and phage resistance, but also bacterial growth inhibitors such as streptomycin or sodium azide can be used for detecting recombinants in a bacterial population (Lederberg, 1950). Replica plating and indirect selection of mutants, developed by Lederberg and Lederberg (1952), add to the ease and accuracy of detecting bacterial recombinants.

Sexual differentiation and unidirectional transfer of genetic material was suggested by Hayes (1952b) and confirmed by more detailed experiments by Jacob and Wollman (1956) and Hayes (1957). The passage of genetic material from donor to recipient was established by Hayes (1953a) and the unidirectional transfer of this material was confirmed by other workers (Weinberg, 1960; Skaar and Garen, 1956; Cavalli-Sforza et al., 1953).

The non filterability of the agents of genetic recombination in E. coli was first discovered in 1950 (Davis, -1950). Cell-to-cell contact is necessary for recombination to take place, as cell free preparations showed no recombinant activity (Lederberg et al., 1951).

The sexual compatibility scheme was proposed by various experimenters at about the same time. Lederberg et al. (1952) proposed that F- x F- was completely infertile, while F+ x F- and F+ x F+ were both fertile. However, the latter cross is less productive. Hayes (1953b) reported the same relationship, defining strains which were incompatible or sterile between themselves as F- and those strains which were self-compatible and give offspring from a cross with F+ or F- as F+.

At the same time, Hayes suggested that the F+ property was transmissible by an agent called F, and thus, 100 per cent of the offspring were F+. The nature of this F agent will be discussed below.

One of the most important discoveries concerning bacterial conjugation was the isolation of High Frequency Recombination (Hfr) mutants (Hayes, 1953a). Use of these mutants increases the frequency of recombination from 10^{-6} to 10^{-2} or 10^{-3} per parental cell. The recombinants arising from an F+ x F- cross are believed to be due to the small proportion of Hfr mutants in the F+ population (Adelberg and Burns, 1960). Pittard et al. (1963) reported that although the frequency of recombination using F+ is less than 0.1 per cent, Hfrs may produce up to 100 per cent recombination.

The F factor is an extrachromosomal piece of genetic material which can exist in two alternative states: an autonomous cytoplasmic state or an integrated state. Such a genetic element has been defined as an "episome" by Jacob and Wollman (1961). In the autonomous state, the episome is free in the cytoplasm and multiplies independently of cell division; in the integrated state, the episome

is incorporated and replicates with the chromosome (Sneath, 1962). The states may fluctuate (Richter, 1956). More than one F particle per F+ cell is believed to exist, but the exact number is still uncertain (Sneath, 1962). The F particle is replicated at higher levels than genes (Bernstein, 1958). So, not only do the factors determining fertility appear to be under genetic control, but also the rate at which they are replicated.

Thus, sexual differentiation appears to be under the control not of the cellular genome, but of an episomic factor. In the F-, the chromosome is formally closed and the cell contains no sex factor. The F+ has a formally closed chromosome and an autonomous sex factor (Jacob et al., 1960).

F- phenocopies among F+ and Hfr populations may occur under three circumstances: (1) excessive aeration; (2) heavy surface agar growth; and (3) spontaneously (Clark and Adelberg, 1962; Hayes, 1953a). The F- phenocopies are incapable of forming recombinants with F- strains.

One of the characters generally ascribed to episomes is that they may be excluded from their host cell. This may occur spontaneously or in certain artificial environmental conditions (Gundersen et al., 1962). F+ cells may be converted to F- by treatment with certain metallic ions, including cobalt and nickel (Hirota, 1956) and even more effectively by treating the F+ culture with acriflavine or acridine orange (Hirota and Iijima, 1957). The susceptibility of F+ cultures to such disinfection is considered proof that the F factor exists in a cytoplasmic state in F+ cells. In contrast to this, in an Hfr, which is resistant to acridine treatment, the F factor is bound to the chromosome (Hirota, 1960). The action of the acridine dyes is to

eliminate the F factor, perhaps by stopping further multiplication of the F factor or by agglutinating the F particles by combining with the acridine cation. Growing cells exposed to acridine orange eventually produce bacteria without F and this loss is permanent (Hirota, 1960).

The attached F factor in the Hfr state is not transmissible except as a chromosome locus (Jacob and Wollman, 1958a; Adelberg and Burns, 1960). The F factor breaks the circular chromosome when it attaches and becomes an end of the chromosome, yielding a conventional linear linkage. The attached F factor becomes the distal end of the chromosome, while the markers at the other end form the origin, which is transferred first to the recipient. The attached F factor is the last marker to enter the F-; therefore, due to spontaneous breakage of the chromosome, the passage of the F factor is rare. Those recombinants which do receive the sex factor generally are Hfr (Sneath, 1962). The attached F factor has been successfully co-transduced with chromosomal markers, confirming the attachment of the F particle (DeWitt and Adelberg, 1962). The transduced males in this case resemble the Hfr parent in their sexual behavior.

The order of chromosome transfer is the same in any single isolated Hfr. However, other Hfrs from the same F+ population may differ in the order of the chromosome transfer and the frequency of various markers (Jacob and Wollman, 1958a; Lederberg, 1957; Wollman et al., 1956). In other words, the sequence varies with the Hfr (Skaar and Garen, 1956). A double male which has two attached F factors has been discovered. It transfers genetic material to a recipient in the form of two nonhomologous linkage groups (Clark, 1963).

No Hfr has been isolated which can transfer at high frequency all of the general markers known (Jacob and Wollman, 1958a).

The F factor may not only become attached to the chromosome, but may undergo genetic recombination with the chromosome of the Hfr cells of E. coli and then return to its autonomous state. It is then commonly referred to as an F' (F-prime) and can pass this sex factor and chromosomal markers to recipients. The chromosomal genes undergo genetic recombination with homologous parts of the recipient chromosome (Pittard and Adelberg, 1964). This phenomenon is commonly referred to as F-duction or sex-duction (Clark and Adelberg, 1962).

Although in most experiments the female and male strains are used in such dilutions as to allow the mating of only one Hfr with a single female cell, the possibility of triparental mating has been reported. Triparental mating has been demonstrated both by simultaneous mating of three cells and by further mating of the zygote with a second male (Fischer-Fantuzzi and DiGirolamo, 1961). This implies that the fertilized female does not acquire an immunity to further recombination. In the same study, an observed frequency of Hfr x Hfr crosses was explained by an assumption that the female cell acts as an attraction center.

Another phenomenon which may accompany bacterial conjugation is zygotic induction (Wollman and Jacob, 1957; Jacob and Wollman, 1958b, Jacob and Wollman, 1956). This occurs when a male lysogenic with an inducible prophage is mated with a non-lysogenic female. The transfer of this inducible prophage from the male to the female is immediately expressed through the induction of the prophage, release of phage, and consequently, the lysis of the recipient. This induction has been

explained by the absence of a repressor in the cytoplasm of the F- (Jacob and Wollman, 1961). This phenomenon has revealed much about the mapping of prophage, the mechanism of chromosome transfer, and cellular regulation, and provides a means of detecting the prophage as a chromosome marker (Clark and Adelberg, 1962).

Bacterial recombination can be divided into progressive stages, each constituting an indispensable step in the eventual formation of recombinant offspring. Three particular stages were proposed by Clark and Adelberg (1962):

(1) Formation of specific pairs resulting from the random collision of the two cells of the opposite mating types. This step can be detected under the light microscope where cell-to-cell contact can be seen in mixtures of F+ and F- or Hfr and F- cells (Lederberg, 1956b). This step is accomplished immediately upon the mixing of the opposite mating types.

(2) Formation of effective pairs. This is defined as a specific pair in which all conditions necessary for genetic transfer is in progress. Upon electron-microscopic examination of specific pairs, "tubes" joining the recipient and donor cells have been reported (Anderson *et al.*, 1957), and it has been suggested that this represents an effective contact between two cells. However, the necessity of subjecting cells to high vacuum and high desiccation in electron microscopy leaves uncertainty as to the nature of this tube. Fisher (1961) feels that the conversion of specific pairs into effective pairs may represent the alignment of partner cells into specific configuration or the formation of the above mentioned conjugation bridge, or it may even represent the formation of a hole

in the rigid part of the cell wall between two cells that would allow chromosome passage. However, tests to detect such a hole as in the latter alternative have proved inconclusive. The formation of effective pairs is accomplished in twenty-five to thirty minutes (Jacob and Wollman, 1958a).

(3) Chromosome transfer from donor to recipient. This step has been the object of intensive investigation and is the step providing the most information for geneticists. Approximately two hours are required for the completion of chromosome transfer (Wollman and Jacob, 1958).

Genetic transfer consists of the oriented and progressive penetration of the Hfr chromosome into the F- bacterium. Always the same extremity or origin "O" of the Hfr chromosome which penetrates first is followed in a predetermined order by the various genetic determinants to the distal end "R" (Wollman et al., 1956). A gradient of markers results if the transfer is allowed to proceed naturally, where the proximal markers entering first are transferred in greater frequency than the later markers due to spontaneous breakage during chromosome transfer (Lederberg, 1955; Wollman and Jacob, 1958).

The result is an incomplete zygote or merozygote. Chromosome transfer can be stopped by artificial means of breaking the pairs apart or selectively killing the Hfr with phage. Only markers having already entered the F- will participate in recombination (Wollman et al., 1956; Lederberg, 1955). Analysis of zygotes at given times can give a genetic map in time units (Lederberg, 1959). This is the means that the single linkage group of E. coli Hfr types has been determined (Cavalli-Sforza and Jinks, 1956; Jacob and Wollman, 1958a; Lederberg,

1947; Clowes and Rowley, 1954) and has led to the circular genetic map for E. coli (Taylor and Thoman, 1964) and Salmonella (Sanderson and Demerec, 1965).

These genetic maps of the bacterial chromosome have been reinforced by physical studies of chromosome transfer. Most important of these were those determining the effect of radioactive ^{32}P and ultraviolet light on recombinant formation (Jacob and Wollman, 1958a). Proof was presented that ^{32}P decay does indeed exert its influence on the genetic material and that the ^{32}P does not only effect integration, but also chromosome transfer by breaking the DNA of the Hfr chromosome, which leads to an even sharper gradient of genetic markers. This confirms the genetic maps determined by previous workers. Similar results were obtained by Wood and Markovich (1964).

The actual mechanism of chromosome transfer is still unclear, It is believed that the bacterial chromosome is transferred as a result of its integration with a sex factor (Adelberg and Pittard, 1965). Studies with Hfr, F', and F+ have shown that transfer is dependent on the replication of the sex factor along with that of the chromosome or chromosomal segment attached to it. The exact role of DNA replication, however, is disputed. Bouck and Adelberg (1963) propose that replication of DNA is necessary only for the initiation of the transfer. More evidence that chromosome transfer can take place in the absence of DNA synthesis was presented by Suit et al. (1964) supporting the fact that transfer can occur as soon as a previous cycle of DNA replication is completed and concomitant DNA synthesis is not obligate for the transfer process. However, it is difficult to be certain that there is total absence of DNA synthesis in the

experiments performed, particularly those in which DNA synthesis is inhibited by thymine starvation (Freifelder, 1965). On the other hand, Jacob and Brenner (1963) have proposed an alternative on the basis of their replicon theory that the Hfr chromosome and integrated sex factor are replicated by a bacterial replication system. They suggest that chromosome transfer requires concomitant replication of the Hfr chromosome so that one of the replicas is passed into the F- as it is formed. Support for this model has also been reported (Rolser and Konetska, 1964; Ptashne, 1965). It is suggested that in either model, the sex factor attaches to the cell membrane and governs the formation of conjugal receptors on the cell surface directly opposite the attachment site (Adelberg and Pittard, 1965). Conjugation, then, triggers both transfer of plasmids, along with any DNA which has been integrated with it.

Fulton's (1965) study of continuous chromosome transfer revealed that E. coli K12 is not only capable of transferring its entire genetic linkage group when due precaution is taken against chromosome breakage, but may initiate a second round in the same order as the first. This is considered evidence that the donor does not open its chromosome into a linear form; rather, it in some way creates an origin while remaining continuous. Fulton considers this support that chromosome transfer is carried out according to Jacob and Brenner's model.

Free energy is required by the donor participating in bacterial conjugation while the F- is passive (Fisher, 1957a). The energy is utilized by the donor cell in effective contact establishments, as well as in genetic transfer to the F-. The Krebs cycle plays a

major role in providing this energy, as glucose or any other Krebs cycle intermediate stimulates zygote formation, while inhibitors of this cycle also inhibit zygote formation.

In addition to the above three steps in bacterial mating, one could add two steps common to transduction and transformation as well as conjugation. These are the integration of the genetic material and the segregation and phenotypic expression of the recombined material to form the haploid recombinant (Jacob and Wollman, 1958a). These last two steps take place in the recipient (which has become the zygote) and extend to the formation of a clone of the recombinant type (Wollman and Jacob, 1958). The donor cell is dispensible once this stage of bacterial recombination has been reached. It is at the integration state where evidence suggests that bacterial DNA carries host specificity determinants so that the degree of success of the received episome or genetic material depends upon the nature of the recipient and donor being used (Arber and Morse, 1965). It is at this stage that post-zygotic elimination may take place (Weinberg, 1960), and the loss of certain chromosome segments before or after fertilization or both was deduced from the study of the zygotes. Various studies of the resulting recombinants tell much about the genetic mechanisms involved in bacterial recombination.

There has been a considerable number of studies concerning the kinetics of bacterial conjugation. In general, it may be said that effective cell pair formation is a function of (a) parental numbers, the ratio of parental bacteria, and the volume of the system, and (b) the time of mixing of the parental bacteria (Nelson, 1951). The number of zygotes formed in a given time is a function of chance

contacts and the speed of the chromosome transfer. The kinetics can, therefore, be studied in two stages, effective cell contact and chromosome transfer. A more complex interpretation has been presented by Kunicki-Goldfinger and Czerwinska (1964) and Fisher (1961).

Environmental conditions have various effects on bacterial conjugation. The major effect of pH and parental population is not on chromosome transfer, rather on contact formation (Hayes, 1957). Fisher (1961) found that collisions were independent of temperature, pH has been proposed to change the cell surface so that a higher or lower number of random collisions take place (Hayes, 1957). The optimum pH reported is from 6.1 (Fisher, 1957b) to 6.5 to 7.5 (Czerwinska, 1964). A negative influence of temperature increase, pH, and osmotic pressure has been reported (Czerwinska, 1964).

The effect of various chemicals on the recombination rate in E. coli has also been studied. Clark (1953) reported alteration of the rate of prototroph formation by addition of various chemicals to the nutrient substrate. Hydrogen peroxide and sodium azide have been reported to stimulate recombination (Clark et al., 1950). The discovery that periodate compounds inhibit bacterial recombination was very significant in determining the mechanism of cellular contact (Sneath and Lederberg, 1961).

Ultraviolet light and X-irradiation have been found to enhance recombination frequency in E. coli (Hayes, 1952; Haas et al., 1948; Clark et al., 1950). This effect is apparently on the recombination occurrence, rather than on chromosomal transfer (Fisher, 1961).

Several reviews of bacterial recombination and its genetic implications have been published and provide organization to the subject of bacterial conjugation (Ravin, 1960; Wollman et al., 1956; Hayes, 1953a; Hayes 1960; Clark and Adelberg, 1962; Adelberg and Pittard, 1965).

Bacterial Conjugation in Species Other Than E. coli

Mating systems analogous to that found in E. coli have been reported for various other genus species. Such conjugation takes place not only within these species, but also among them.

Genetic recombination in Pseudomonas aeruginosa was first reported by Holloway in 1955, and an infectious fertility factor similar to the F factor of E. coli K12 was reported and termed FP+ (Holloway and Jennings, 1958). The mating of FP+ and FP- superficially appears similar to the mating of the F+ and F- of E. coli K12 (Loutit and Pearce, 1965).

Bacterial conjugation in Serratia marcescens is somewhat less well defined. Genetic recombination takes place and the reaction has properties similar to conjugation in E. coli; however, the donor-acceptor relationship does not appear to be identical with the F+-F- relationship in E. coli (Belser and Bunting, 1956).

A type of recombination between mutants of Vibrio cholera was reported by Bhaskaran in 1960 and a fertility factor has been shown to be infectively transmitted (Bhaskaran and Tyer, 1961).

Interspecies crosses also occur. Successful mating of Salmonella typhosa and Serratia marcescens has been described (Falkow et al., 1961). This is particularly significant because of the divergent

DNA base ratios of these parental strains and the lack of a demonstrable common system of mating polarity.

E. coli can participate in bacterial conjugation with several other species. Infection of strains of Salmonella, Shigella, Serratia, and Klebsiella with the F factor has been observed (Makela et al., 1962). Large differences were found in the ability of the strains to be infected with F.

In another test, all strains of Shigella flexneri tested were found to form recombinants with E. coli, resulting in hybrids (Luria and Burrous, 1956). The Shigella behaved as recipient in these crosses and was transformed to F+ by incubation with an F+ culture. The frequency of recombination was lower than in similar crosses in E. coli although the transfer was the same, this suggesting incomplete homology between the two species. Such a mating of E. coli and Shigella flexneri can result in the restoration of virulence to an avirulent strain of Shigella (Formal et al., 1965; Falkow et al., 1963).

E. coli genes with the segregation and transfer properties of F-linked markers may be transmitted to Proteus (Falkow et al., 1964). These strains also have different overall DNA base compositions and the acquired F-linked genes may be correlated with an addition of a DNA fraction with the molecular weight and base composition of E. coli. The genetic material is not integrated and the episomally infected Proteus cells do not exhibit the surface changes which normally accompany the presence of F. No Proteus F+ has been isolated.

Of more direct significance to this paper are the hybrids resulting from E. coli and S. typhimurium. The first successful mating

between the two species was accomplished utilizing S. typhimurium LT-7 mut as the recipient and an E. coli Hfr (Miyake and Demerec, 1959) and confirmed by Baron et al. (1959). In a test to determine the fertility of a number of Salmonella strains with E. coli K12 Hfr, it was found that fifty-two per cent of the Salmonella strains tested were fertile (Ørskov et al., 1961), whereas only thirty-one per cent of the E. coli test strains were fertile (Ørskov and Ørskov, 1961).

Miyake reported that the mut factor was imperative for such crosses (Miyake, 1959). A population of mut Salmonella is found to be heterogeneous with respect to its ability to serve as recipients with E. coli. Those having high fertility (and the strains derived from them) are termed "fertile" (fer), while those cells with low fertility are called "infertile" (infer) (Miyake, 1962). Miyake feels that the mutation from infer to fer is increased by the presence of the mut gene, and this would explain why only strains carrying mut are compatible with E. coli. The mutability factor of LT-7 makes that strain more mutable than LT-2 and auxotrophic mutants are isolated more easily (Demerec et al., 1958). However, these mutants also revert easily. The role of mut suggests that a genetic determinant may be involved in the ability of certain S. typhimurium cells to form hybrids with the E. coli donor.

The back-cross of E. coli-S. typhimurium hybrids with the E. coli Hfr results in high frequency recombination (Baron et al., 1959b). The exact reason for this increased recombination frequency is uncertain; but most believe the increase in recipient ability is due to the presence of the integrated genetic material and a resulting artificial chromosome homology (Johnson et al., 1964b).

The fact that recombination can occur is evidence for some degree of genetic homology between Salmonella and E. coli. Recombinant analysis and interrupted matings, along with molecular hybridization experiments demonstrated that this genetic homology is incomplete (Falkow et al., 1962). Although the gross genetic order and distances of the genetic maps of the two species are the same (Zinder, 1960a), there is a defect, perhaps a deletion, in the lactose region of Salmonella (Miyake, 1962).

This incomplete homology is confirmed by transduction experiments with the E. coli-S. typhimurium hybrids. Apparently, sufficient evolutionary diversity has occurred to restrict transduction even for those genes common to both organisms (Zinder, 1960a). Using phage P22 as the vector, when the hybrid transducing fragments are mostly E. coli genetic material, there are either no or only few recombinants; whereas, when the transducing fragments carry Salmonella markers, the expected number of transductants are found (Demerec and Ohta, 1964). This provides a means of differentiating between E. coli and S. typhimurium regions of the hybrid chromosome and provides a means of genetic mapping. The transducing restriction is not due to the vector, as P22 can carry E. coli or Salmonella genetic material or a composite of both. It appears that the integration in Salmonella of E. coli genetic material is one-tenth that of the S. typhimurium fragment (Demerec and Ohta, 1964). Apparently, there is sufficient molecular variance in the genetic structure of the two species to cause sufficient diversity of the structure to prevent necessary pairing over short distances prior to recombination (Zinder, 1960a).

It was recently discovered that a mutagenic agent can produce analogous recipient ability in S. typhimurium LT-2 as the mut in LT-7 (Eisenstark, 1965). The mutagen utilized was N-methyl-N'-nitro-N-nitrosoguanidine (Eisenstark et al., 1965). There was evidence presented that the mutagen acts on the recipient only and induces fertility (fer) in recipient cells.

The F factor has been passed from E. coli K12 to S. typhimurium and this F agent made that S. typhimurium fertile (Miyake, 1959) and capable of passing this F agent back to E. coli (Zinder, 1960b). Salmonella Hfrs have been isolated from the offspring of an E. coli K12 Hfr x S. typhimurium cross (Makela, 1963; Zinder, 1960b). This is a very useful tool, because otherwise, the naturally occurring Salmonella are believed to be F-, which can act as recipients, but unable to initiate conjugation and act as donors (Makela, 1963).

It has become possible to analyze the recombination in this interspecies system and the mechanism of conjugation seems to be like that of the E. coli mating system. The kinetics also appear to be the same (Johnson et al., 1964a). The S. abony Hfr strain exhibits the oriented chromosome transfer that characterizes E. coli K12 Hfr donors and appears to have the same circular order of seven markers studied (Makela, 1963).

It now seems that all enteric strains studied can be fertile in certain crosses, though sterile in others. The hybrids have novel biochemical and antigenic characters, as well as virulence. The possibility of interspecies recombination contributes much to the derivation of an evolutionary scheme for bacteria.

Episomes

A review of the properties of episomes other than the F factor might lend some understanding to the functions of the F factor. There are a number of properties considered common to all bacterial episomes: they may be transmitted by infection; they may be able to control the appearance of certain substances on the cell surface; and when in the cytoplasmic state, they may be susceptible to disinfection by acridine dyes (Sneath, 1962). The well established episomes are the F factor, temperate bacteriophage, colicin factor (col), and the resistance transfer factor (RTF). The FP fertility factor of Pseudomonas is also believed to be an episome.

Adelberg and Pittard in 1965 reviewed the properties of autonomous genetic elements or plasmids. In addition to the properties given above, these elements are composed of DNA and bring about their own transfer through the conjugation which they promote. These authors called the elements "autotransferables" and included RTF, col, and the F factor. Some of these appear to have incorporated segments of the bacterial chromosome which are capable of pairing with homologous regions of the host genetic material. Crossing over in paired regions brings about the integration of the host chromosome with the autotransferable element, so that when conjugation occurs, the entire segment is transferred. Autotransferable elements which promote chromosome transfer in this manner are called "sex factors."

Lysogeny is a state of a bacterial cell infected with a temperate bacteriophage existing as a prophage attached to the bacterial chromosome (Lederberg and Lederberg, 1953). When the prophage becomes autonomous, vegetative phage production ensues, resulting in the lysis

of the cell and the release of bacteriophage (Jacob and Wollman, 1961). Although the prophage is an episome, it does not exhibit some of the properties of the other known episomes.

Colicins are a variety of diffusible antibiotic substances which are all of a protein or polypeptide nature and are produced by many coli and related bacteria. Their spectra of activity may differ, but they are very specific in their action toward other strains of the family Enterobacteriaceae (Fredericq, 1958).

All colicin produced by a colicinogenic culture is synthesized and released by a small fraction of the bacterial population and that synthesis is lethal (Fredericq, 1963). Only the cells which do not yield colicin are viable, while the others are killed by the synthesis of colicin.

Non-colicinogenic Salmonella strains have been found to acquire col when grown in mixed culture with Escherichia or Shigella (Smith and Stocker, 1962). The colicin factor has been found to induce chromosome transfer in S. typhimurium LT-2 (Smith and Stocker, 1962; Ozeki and Haworth, 1961) and E. coli (Clowes, 1961) in a manner similar to the F factor. Colicins modify the cell surface of its host, as does the F factor. (Fredericq, 1963). There are reports of col I actually causing pairing of cells (Clowes, 1961) and effective col transfer (Monk and Clowes, 1964) in the absence of the F factor. Other colicins are believed to be incapable of bringing about this pairing, however.

The F factor present in a colicin donor reduces the transfer of col I. However, when the F is present in non-col recipient, col I is increased (Monk and Clowes, 1964). There have been proposals that

col may be epistatic to F, at least to the point of interfering with functions related to chromosome transfer (Nagel de Zwaig and Anton, 1964).

Multiple drug resistance was first brought to light because of its medical significance. This resistance factor has the properties of episomes, however, with no known attachment point on the chromosome. The factor harbors resistance to sulfonamide, streptomycin, chloramphenicol, and tetracycline. This factor can be transmitted by cell-to-cell contact among members of Enterobacteriaceae regardless of sex (Watanabe and Fukasawa, 1961). There is a wide range of bacterial genera which act as acceptors, leading to a type of infective heredity. The frequency of transfer varies with the donor and recipient. The transferred multiple drug resistance can be further transferred to other sensitive strains indefinitely (Watanabe, 1963).

Resistance factors do not confer maleness or transferability of the chromosome to F- strains of E. coli K12 (Watanabe and Fukasawa, 1961); however, the two factors may be closely related. A type of normally non-transferable resistance factor can be made capable of transmission by conjugation through interaction with the F factor in the donor cell (Harada et al., 1964).

On the other hand, the infection of E. coli male cells with RTF results in the inhibition of several distinct functions of the F factor: mating, capacity to transfer the chromosome by conjugation, production of the f+ antigen, and the formation of receptors for male-specific bacteriophage f1 and other RNA phages (Hirota et al., 1964; Watanabe and Fukasawa, 1962; Meynell and Datta, 1965). These results

suggest that the R factor controls a key mechanism in the synthesis of "F substances" formed on the cell surface by the F factor.

The presence of the F factor in the recipient has been shown to slightly repress the acceptance of RTF; and, conversely, RTF present in the recipient slightly reduces the acceptance of the F factor, F', and the host chromosome (Watanabe and Fukasawa, 1962).

The property of episomes most important to this thesis is that in each case the surface of the cell is altered by the presence of the episome. It is possible that these surface changes are closely related to the surface properties accounting for successful cell mating in the F system.

Sex-specific Bacteriophage

Susceptibility of a bacterial strain to bacteriophage is largely a function of the cell surface of that bacteria. Therefore, the discovery of phage specific for donor or recipient cells provides information about unique surface structures that may account for the roles of donor and recipient cells in conjugation.

Loeb (1960) reported a bacteriophage, $\phi 1$, that is highly specific for F+ and Hfr strains of E. coli. Two other subgroups of ϕ bacteriophage were discovered in 1961 (Loeb and Zinder, 1961). Using $\phi 2$ for a detailed study, it was found that this bacteriophage contains RNA, not DNA, as its nucleic acid. This small phage gives a large yield of phage per bacterium (about ten thousand) and resembles DNA bacteriophage in its general features. The failure of the ϕ phages to grow on female bacteria is due to their failure to attach to them, so the differentiation is in the adsorption phase.

Another RNA phage, MS2, attacks F⁺ strains of E. coli, but not corresponding F⁻ strains (Davis et al., 1961). This phage is similar to the above f2 phage of Loeb and Zinder.

The RNA phage, μ_2 , also has characteristics very similar to f2 (Dettori et al., 1961). It lyses both F⁺ and Hfr types of E. coli but is unable to lyse female cells with no known exceptions to the rule. μ_2 does not adsorb to F⁻ cells, while adsorbing very rapidly to F⁺ and Hfr cells. Again, the lack of a specific receptor for μ_2 is the explanation for the resistance of the F⁻ strains (Dettori et al., 1963). This phage has been characterized in detail (dePetris and Nava, 1963; Ceppellini et al., 1963).

The RNA phage M12 was isolated by Hoffschneider in 1963. It also is a male specific phage of E. coli.

Phages specific for female strains have also been isolated. The temperate phage "tau" forms plaques on F⁻, but not on F⁺ strains of E. coli K12 (Hakura et al., 1964). This phage contains DNA. It adsorbs equally well to F⁻ and F⁺ strains, indicating that a later step in the growth cycle is being inhibited. It seems possible that the F factor directs the synthesis of a cytoplasmic repressor that inhibits multiplication of phage "tau"

Another DNA E. coli F⁻ phage is ϕ (Dettori et al., 1961). This phage is poorly adsorbed on both F⁺ and Hfr cells, while adsorption on F⁻ cells is comparable to that of phage μ_2 on male cells.

The DNA phage SP6, has been found to be specific for F⁻ strains of Salmonella (Zinder, 1961). As the above F⁻ phage, it attaches to all mating types with progeny resulting from F⁻ only. It fails to attach to any E. coli.

Another type of sex-specific bacteriophage of E. coli has been described by Watanabe and Okada (1964). This virulent phage, W-31, forms large clear plaques on F- and stable Hfr strains, but small turbid plaques on F+ strains. It is said to be quasi-specific for females. Adsorption occurs equally well in F- and F+, suggesting that some step after nucleic acid injection is suppressed by an autonomous F, but not by an integrated F.

Changes in susceptibility and resistance to ϕ and μ_2 are paralleled by changes of mating types, and there is a negative correlation over a wide range between the degree of susceptibility to the two phages. The possibility of infection of E. coli F- cells during conjugation with μ_2 -infected males has been investigated and not ruled out (Sironi et al., 1964).

These sex-specific bacteriophage can be utilized in numerous experiments concerning the bacterial mating system. The relationship of the sex-specific phage receptor sites to the cell surface properties of donor and recipient cells is of particular interest.

Surface Properties and Conjugation

The previously described means of inhibiting bacterial conjugation by treatment of the Hfr with periodate compounds by Sneath and Lederberg (1961) can be correlated with phage susceptibility of the mating types. It has been interpreted that the action of periodate is in the alteration of a surface substance specifically associated with the conjugal activity of male cells. Treatment of Hfr and F+ cells with sodium periodate results in a sharp decrease in μ_2 adsorption, while O adsorption was practically unaffected and in some strains

significantly increased by this treatment. F- strains do not adsorb μ_2 before or after similar treatment and the adsorption of ϕ was unaffected (Dettori et al., 1961). This provides further evidence of the differences in surface structure between bacterial cells of different mating types.

The basis of phage specificity, as mentioned above, is in most cases dependent upon the availability of a specific receptor on the cell wall. The receptors for the male-specific phages are apparently missing in female cells. The ϕ receptor is not periodate sensitive and also present in small amounts on the surface of male cells. Dettori and his co-workers (1961) proposed that perhaps the explanation is that the receptor for ϕ_1 is in no way sex specific, but is "covered" by the μ_2 receptor in a male cell, while fully uncovered in female cells. The amount of ϕ_1 receptor covered or uncovered could vary with the environmental conditions.

Clones of E. coli have been isolated from an Hfr normally sensitive to a male phage and resistant to ϕ which are resistant to both types of phage (Gallucci and Sironi, 1964). The resistance of these clones to the male-specific phage is due to the lack of adsorption of the phage. The contemporaneous loss of sensitivity of phage and of the ability to conjugate leads to the consideration of possible identity of the phage receptor and the "conjugal substance"

This leads to a discussion of what this conjugal substance might consist of. This substance must play an integral part in conjugation.

Kern (1962) reported inhibition of conjugation with preparations of E. coli Hfr or F- cell walls. Maximum inhibition occurred in the

early stages of conjugation and the cell wall inhibition was lacking at thirty minutes. Lancaster et al. (1965) also found that cell walls of fertile male and female E. coli strains inhibit recombination between compatible cells providing the cell walls were added to the mating mixture before the formation of stable mating pairs. Fisher (1961) reported that pairing does take place between the Hfr and F- E. coli cell wall fragments. This is consistent with the idea that cell walls exert their effect during conjugation by competition for complementary sites involved in the initial contact of the mating cells.

Lancaster et al. designed a procedure for obtaining the conjugation factor by hot phenol extraction, as well as devised a means of assaying the conjugation factor activity. It was found that cell walls from Hfr, F-, and F+ cells are very similar in their ability to inhibit conjugation. E. coli B cell walls inhibited conjugation to a lesser extent than did other recipients. This is a reflection of the B's reduced ability to recombine with E. coli K12 donors. Probably E. coli B, although acting as a recipient in crosses with E. coli K12 donors, lacks the female conjugation factor of E. coli K12 recipient strains. The workers deduced that male cells of E. coli contain both male and female conjugation factors, since males can be converted to F- phenotypes with excess aeration.

Because both male and female conjugation factors were extracted by the same procedure, it was possible that the material extracted from the males might actually be the female conjugation factor. However, Lancaster et al. found that there was a decrease in inhibition because of neutralization of male activity by female cell walls in the same mixture, indicating that there is not only female,

but also a male conjugation factor. Extraction from fertile cells of substances involved in the conjugation process provides a means of investigating directly the relationship of various sex-related surface characters to the conjugation factor itself. Among these characters are sex-specific ϕ receptor sites, male specific antigen, and male pili.

Conjugation can take place between protoplasts of compatible mating strains of E. coli. These protoplasts are usually produced by growing cells in penicillin and maintaining them in medium containing magnesium and a high sucrose concentration (Lederberg, 1956a). Hagiwara (1958) and Lederberg and St. Clair (1958) reported that successful genetic recombination could take place when either or both of the compatible mating pair were protoplasts. Recombination of protoplasts was able to be detected by plating the mating mixture on agar where the protoplasts revert to rods which are capable of dividing. Otherwise, they would not divide, but simply increase in volume. Lederberg and St. Clair deduced that the wall defect imposed by the penicillin has no influence on either the genetic continuity or phenotypic manifestation of this surface-related property. There are strong hints that much residual cell wall material persists on penicillin protoplasts and perhaps some of this residue is the conjugation factor.

Maccacaro (1955) reported several surface properties of the fertile mating types of E. coli. He found the agglutinating pH of F- strains much more acidic than that of F+ strains. F- strains also have far greater affinity for the basic dyes than do F+ strains. There is greater spontaneous instability in broth cultures of fertile strains, compared with the corresponding sterile strains. These

properties suggest there are differences in surface charges. Because Maccacaro could not detect any morphological equivalent of fertility at the level of the cell surface, he proposed that fertility might be nothing more than an epiphenomenon of surface charges. Further studies of Maccacaro and Comolli (1956) lead them to believe F+ and F- are characters resulting from a different degree of the same property based on the finding that in a cross of F+ x F+, one of the two strains behaves mostly as F+ and the other as F-, the F+'s having different strengths. Their conclusion that compatibility and surface properties are highly correlated seems soundly based on the following points:

(1) There is a uniform difference in surface properties between F- and F+ variants of every tested strain. (2) Compatibility and surface properties are transmitted by infection from F+ to F- along the same time course. (3) Screening of infected strains for cultural behavior yields results that are fully consistent with scoring for compatibility.

The belief that F- cells are more electronegatively charged than phenotypically identical F+ cells was confirmed by micro-electrophoretic studies (Turri and Maccacaro, 1960). These differences were shown, to a lesser degree, by strains in which the character fimbriae, although genotypically present, was not phenotypically expressed (Fim(+)).

A unique staining reaction during conjugation was reported by Zinder (1960b). A reddening appeared at the junction of compatible E. coli F+ and F- on EMB agar. This staining did occur in crosses of Hfr and F- cells. This reaction was not clearly understood, but seemed to involve some surface interaction which caused sufficient

damage to allow eosin to penetrate the cell and stain the acid cytoplasm.

An antigen termed f^+ distinguished by Ørskov and Ørskov (1960) has been correlated with the F state. Antiserum was produced using heated Hfr, F^+ , and F^- cultures as antigens. F^+ and Hfr strains were found to react identically in the serological tests. It was found that the Hfr or F^+ antiserum, after adsorption with the F^- cells, agglutinated the F^+ and Hfr cells. A few cases of the f^+ antigen cultures lost their F^+ or it was not present or detected, and this state was designated as $F-(f^+)$. However, no F^- cultures were found to agglutinate the f^+ antiserum. There was a close correlation between the F particle and the f^+ antigen.

The discovery by Sneath and Lederberg (1961) that periodate devirilizes the male mating types of E. coli K12 is believed to be a significant step in determining the conjugal substance. These devirilized cells were found still capable of acting as females. Female mating types were not affected by periodate. The devirilized cells recovered their virility after growing about two hours in broth. The action of periodate appeared to be to prevent the formation of mating pairs, presumably by altering the surface properties of the male cells. The effect was consistent with an oxidation of the glycol links in a polysaccharide on the surface of male cells.

Bacterial pili recently entered the search for the identity of the conjugation factor. These appendages were observed by several independent workers during other studies (Houwink and Van Iterson, 1961; Anderson, 1949; Noda and Wyckoff, 1952; Weibull and Hedwall, 1953; Smith, 1954). Weibull was the first to characterize bacterial

pili, finding the "filaments" quite stable. The filaments were further characterized by Maccacaro and Angelotti (1955). The filaments radiate from the cell in all directions, giving the impression of rigidity and fragility, and look as if they are inserted in the cell membrane through the cell wall. These workers estimated that these filaments nearly double the surface area, but leave almost unaffected the volume of the bacterial cell. Electron microscope photographs showed sixteen hour broth cultures richly endowed with these filamentous appendages. Pili are distinguished from flagella by being thinner, straighter, and more numerous than flagella (Duguid et al., 1955). They are fragile, being removed by mechanical agitation in high speed mixers, but grow back spontaneously. Pili are of uniform diameter (eighty to one hundred angstroms), are visible only under the electron microscope, and may be readily obscured by slime and debris. They are believed to be proteinaceous in nature (Brinton, 1959). The most recent review of the properties of bacterial pili was presented by Brinton in 1965.

Duguid and Gillis (1956) found pili present in most strains of Salmonella, E. coli, and Shigella. Duguid et al. (1955) found fimbriae with flagella in twenty-five test strains and independently in six non-motile strains.

Hemagglutination of various red blood cells by pili was reported by Duguid and his co-workers, as well as by Constable (1956). They may also agglutinate other objects, such as sperms, yeast cells, and inactivate some viruses. This has led to the belief that fimbriae may serve as organs of attachment.

Pili have been purified and used as specific antigens (Gillis and Duguid, 1958). The antigenic composition of fimbriae was found to be the same in all Sh. flexneri strains regardless of the O serotype. The antiserum agglutinated E. coli fimbrial strains, but the Sh. flexneri fimbriae had a major specific antigen different from that of the major specific antigen of E. coli. However, Sh. flexneri serum did not react with fimbriated strains of Salmonella or Proteus.

Penicillin spheroplasts of E. coli K12 were found to have fimbriae on their surfaces (Maccacaro and Turri, 1959b). These spheroplasts, whether normal or lysed osmotically, retained the HA properties with the specificity of the cells from which they were obtained. The electron microscope showed these pili to be identical in number and dimensions with those visible on the cells.

Certain differences in the metabolism of fimbriated and non-fimbriated strains have been reported (Dettori and Maccacaro, 1959; Maccacaro and Dettori, 1959); however, conflicting results indicate no differences in the rate of either aerobic or anaerobic metabolism correlated with pili (Wohlhieter et al., 1962).

Maccacaro et al. (1959) proposed that there are three states in which cells can exist in relation to fimbriation: Fim*(genotypically and phenotypically fimbriated); Fim(+) (genotypically fimbriated and phenotypically not fimbriated); and Fim- (genotypically and phenotypically not fimbriated). Although a chromosomal location for Fim has been deduced from recombination experiments (Maccacaro et al., 1959; Brinton et al., 1961), there seems to be an extrachromosomal entity controlling the production of fimbriae and the expression of the character. The transduction of piliation with other markers and the

instability of pili transductants are not consistent with a stable, exclusively chromosomal determinant at the suspected location (Brinton et al., 1961) and some episomal characteristics have been observed. Piliation has been successfully transferred from E. coli to S. typhosa (Brinton and Baron, 1960).

A new type of bacterial pilus genetically controlled by the fertility factor in E. coli K12 was recently described by Brinton et al. (1964). Previous attempts to correlate pili with the fertility of male bacteria were unsuccessful because it was observed that females could have many pili and some F⁺ strains have very few pili. On the other hand, such a possibility could not be disproved because none of the male strains were ever completely without pili. Brinton et al. used the M12 male-specific bacteriophage to distinguish the F pili on male bacteria from others on the same cells. The presence of F pili was directly related to susceptibility to the male-specific phage. All Hfr and F⁺ strains had F pili, while the F⁻ strains had none. Type I pili were found on all test cultures and could not be distinguished from F pili except in the presence of phage M12. There were as many as one to four hundred Type I pili per cell; but only from zero to five F pili per cell were found, with one or two most common. There appeared to be no preferred sites of attachment of the F pili, as they were located at the sides and ends of the cells with equal frequency. The correlation of F piliation with the presence of the F factor was illustrated by the disappearance of F pili when the F factor was removed and the appearance of F pili when the factor was added.

Brinton and his co-workers proposed the F pili might be identical to the Ørskov f⁺ antigen. Similarities suggested were:

- (1) They both occur on F⁺ and Hfr, but not F⁻ cells.
- (2) The average number of F pili per cell is only one; therefore, agglutination would be weak.
- (3) The F pili may be masked by Type I pili.

Various mutants of the male properties have been isolated. Strains have been isolated which are fertile, but resistant to male phage; sterile, but susceptible to male phage; and sterile, with the f⁺ antigen. This, however, does not disprove the role of F pili because no fertile strains without F pili have been isolated.

Brinton et al. also presented evidence that F pili are necessary for chromosome transfer. If the pili are broken off before mating, the recombinants are 0.1 per cent of those of the F piliated control. The F pili are generally regenerated; but, if not, there is no chromosome transfer. The relationship of pili to the conjugation bridge previously reported (Anderson et al., 1957; Bladen, 1963) is unclear. The pili could transfer with or without the bridge. In most published electron-micrographs of conjugating bacteria, pili can be seen on the male partner, usually reaching from the male to the female. Brinton suggests that the conjugation bridge may be an artifact of the electron microscope.

F pili have been found to form complexes with the male-specific phage, f₂ (Valentine and Strand, 1965). Addition of F pili to a phage lysate inactivates as much as 50 to 75 per cent of the phage. This inactivation can be reversed by blending the phage-pili complexes before the phage assay. This inactivation was explained by a reversible

attachment to the F pili. The purified pili were characterized and described as being sedimented by centrifugation at 100,000 G for thirty minutes and readily precipitated with $(\text{NH}_4)_2\text{SO}_4$. By these criteria, the F pili are similar to other types of pili with the exception of adsorbing phage.

A means of assaying F pili, or male substance, was devised by Ippen and Valentine (1965). This was done by adding radioactive f2 phage to the F pili, running this through a membrane filter to remove the unadsorbed phage, and determining the relative amount of F pili present by the number of phage adsorbed.

These workers also reported that F pili synthesis parallels the growth of a culture of male cells. Although apparent in detectable quantities during the lag phase, pili synthesis increases most rapidly during the exponential phase of cell growth, reaching a plateau during the early stationary phase and remaining at a relatively constant rate for at least twenty-four hours. This observation again illustrates the stability of the F pili and indicates their resistance to destruction by cellular enzymes during this period. A large number of free pili, as well as those still attached to the cells, are found in a culture,

Various F+, F', Hfr, and F- bacterial strains were tested by the filtration assay for F pili. All male strains tested synthesized the F pili; whereas, the female strains did not. The highest values were found with male E. coli K12 strains. F pili had been identified in E. coli, Salmonella, Shigella, Serratia, and Proteus (Brinton, 1964) and Ippen and Valentine's results agreed with those findings.

Several surface factors, then, have been suggested to play a role in determining sexual mating types. However, the correlation among the various factors is still unclear.

Antigens and Conjugation

The immunological properties of Enterobacteriaceae are quite complex. Probably the most widespread and non-specific antigen is a heterogenetic enterobacterial antigen that is common to many different species of the family (Whang and Neter, 1962). Also, several O and some H antigens have been found in a number of species of Salmonella, Shigella, Escherichia, and Proteus (Saphra and Wasserman, 1945), indicating that there are numerous antigenic interrelations and transitional forms among the individual types, as well as among groups, species, and genera.

Most useful for identification and differentiation have been some specific antigenic reactions. Among these is the commonly studied O, or somatic antigen, characterized in Salmonella and E. coli. A study to determine the nature of this antigen has led to the conclusion that this antigen consists of a combination of polysaccharide and a "native" haptene, the native haptene being found apart from the endotoxin where the biosynthesis might initiate within the cell (Milner et al., 1963; Anacker et al., 1964).

Shands (1964) found the somatic antigen of E. coli to be fibrillar in structure and located in considerable quantities on the surface of the bacterium. Furthermore, it can extend up to 150 μ beyond the confines of the cell wall. The O antigens are heat stable.

In addition to the O antigens, there are K antigens associated with the cell structure in E. coli. This K antigen may be of the A, B, or L type. The A antigen may interfere with O agglutination, even after two-and-one-half hours heating at 100°C. This is commonly called the capsular antigen (Sahab, 1961). In contrast to the A and O antigens, the B antigen decreases in agglutinating ability above 65°C (Tripodi and Baily, 1963). The L antigen is a thermolabile somatic antigen that is associated with the envelope of E. coli.

The flagellar antigen H is found in many strains of Enterobacteriaceae, although it is most widely utilized in the genus Salmonella. These antigens may occur in two alternating phases in the same strain of Salmonella (Lederberg and Iino, 1956). In contrast to Salmonella, E. coli is monophasic (Ørskov and Ørskov, 1961).

There are several good reviews of the immunological properties of Enterobacteriaceae, including the classic one authored by Kauffman in 1947.

The genetic basis of cellular antigens has been probed. There are two loci responsible for H antigens, accounting for the phase variation in Salmonella (Lederberg and Iino, 1956). Recombination of serotypes by transduction with phage particles of the flagellar loci, which are presumably closely linked, occurs (Lederberg and Edwards, 1953; Stocker et al., 1953). The transfer of motility from E. coli K12 to E. coli B, as well as among other enterics, appears to take place by conjugation (Furness and Rowley, 1955). A very good study of the action of both the H and O antigens of E. coli during bacterial conjugation was presented by Ørskov and Ørskov in 1962.

The Vi, somatic, and flagellar antigens of S. typhosa have all been transferred to S. typhimurium and genetically mapped in this way (Johnson et al., 1965). In these tests, there appeared to be a relationship between the Vi antigen of Salmonella and the K antigen of E. coli.

The role of cellular antigens in initiating conjugation has been studied. Antigenic differences between F- and F+ cells were first detected by Maccacaro (1956). He suggested that the F+ cells were deprived of a capsular antigen, which seemed to be of the thermo-labile B type. However, Ørskov and Ørskov (1961) could find no correlation between fertility and any single common antigen and decided fertility depends upon common origin, rather than antigenic structure. About the same time, Ørskov, Ørskov, and Kauffman (1961) found that random Salmonella test strains which were fertile with E. coli K12 Hfr came from all different Salmonella O groups. The number of fertile did seem higher among the higher O groups, with 24 per cent of the A through D groups being fertile, while 64 per cent of the E and higher groups were fertile. These workers posed the possibility that this could correspond to differences in the sugar complexities in high and low O groups described by Kauffman. Still, no direct correlation between any specific serological type or the presence or absence of any O, K, or H antigens and fertility could be detected in the strains studied.

The review of literature makes clear certain known facts about sexual conjugation in bacteria. For conjugation to take place, it is necessary to have a pair of compatible cells, capable of forming recombinants with one another. The compatible cell pair upon random

collision can form an effective pair, followed by chromosome transfer and the formation of the merozygote.

The exact nature of this effective contact is yet unclear. It is believed that some means of attraction and attachment between the male and female cells must be responsible.

Certain cellular characteristics have been attributed to male cells. In addition to a difference in surface charge from that of F- cells, the male cells are capable of adsorbing RNA male-specific bacteriophage, which F- strains do not adsorb. Periodate's inhibition of conjugation is due to its action on some substance on the male cell surface. The Ørskovs' discovery of an f+ antigen has confirmed the presence of a unique substance on the surface of male cells. None of the common antigens have been correlated with fertility in test strains. F pili, a special type of pili found only on the surface of male cells, are now believed to play an important role in conjugation. However, the exact chemical composition and role of the conjugal substance or F pili on the male cells remain unknown.

Effective contact and recombination can occur between E. coli and S. typhimurium. The hybrid of such a cross exhibits increased fertility in a back-cross with its parent Hfr. Increased genetic homology is one explanation for the fertility of these hybrids. However, any change in the antigenic make-up of the hybrid which might account for increased fertility has not been investigated.

MATERIALS AND METHODS

Bacterial Strains

The S. typhimurium and E. coli strains used were obtained from the stock culture collection of the Virology and Microbial Genetics Laboratory, Kansas State University. Table I, page 40, presents information concerning the strains used.

The test hybrids are listed under the species of their F- parent, as they retain many of the F- properties. Strain 3036 is a hybrid from a S. typhimurium F- and an E. coli Hfr, which is fertile in backcrosses with the E. coli Hfr. Strain 3018 is a hybrid from an E. coli F- and a S. typhimurium Hfr, which is capable of forming recombinants (is "fertile") with the S. typhimurium Hfr. The hybrids were produced previously in the laboratory according to the method described by Eisenstark (1965).

Media

Nutrient broth. Nutrient broth was used for liquid growth media, while nutrient agar was used as the solid medium. The nutrient broth contained eight grams Nutrient Broth (Difco) and five grams sodium chloride dissolved in one liter distilled water. The nutrient agar contained eight grams Nutrient Broth, five grams sodium chloride, and fifteen grams Bacto-Agar (Difco) dissolved in one liter distilled water.

Physiological saline. Physiological saline, which was used for dilutions and antigen preparations, consisted of 0.85 per cent sodium chloride solution in distilled water.

TABLE I
BACTERIAL STRAINS AND THEIR DESCRIPTION

Culture	F state	Fertility with Hfr of opposite genus	Description
<u>S. typhimurium</u>			
1902	F-	infertile	<u>leu24</u>
3036	F-	fertile	hybrid TC <u>leu24</u> x Hfr CS101
1826	Hfr		S249 Hfr 21 (PLt22hi)+B2
<u>E. coli</u>			
1833	F-	infertile	C600
3018	F-	fertile	hybrid CT 1472 x 1738
1747	Hfr		CS101

Motility test agar. Semi-solid Motility Test Agar (Difco) was prepared by dissolving twenty grams of the motility medium in one liter distilled water.

Gel diffusion agar. Both Wilson and Pringle's (1954) and Mansi's (1957) gel diffusion agars were prepared. Wilson and Pringle agar contained 0.85 per cent Ionagar #2, 0.01 per cent Merthiolate, and .002 per cent methyl orange in distilled water. Mansi agar was made in essentially the same way except for the addition of 0.85 per cent sodium chloride.

Antigen Preparation

After testing all six cultures for motility by stabbing motility test agar and finding them actively motile, it was decided to produce both H and O antisera against each of the strains. The procedure for antigen preparation was essentially that outlined by Campbell et al. (1964).

O antigen preparation. Large flat bottles containing one hundred ml solidified agar on one side were inoculated with the organism by spreading one to two ml of an eighteen hour broth culture onto the agar surface. The bottles were then incubated at 37°C for eighteen to twenty-four hours and the cells washed off the surface of the agar with twenty to thirty ml sterile saline and collected with a bulb and sterile pipette. The cell suspension was placed in the steamer for two and one-half hours. After removal from the steamer, the suspension was centrifuged at three thousand rpm for thirty minutes at 5°C in a Servall centrifuge. The cells were then diluted with 0.5 per cent

formalinized saline to the concentration of approximately 10^9 cells per ml using McFarland's nephelometer tubes. The sterility of the antigen preparation was tested by mixing a drop of the suspension into five ml nutrient broth and incubating the broth for forty-eight hours at 37°C . The antigens were then placed in sterile bottles and stored at 5°C .

H antigen preparation. A flask containing five hundred ml nutrient broth was inoculated with one ml of an eighteen hour broth culture. The flask was incubated and aerated at 37°C . After eighteen hours incubation, two hundred fifty ml of 1 per cent formalinized saline was added, giving a final concentration of approximately 0.33 per cent formalin. The flask was stoppered and allowed to stand at room temperature several days. The cells were then centrifuged at three thousand rpm for thirty minutes at 5°C and resuspended in twenty to thirty ml of 0.5 per cent formalinized saline. The suspensions were also standardized to 10^9 cells per ml and tested for sterility as the O antigens were.

Antisera

White rabbits were used for the production of antisera. These rabbits were given intravenous injections of the above antigens in increasing doses of 0.1, 0.25, 0.5, 0.75, and 1.0 ml at two day intervals. A trial bleeding was carried out on the tenth day after the last injection and if the titer was not sufficient, a booster of one ml antigen was given and the final bleeding took place approximately one week following the booster.

The blood was collected in sterile tubes and processed as follows. The fresh blood was allowed to stand at 37°C for about an hour, or until the clot had formed. The clot was then loosened from the sides of the tube with a sterile wooden applicator stick and the blood was placed at 5°C overnight. The clot was then spun down by centrifugation at 1,500 rpm for fifteen minutes at 5°C in an International centrifuge. The clear serum was removed using a bulb and sterile pipette, placed in sterile vials, and stored in the freezer.

Tube Agglutination Tests

Test antigens were prepared as above except that sterility was not imperative. Also, the H antigens were not centrifuged; rather, the concentration of an overnight broth culture provided a sufficient concentration of cells after the addition of formalinized saline.

The basic procedure used for tube agglutination tests was that of Campbell et al. (1964). Dilutions of the antiserum were made in Kahn tubes, beginning with a 1:10 dilution (0.2 ml serum into 1.8 ml saline) and subsequent 1:2 serial dilutions (one ml transferred into one ml saline) up to a dilution of 1:5,120. One ml was discarded from the last tube. One ml antigen was then added to each tube. A control tube was set up for each system, containing only the antigen and saline. The tubes were placed in a 56°C waterbath. The H agglutination tubes were read after two to three hours, the O tubes after six to twelve hours. The tubes were allowed to stand overnight at room temperature and again observed, as some questionable results were clarified after some hours of standing. Once the original

antiserum had been titered, the number of dilution tubes in later tests could be adjusted accordingly.

Agglutinin-Adsorption

The major step in the agglutinin-adsorption procedure is the production of the adsorbed serum. The procedure followed was that of Edwards and Ewing (1962).

The adsorbing antigens were prepared similarly to the antigens previously described. The O antigen was heated, centrifuged, and the cells from one large bottle resuspended in one ml of 0.5 per cent formalinized saline. At the same time, thirty to forty ml of H antigen were centrifuged and resuspended in one ml of formalinized saline. Two such preparations of each the O and H antigens were prepared for the appropriate serum sample to be adsorbed.

The adsorption was carried out according to the "double-adsorption" scheme. The O serum was diluted 1:5, the H serum 1:10, and one ml of these dilutions mixed with one ml of the appropriate concentrated adsorbing antigen preparation. This mixture was incubated at 50°C for one to two hours and centrifuged to spin down the antigen-antibody complex. The clear supernatant was removed and this supernatant mixed with the remaining ml of adsorbing antigen. The mixture was again incubated at 50°C for one to two hours and placed at 5°C overnight. The mixture was then centrifuged, the adsorbed serum removed, placed in sterile vials, and stored in the freezer.

The testing of the adsorbed serum was carried out using the normal tube agglutination test. However, 1:5, 1:10, 1:15, and 1:20 were the initial dilutions of the antiserum followed by two-fold dilutions.

Antigen Typing

Conventional antigenic typing of the six strains was carried out with Salmonella and E. coli typing sera procured from Baltimore Biological Laboratory. The typing was carried out according to instructions accompanying the sera. O typing was performed with both the S. typhimurium and E. coli strains, while only the S. typhimurium were H typed.

Gel Diffusion Precipitation System

The microscopic adaption of Ouchterlony's double gel diffusion technique (1948) was used. One and one-half ml agar preparation was placed on a clean microscope slide between two number eight rubber bands placed three cm apart around the slide. These rubber bands had previously been sealed to the slide with small amounts of the agar. Wells were made after the agar had solidified with a number 1 cork borer, according to the pattern illustrated in Fig. 1, page 46. The wells were rimmed and sealed with a very small amount of the agar preparation, this to prevent seepage of the antigen or antiserum.

For the gel diffusion test, the wells were filled respectively with antigen and antiserum. The prepared slides were then placed in a humidified chamber consisting of an enclosed plastic container containing damp paper towels. The slides were incubated in this chamber at room temperature and observed daily.

Different types of antigens were tested for use in these tests. Live and formalinized cells were used for antigens against the H antisera. Live and heated cells plus sonicated cell extracts of heated cultures were used for antigens against O antisera.

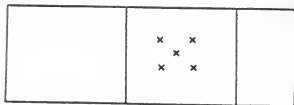


Figure 1. Pattern for the microscopic gel diffusion precipitin tests. Antiserum was placed in center well, the antigen in surrounding wells.

The sonic extract was prepared from the previously prepared O antigen. Five ml of the concentrated, heated cell suspension were placed in a small stainless steel test tube. The suspension was then sonicated at 2.5 amperes for ten minutes in a Branson S-75 sonifier. The tube was held in an ice bath while sonification was taking place. The suspension was allowed to sit overnight at 5°C and centrifuged at 10,000 rpm for thirty minutes at 5°C. The supernatant was removed and labeled Extract 1. The sediment was resuspended in five ml saline and sonicated in the same manner as before, the supernatant being labeled Extract 2. These extracts were used soon after preparation and were kept at 5°C until their use.

Electron Microscopy

Overnight broth cultures of the six test strains were used for electron-microscopic specimens. The broth culture was diluted into 1 per cent ammonium carbonate to the proper concentration. It was decided not to wash the cells because of the reported fragility of bacterial pili, which were of particular interest in this study.

A drop of the cell suspension was placed on a two hundred mesh stainless steel grid which had been previously coated with collodion. The excess moisture was drawn off the grid after thirty seconds.

The grids were shadowcast with 80% platinum-20% palladium in a vacuum device. The grids were placed at a known angle from the source of the rare metals. The specimens were then viewed under a RCA EMU2 electron microscope with Canalco accessories and photographs taken.

RESULTS

Agglutinin Adsorption

The serological tests were designed to gain three main objectives. It was desired first, to confirm the f+ antigen described by Ørskov and Ørskov (1960); second, to detect a possible common antigen between the E. coli and S. typhimurium Hfr strains, 1747 and 1826; and third, to detect any unique antigenic reactions of the "fertile" strains, 3018 and 3036, which had been selected as a result of the hybridization procedure.

Preliminary titering of the antisera revealed that the various antisera had titers sufficiently high for use in the proposed experiments. The antigenic relationship of the six test strains was determined by adsorbing the antisera and then testing the adsorbed antisera for agglutination with the appropriate antigens.

The investigation of the H antigens was less intense than that of the O antigens. No peculiarities arose in the H antigen tests. They appeared to be species specific, and thus adsorbed by other members of the same species. Table II, page 49, presents results illustrating the H antigen species specificity and the lack of detection of any unique antigens in the test strains.

The results of the O tests provided more interesting information subject to examination. The results of these tests are presented in Table III, page 50. Three reactions should be particularly noted. First, the Hfr sera of both E. coli 1747 and S. typhimurium 1826 reacted with their own antigen after adsorption with the respective F- strain. Second, the E. coli Hfr agglutinated the adsorbed

TABLE II
RESULTS OF AGGLUTININ-ADSORPTION TESTS USING
FORMALINIZED CELL SUSPENSION AS ANTIGENS*

Antiserum	Adsorbing antigen	Test antigen					
		<u>E. coli</u>			<u>S. typhimurium</u>		
		F- 1833	<u>fer</u> 3018	Hfr 1747	F- 1902	<u>fer</u> 3036	Hfr 1826
<u>S. typhimurium</u>							
F- 1902	control	0	0	0	+	+	+
	1826	0	0	0	0	0	0
	3036	0	0	0	0	0	0
<u>fer</u> 3036	control	0	0	0	+	+	+
	1902	0	0	0	0	0	0
	1826	0	0	0	0	0	0
Hfr 1826	control	0	0	0	+	+	+
	1902	0	0	0	0	0	0
	3036	0	0	0	0	0	0
<u>E. coli</u>							
F- 1833	control	+	+	+	0	0	0
	1747	0	0	0	0	0	0
	3018	0	0	0	0	0	0
<u>fer</u> 3018	control	+	+	+	0	0	0
	1833	0	0	0	0	0	0
	1747	0	0	0	0	0	0
Hfr 1747	control	+	+	+	0	0	0
	1833	0	0	0	0	0	0
	3018	0	0	0	0	0	0

* + indicates agglutination; 0 indicates no agglutination.

TABLE III
RESULTS OF AGGLUTININ-ADSORPTION TESTS USING
HEATED CELL SUSPENSIONS AS ANTIGENS*

Antiserum	Adsorbing antigen	Test antigen					
		<u>E. coli</u>			<u>S. typhimurium</u>		
		F- 1833	<u>fer</u> 3018	Hfr 1747	F- 1902	<u>fer</u> 3036	Hfr 1826
<u>S. typhimurium</u>							
F- 1902	control	0	0	0	+	+	+
	1747	0	0	0	+	+	+
	3018	0	0	0	+	+	+
	1826	0	0	0	0	0	0
<u>fer</u> 3036	control	0	0	0	+	+	+
	1747	0	0	0	+	+	+
	1833	0	0	0	+	+	+
	3018	0	0	0	+	+	+
	1826	0	0	0	0	0	0
	1902	0	0	0	0	0	0
Hfr 1826	control	0	0	0	+	+	+
	1747	0	0	0	+	+	+
	1833	0	0	0	+	+	+
	3018	0	0	0	+	+	+
	1902	0	0	+	0	0	+
	3036	0	0	+	0	0	+
<u>E. coli</u>							
F- 1833	control	+	+	+	0	0	0
	1747	0	0	0	0	0	0
	3018	0	0	0	0	0	0
<u>fer</u> 3018	control	+	+	+	0	0	0
	1747	0	+	0	0	0	0
	1833	0	+	0	0	0	0
Hfr 1747	control	+	+	+	0	0	0
	1833	0	0	+	0	0	+
	3018	0	0	+	0	0	0

* + indicates agglutination; 0 indicates no agglutination.

S. typhimurium Hfr antiserum and vice versa. Third, the fer E. coli 3018 antiserum gave further agglutination of its antigen following adsorption with either E. coli strain 1747 or 1833. Other than the exceptions just mentioned, the O antigens also showed species specificity.

There was some difficulty with auto-agglutination of the Salmonella 1826 and 3036 antigens. Care, therefore, had to be taken to use fresh antigens of these strains for agglutination tests.

Adsorption appeared incomplete in some instances. An explanation for this is not available, as the recommended procedure for adsorption was followed, and an excess of adsorbing antigen was used.

O and H Antigen Typing.

The flagellar and somatic antigen types were determined to provide a more complete analysis of the antigenic make-up of the six test strains. Of particular interest was the typing of the hybrids as compared with their parents.

The E. coli cultures 1747 and 1833 were found to be in the poly-A group of the E. coli O typing system. Further testing showed them to be O:127 strains. The E. coli hybrid 3018, however, showed exceptional antigenic typing. It was found to agglutinate not only E. coli antisera, but also Salmonella antisera. It was impossible to identify the antigen, as the hybrid reacted with several of the individual Salmonella antisera.

The S. typhimurium cultures 1826, 1902, and 3036, were found to give the expected positive reactions with the poly-O Salmonella serum. They were further found to be of the Salmonella O group B.

They reacted with flagellar antisera i and 1:7. These reactions coincide with Edward and Ewing's (1962) typing scheme for S. typhimurium: somatic antigens 1,4,5,12 and flagellar antigens i:1:7.

None of the Salmonella strains showed agglutination of E. coli O antisera. Therefore, the antigenic typing of the hybrid 3036 was apparently not altered by the fact that it contained E. coli genetic material from its male parent.

Microscopic Gel Diffusion Test

The gel diffusion precipitin test has been used successfully in serological studies of various bacterial genera. Some of the strains analyzed in this manner were Brucella (Sulitzeanu, 1958), Vibrio fetus (Risti, 1959), and Pasturella (Chen and Meyer, 1955; Davies, 1956) in addition to the widely used Diphtheria toxin test.

Olitzki and Sulitzeanu (1957, 1958) reported precipitin lines using acetone-killed cells and sonic extracts of acetone-killed Brucella cells. However, they found no lines using live cells as antigens. It was hoped, therefore, that the double diffusion microscopic test might be adapted to determine qualitative antigenic differences among the cellular extracts of the six test strains.

No differences in the efficiencies of the two agar preparations could be noted. However, Wilson and Pringle's agar was used most often.

No lines appeared with the live or heated cell suspensions being used as antigens. After this discovery, it was decided not to concentrate on the H antigens in this study, as the agglutination-adsorption results with these antigens had proved inconclusive.

Precipitin lines did form on the slides where sonic cell extracts were placed in the antigen wells against the antiserum. These lines were visible in approximately five days. All antisera were tested with all of the six sonic extracts of the test cultures. In all cases, the antiserum was placed in the center well and the antigens in the surrounding wells.

Both Extracts 1 and 2 gave positive reactions; however, the results of the two extracts were not always identical. Therefore, the results presented here are the combined results of both extracts.

The results of the tests were not recorded photographically, but rather analyzed and recorded as positive or negative, as the tests were intended to be strictly qualitative. The results of the gel diffusion tests utilizing sonic extracts of heated cell suspensions as antigens are recorded in Table IV, page 54.

The appearance of a precipitin line on a slide was considered evidence that the antiserum and antigen were in proper proportions, as all sonic extracts were prepared in the same manner and were believed to be of comparable concentrations.

Some halos were observed surrounding the wells of certain antisera. This has been reported to occur with serum that is aged (Bösel et al., 1965). These were easily distinguished from the specific immunoprecipitates.

These reactions did not show species specificity to the degree that was found in agglutinin-adsorption, although some did occur. Most reactions appeared to be strain specific.

There were two reactions of interest in these tests. First, was the interaction of the E. coli and S. typhimurium Hfrs. The E. coli

TABLE IV
 RESULTS OF MICROSCOPIC DOUBLE GEL DIFFUSION TESTS
 USING SONIC EXTRACTS OF HEATED CELL CULTURES
 AS ANTIGENS*

Antiserum	Bacterial sonic extract					
	<u>E. coli</u>			<u>S. typhimurium</u>		
	F- 1833	<u>fer</u> 3018	Hfr 1747	F- 1902	<u>fer</u> 3036	Hfr 1826
<u>S. typhimurium</u>						
F- 1902	0	+	0	+	0	0
<u>fer</u> 3036	0	0	0	0	+	0
<u>Hfr</u> 1826	0	+	+	+	0	+
<u>E. coli</u>						
F- 1833	+	+	0	0	0	0
<u>fer</u> 3018	+	+	+	+	+	+
<u>Hfr</u> 1747	+	0	+	0	0	+

* + indicates a visible precipitin line appeared in five days.

Hfr 1747 antiserum precipitated the S. typhimurium Hfr 1826 sonic extract; and, in turn, the S. typhimurium Hfr 1826 antiserum precipitated the E. coli Hfr 1747 sonic extract. Second, it should be noted that the antiserum of fer 3018, derived from an E. coli F- and S. typhimurium Hfr, reacted with all six of the test antigens. The significance of these findings will be discussed later.

Electron-Microscopic Examination

The electron-microscopic examination of the specimens was intended to provide information supplemental to the serological studies. The physical appearance of the cultures was studied and the appendages present given particular attention. The objective was to seek out any unique physical characteristic that might be correlated with the sexual state of the strain.

Figures 2 through 8, pages 58 and 59, are photographs selected to be representative of the cell types observed. All combinations of pili and flagella on cells of a single culture were observed. There were cells with neither flagella nor pili, as well as cells with both flagella and pili.

The flagella observed were long, wavy filaments and appeared typical of previously published photographs of flagella (Smith, 1954; Houwink and Van Iterson, 1961). There was no noticeable difference among the flagella of the six test strains.

The number of pili per cell varied a great deal within each strain. Each culture contained cells with no pili visible, as well as cells with numerous pili covering the cell surface. The pili also appeared typical when compared to photographs published by other

workers (Houwink and Van Iterson, 1961; Brinton, 1959; Weibull and Hedwall, 1953; Duguid et al., 1955; Maccacaro and Angelotti, 1955; Duguid and Gillis, 1956).

No physical differences could be determined in the number or appearance of pili among the strains. No other distinguishing cellular characteristics were evident.



Figure 2. Electron micrograph of E. coli Hfr 1747.



Figure 3. Electron micrograph of E. coli F- 1833.

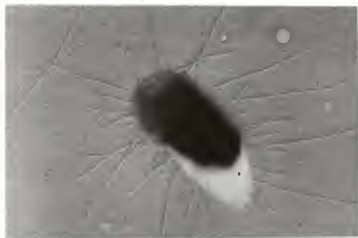


Figure 4. Electron micrograph of hybrid 3018, derived from an E. coli F- x S. typhimurium Hfr cross.



Figure 6. Electron micrograph of S. typhimurium F-1902



Figure 7. Electron micrograph of S. typhimurium Hfr 1826.



Figure 8. Electron micrograph of hybrid 3036, derived from a S. typhimurium F- x E. coli Hfr cross.

DISCUSSION

There was nothing in the results of the H antigenic tests to suggest that these antigens have a significant role in the sexual types involved in bacterial conjugation. Neither did the electron-microscopic examinations reveal any outstanding characteristics of the flagella of any of the strains. Therefore, it was concluded that flagellar structure is not correlated with the sexual state.

The attempt to relate the sexual state to cell structures visible under the electron microscope was also unsuccessful. It should be mentioned here that aerated broth cultures were used as specimens for the examination. It has since been learned that aeration decreases the production of pili. This could account for the fact that pili were not abundant on most of the cells viewed. Regardless of this fact, however, enough piliated cells were observed to be able to deduce that there was no number or structure of pili characteristic to any one strain or sexual type.

The O antigen used for the preparation of the antiserum and test antigens were prepared in much the same manner as described by Ørskov and Ørskov. The basis for detecting the f+ antigen was the removal of the common somatic antigens from the Hfr antiserum by adsorption with the related F- and then testing for agglutination with the Hfr antigen. Any agglutination at this point was assumed to be due to the f+ antigen-antibody complex, provided, of course, that there was no further agglutination by the F- used for adsorption of the serum. As the Ørskovs reported, this f+ agglutination is weak, and any agglutination, even at the dilution of 1:5 or 1:10 of the adsorbed antiserum

was considered a positive reaction. Both S. typhimurium and E. coli Hfrs were tested against the adsorbed Hfr antiserum of the opposite genus in this manner.

Naturally, results based on the observance of such weak agglutination might be questioned. However, based on the above understanding of the nature of the f+ antigen, it seems safe to conclude that the agglutination of the E. coli and S. typhimurium Hfrs by their respective adsorbed antisera represented the f+ antigen.

Therefore, the agglutination of the E. coli Hfr 1747 antiserum by the 1747 antigen following adsorption with E. coli F- 1833 must be due to the presence of an f+ antigen on the E. coli Hfr cells. At the same time, the agglutination of the S. typhimurium Hfr 1826 serum by 1826 antigen after adsorption with S. typhimurium F- 1902 indicates a f+ antigen on the S. typhimurium Hfr cells also.

The above described reaction was observed also when the Salmonella Hfr antiserum was adsorbed with the corresponding S. typhimurium hybrid 3036. This hybrid, thus, appeared to act identically to the F- parent in removing the somatic antibodies and allowing the f+ antigen to be expressed. However, the E. coli hybrid did not always act identically to the E. coli F-.

Not only did the Hfrs react with their own adsorbed antiserum, but the E. coli Hfr 1747 also reacted with the adsorbed S. typhimurium Hfr serum, while S. typhimurium 1826 reacted with adsorbed E. coli Hfr serum. It appears, therefore, that related f+ antigens are present on E. coli and S. typhimurium male cells. This is compatible with the belief that the F factor, which was originally introduced into Salmonella strains from E. coli male cells, is responsible for the

presence of the f+ antigen on the cell surface. It seems reasonable, then, that they should share a common f+ antigen.

The gel diffusion tests had the disadvantage that the antisera used were not adsorbed. This made it impossible to detect the f+ antigen within the species due to the somatic antibodies present. However, it was felt that because of the nature of the tests and the antigen used, these might provide some useful information.

The gel diffusion tests did, indeed, provide some interesting results. One of these was that the E. coli Hfr sonic extract formed precipitin lines against the S. typhimurium Hfr antiserum, and vice versa between the S. typhimurium Hfr antigen and the E. coli Hfr antiserum. This adds to the evidence that a common antigen does exist between the male strains of the two species.

Therefore, the gel diffusion tests, in conjunction with the agglutinin-adsorption tests, fulfilled the first two objectives of the serological tests. First, the f+ antigen of E. coli previously described by the Ørskovs, as well as a similarly acting antigen in S. typhimurium male cells, was confirmed. Second, such antigens in the two species were found to be immunologically related.

The third objective, the analysis of the antigenic make-up of the "fertile" hybrid strains, was approached in much the same manner as the detection of the f+ antigen. The agglutinin-adsorption results of interest in this case, however, were those of the F- parent antiserum adsorbed with its respective hybrid and tested for agglutination with the F-, and the hybrid antiserum adsorbed with the parent F- and tested with the hybrid. In this way, it was possible to detect any antigen present in one strain and not the other.

The hybrid of E. coli, 3018, gave agglutination with its own antiserum, not only following adsorption with its E. coli F- parent 1833, but also following adsorption with the E. coli Hfr, 1747. This indicated that the hybrid harbored antigens found neither in the F- nor Hfr E. coli. It seems plausible that this antigen had been acquired from the S. typhimurium male parent. There was no evidence of agglutination of 3018 by various S. typhimurium antisera in the agglutinin-adsorption tests, however.

It is at this point that the action of fer 3018 in the gel diffusion test and in antigenic typing should be noted. This strain's antiserum resulted in precipitation lines with all of the six test strains, both E. coli and S. typhimurium, suggesting that the unique antigenic fraction might be Salmonella. More evidence that the E. coli hybrid had gained a Salmonella antigen was its peculiar antigenic typing. This strain gave strong agglutination with Salmonella, as well as E. coli, typing antisera.

The possibility of this strain having altered bacteriophage susceptibility along with the antigenic change was investigated. Bacteriophage typing revealed that this strain had acquired susceptibility to certain Salmonella phage to which its E. coli F- parent was resistant. This confirmed the belief that the new surface antigen was Salmonella in nature.

The hybrid 3036, from the S. typhimurium F-, showed no such unique antigenic structure. Its serum gave no further agglutination following adsorption with either S. typhimurium F- 1902 or Hfr 1826. Its antiserum showed no cross reaction with E. coli antigens, neither

in agglutination nor gel diffusion tests. This hybrid has apparently acquired no E. coli antigens from its E. coli Hfr parent.

These results made it difficult to draw a general conclusion concerning the antigenic make-up of "fertile" hybrids. Antigenic changes are evidently not imperative for fertility, as the hybrid 3036 had acquired no such antigenic fraction, yet was considered fertile in recombination with the E. coli Hfr. It does seem safe to conclude, though, that antigenic conversion by sexual recombination can occur during interspecies crosses, as it is known to occur during intraspecies crosses. This completed the third phase of the serological study, that of analyzing the antigens of the fer hybrid strains.

The immunological survey of a number of E. coli-S. typhimurium hybrids would be necessary before making definite statements concerning their antigenic composition. Both this study, and a more intensive investigation of the antigens of E. coli and S. typhimurium Hfrs and F-'s, could provide interesting information about the role of antigens during bacterial conjugation.

CONCLUSION

Serological and electron-microscopic examination were utilized in an attempt to detect any unique antigenic or physical characteristics which might be correlated with the sexual state of six test bacterial strains. These strains included an Hfr, F-, and "fertile" hybrid of both Escherichia coli and Salmonella typhimurium.

Agglutinin-adsorption and slide gel diffusion tests led to the confirmation of a f+ antigen in both S. typhimurium and E. coli Hfrs. These f+ antigens were found to react with the f+ antiserum prepared from the Hfr of the opposite genus. Thus, a common male antigen appears to be shared by S. typhimurium and E. coli male cells.

No significant or consistent physical characteristics could be determined upon examination of electron micrographs of the six test strains. All cultures exhibited similar cell morphology, with pili and flagella being observed in all test cultures.

The adsorption-agglutinin tests revealed that the hybrid 3018, derived from an E. coli F- and S. typhimurium Hfr, contained some antigenic fraction found in neither the E. coli F- nor Hfr. The slide gel diffusion tests indicated that the hybrid cross-reacted with S. typhimurium, and it thus appeared that the hybrid inherited a part of its antigenic structure from its S. typhimurium male parent. The Salmonella nature of the acquired antigen was confirmed by antigenic typing and bacteriophage susceptibility tests. No such E. coli antigenic fraction could be detected in the hybrid 3036, derived from a S. typhimurium F- and E. coli Hfr. Therefore, no generalization could be made concerning the antigenic constitution of such

hybrids. There was evidence of antigenic conversion occurring during interspecies bacterial recombination.

Certainly, further investigation of the serological properties of various sexual types, as well as the hybrids, of E. coli and S. typhimurium could provide information very helpful towards the comprehension of the mechanism of bacterial conjugation.

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ANTIGENIC AND SURFACE PROPERTIES OF FERTILE
STRAINS OF ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM

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Bacterial recombination occurs between compatible cell types of a F+ or Hfr (donor) and a F- (recipient). The formation of effective pairs is the first step in the formation of recombinants and is followed by chromosome transfer and the integration of the newly received genetic material by the recipient.

Factors responsible for effective pair formation have been investigated. A unique antigenic constituent of male Hfr and F+ cells has been described and termed "f+ antigen." The presence of a special type of appendage, F pilus, has also been attributed to male cells.

Bacterial conjugation takes place in several species of Enterobacteriaceae, and interspecies crosses occur as well. Of particular interest is the successful mating of compatible cell types of E. coli and S. typhimurium and the resulting hybrid recombinants, which have been found to give high frequency recombination (are "fertile") when mated with their parent Hfr.

The objectives of the research described in this thesis were first, to confirm the presence of an f+ antigen on E. coli Hfr cells and seek a similar antigen in S. typhimurium; second, to attempt to determine any antigenic relationship between E. coli and S. typhimurium Hfr strains; and third, to detect any unique antigenic fraction of the hybrids of E. coli-S. typhimurium. In addition to the serological aspects, electron-microscopic examination of the test strains was used to seek any differences in the visible surface structure of the sexual type.

Agglutinin-adsorption and gel diffusion tests were used for serological experiments. Six test strains were utilized: Hfr, F- and "fertile" hybrids of both E. coli and S. typhimurium. Heated cell

suspensions were used as antigens in agglutination tests. Sonic extracts of the heated antigens were used as antigens in the microscope-slide adaption of the gel diffusion precipitation test. Adsorption of the male antisera with the corresponding female cells was used to detect any male antigen present. Adsorption of the hybrid antisera with the parent cell strains was used to detect any unique antigenic fraction of the hybrid. Conventional antigenic typing of the six strains was also carried out.

Agglutinin-adsorption tests indicated the presence of an antigen in S. typhimurium Hfrs as well as E. coli Hfrs not found in F- strains, and these antigens were immunologically related. The same tests indicated also that the hybrid from E. coli F- x S. typhimurium Hfr possessed an antigen present in neither E. coli F- nor Hfr strains. The E. coli hybrid also exhibited exceptional typing with commercial E. coli and Salmonella antisera.

Precipitation lines appeared between the E. coli Hfr sonic extract and the antiserum of the S. typhimurium Hfr and vice versa in the gel diffusion tests. The above mentioned E. coli hybrid antiserum formed precipitation lines with all test antigens, including the S. typhimurium antigens.

Electron-microscopic examination failed to reveal any characteristic cellular morphology or appendage among the sexual types.

The above results led to the conclusion that there is a f+ antigen in Hfr strains of E. coli and S. typhimurium and these antigens are related. The results also indicated that the tested "fertile" E. coli hybrid has an antigen fraction not present in the E. coli strains tested. No such antigenic fraction was detected in the S. typhimurium

"fertile" hybrid. Therefore, no definite conclusions could be drawn concerning the antigenic make-up of such hybrids.

Further serological investigation of the sexual types of E. coli and S. typhimurium, as well as their hybrids, could reveal much about the role and action of antigens during conjugation.