TRANSFER OF PILIATION FROM *ESCHERICHIA COLI* TO *SALMONELLA TYPHOSA* BY GENETIC RECOMBINATION

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SUMMARY

The ability to produce pili, the hair-like, non-flagellar appendages of bacteria, has been transferred from *Escherichia coli* to *Salmonella typhosa* by genetic recombination. The piliated *E. coli* donor was mixed with the permanently non-piliated *S. typhosa* recipient on solid medium which could not support the growth of either. Recombinant forms were selected in the presence of various sugars which the *S. typhosa* parent could not ferment. Hybrid forms fermenting xylose or rhamnose were, with few exceptions, piliated. Since the *S. typhosa* parent was never observed to produce a piliated form spontaneously, the occurrence of pili on hybrids which retained most of the biochemical and all of the antigenic properties of *S. typhosa* must have been due to genetic transfer. Furthermore, the antigenicity of the transferred pili was determined by the *E. coli* donor.

The piliated *S. typhosa* hybrids, which also possessed the Vi antigen, spontaneously produced two other, more stable forms, usually at a high rate. One of these lacked pili and the other lacked the Vi antigen. This rapid breakdown was probably due to the segregation of diploid heterozygotes. An analysis of the segregants showed the piliation character to be linked to arabinose fermentation.

INTRODUCTION

Pili are non-flagellar, hair-like appendages, unrelated to motility, found growing from the surface of many different species of bacteria. They are thinner, straighter, and more numerous than flagella and have a uniform diameter of 80–100 Å. Many piliated strains of bacteria can exist in two forms, the completely piliated (P+) or the completely non-piliated (P—), and these strains may change from one form to the other at a high rate[1–3].

The P+ form of *Escherichia coli* B–L(E), studied by BRINTON[3], however, spontaneously gives rise to stable P— forms which do not revert to P+. Presumably, other strains not having pili have been isolated as stable P— forms from nature. A stable P— strain, thus, could not produce pili unless it were to change spontaneously

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(rare mutation to P+), or unless it could be made P+ by a process of genetic transfer such as bacterial recombination[4] or transduction[5].

The present account is a report on the transfer of the P+ character from a high frequency of recombination (Hfr) strain of *E. coli* K-12 (see ref. 6), to a stable P— strain of *Salmonella typhosa* as a result of genetic recombination[7]. The serological cross-reaction with *E. coli* in certain hybrids from matings between donor (Hfr, P+) *E. coli* and recipient (F— P—) *S. typhosa* observed by BARON *et al.*[8] is explained by the acquisition of pili (pilial antigen) by the *Salmonella* hybrids (F—, P+).

MATERIALS AND METHODS

Bacterial strains

The Hfr parent was the W1895 strain of *E. coli* K-12 (see ref. 6), which was able to ferment the sugars, lactose (Lac+), L-arabinose (Ara+), D-xylose (Xyl+), L-rhamnose (Rha+), L-fucose (Fuc+) when received from Dr. P. D. SKAAR, and D-arabinose (D-Ara+) following selection of a mutant clone able to utilize this sugar. An F— strain (643L+) of *S. typhosa* was isolated from the F° strain 643 by BARON *et al.*[9] as a Lac+ hybrid from an initial mating with W1895 and used as the recipient strain in further crosses. Strain 643L+ was able to utilize lactose as a result of the initial mating, but like the parent strain 643, still required cystine (cys—) and tryptophan (try—) and was unable to utilize any other of the above sugars. It still produced the somatic (O) antigens 9, 12, the flagellar (H) antigen "d", and the Vi antigen, components characteristic of *S. typhosa*. In common with all strains of *S. typhosa* tested, it did not cross-react with antiserum against the *E. coli* K-12 strain.

Media

0.8 % Difco nutrient broth plus 0.5 % sodium chloride was used for both liquid and solid media for purposes of routine transfer or cultivation. Plates and slant or stab tubes were prepared by the addition of 2 % agar to the liquid media.

Minimal medium was made up by mixing separately autoclaved Difco Noble Agar (14 g/400 ml H_2O); physiological saline, 300 ml; KH_2PO_4, 40 ml of 60 g/l; K_2HPO_4, 40 ml of 140 g/l; $(NH_4)_2SO_4$, 40 ml of 20 g/l; $MgSO_4$, 4 ml of 20 g/l and 10 ml of a 20 % solution of the appropriate carbohydrate previously sterilized by filtration. To this medium was added 10 ml of 0.4 % L-cystine and 10 ml of 0.4 % L-tryptophan, to satisfy the nutritional requirements of the *S. typhosa* recipient organism.

Special Eosin–Methylene Blue agar without sugars (Difco, BBL), to which was added 0.4 % of the appropriate sugar (Pfanstiehl) was employed. Phenol Red Broth (Difco) plus 1 % carbohydrate was used for fermentation tests.

Recombination techniques

The parental strains, *E. coli* W1895 and *S. typhosa* 643L+ were centrifuged from 18 h nutrient broth cultures and washed 3 times with saline. 0.1 ml containing $1 \cdot 10^7$ cells of W1895 was spread together with 0.1 ml containing $2\text{–}5 \cdot 10^8$ cells of 643L+ on plates of minimal agar containing cystine (cys), tryptophan (try), and either L-arabinose, D-xylose, L-rhamnose, L-fucose, or D-arabinose as the selective carbohydrate. The *E. coli* parent, W1895, required methionine (M—), while the *S. typhosa* hybrid 643L+, used as the recipient in these crosses, could not utilize L-arabinose

or the other carbohydrates listed. M+Ara+, M+Xyl+, etc. progeny can be detected only on addition of cystine and tryptophan which fulfill the nutritional requirements of the recipient parent. Recombinants were purified by streaking on EMB agar containing the appropriate carbohydrate. In cases of ambiguity, suspect colonies were restreaked further on this medium to establish purity and stability.

Bacterial agglutination

To fourfold serial dilutions of rabbit antisera in saline were added about $5 \cdot 10^8$ bacteria per ml. After 1 h at room temperature (25–30°) the tubes were observed by oblique lighting under low magnification. The presence of visible agglutination was taken as a positive reaction. The procedures described by EDWARDS AND EWING for slide agglutination tests also were employed. Cultures were examined simultaneously in saline and tested with normal serum at the same dilution as that used for the antiserum.

Electron microscopy

Cultures were grown in aerated liquid medium to a concentration of about $3 \cdot 10^8$ cells/ml, fixed for 1 h by the addition of formaldehyde to a concentration of 2 %, and dialyzed against several changes of distilled water to remove inorganic salts. A small drop of the cell suspension was placed on a collodion film overlying a microscope grid and allowed to air dry. Specimens were shadowed with platinum and observed in an RCA EMB microscope. Some cultures were centrifuged and washed twice with distilled water before fixing and drying. This procedure resulted in a cleaner background.

Hemagglutination

Cultures to be tested for their capacity to agglutinate chicken red blood cells were diluted and spread on the surface of nutrient agar plates and incubated at 37° for 18 h. About one hundred individual colonies were combined and suspended in 1 ml of physiological saline solution. Two-fold serial dilutions were made in saline in 5-ml glass tubes. Red blood cells were centrifuged from preparations of heparinized chicken blood and washed 3 times with saline. These were added to each tube to a concentration of about 10^7 cells/ml, and after 3 h at 4° the agglutination test was read. With positive hemagglutination a diffuse sediment appears at the bottom of the tube, while lack of hemagglutination is shown by a sharply defined red button.

RESULTS

Initial observations

When the *E. coli* Hfr was crossed with the *S. typhosa* hybrid, 643L+, the number of recombinants obtained was found to vary depending on the sugar used to detect the progeny. It has been observed also that recombinants selected on plates containing xylose or rhamnose, with few exceptions, were highly unstable forms[8]. These forms could not be purified as stable positives for the sugar on which they had been selected. Two principle types of distinguishable colonies, however, could be observed by means of oblique lighting when a single positive clone from EMB agar was restreaked on nutrient agar: a small, dense colony which was shown to possess the

Vi, O, and H antigens of *S. typhosa* and which cross-reacted with antiserum prepared against living *E. coli* K-12, and a larger, more translucent form, typical of *S. typhosa* 643L+ (non-reactive with *E. coli* antisera but reacting with *S. typhosa* Vi, O, and H antisera). Further restreaking of the dense form gave rise almost entirely to stable translucent colonies, usually within a few subcultures. The dense form was so unstable that the translucent colonial type often arose as a sector within an originally dense colony.

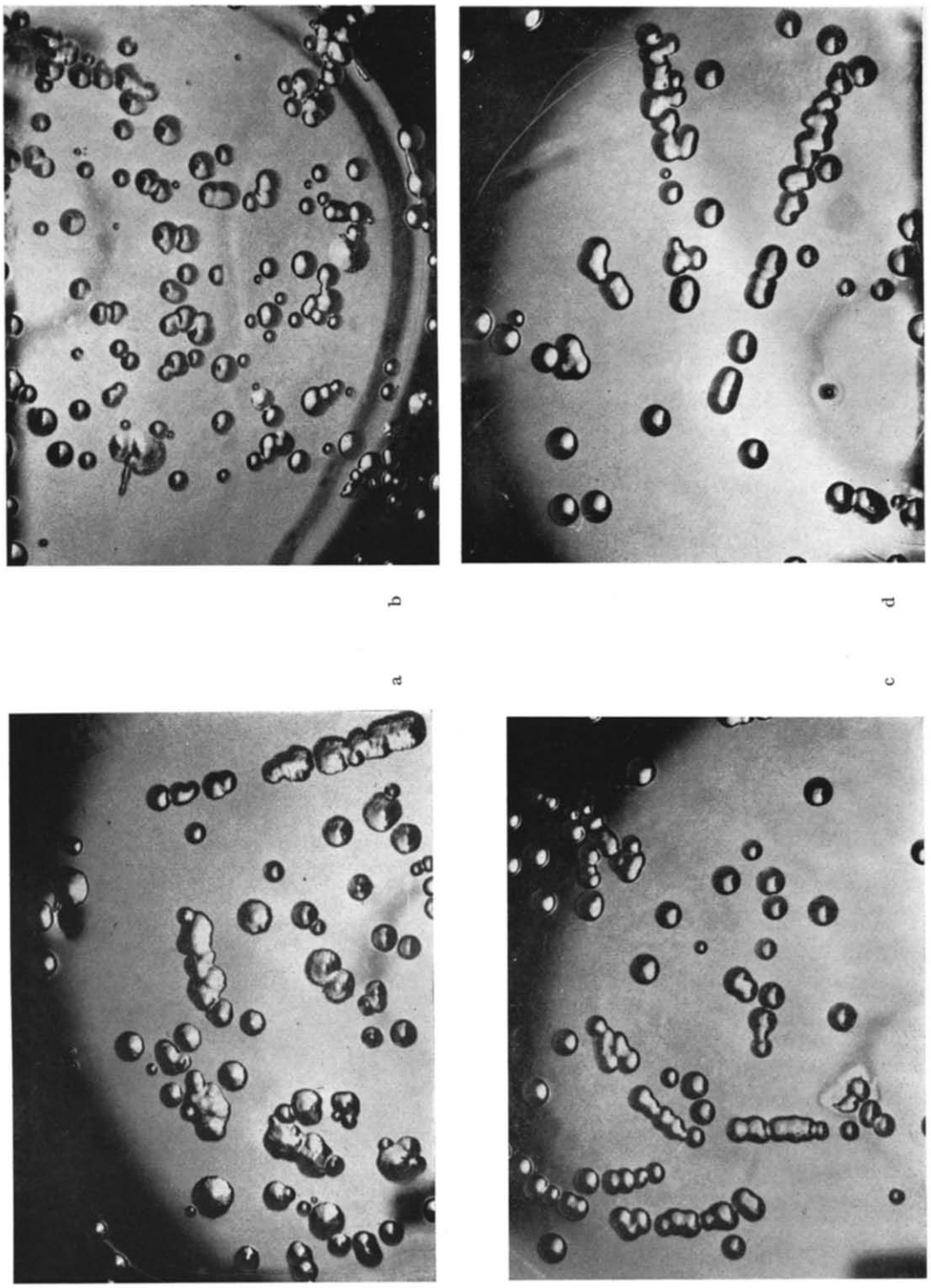
One of the recombinants, strain X30D, selected for the ability to utilize xylose, however, was a small, dense colonial form which remained relatively stable, although, occasionally it did give rise to a considerably larger, more translucent variant, X30T, and also, to a slightly smaller form, X30P.

The properties of these variants which were derived from the hybrid X30D were examined in detail and are compared with those of the parents in Table I. The *E. coli* K-12 Hfr parent was composed of two colonial types, a smooth form and a rough form (Fig. 1). Since no difference between these two types was ever observed with respect to any of the characteristics in Table I, they are both listed in the same column. Both the *S. typhosa* 643 parent and the hybrid *S. typhosa* 643L+ exhibited one main type of colony form which was rather large and glistening when viewed

TABLE I

CHARACTERISTICS OF PARENTAL AND HYBRID STRAINS

Characteristics	*Male parent E. coli K-12 Hfr W1895*	*S. typhosa 643*	*Female parent S. typhosa 643L+*	*Hybrid selected on xylose X30D*	*Segregants from hybrid X30D*	
					X30T	*X30P*
Lactose	+	—	+	+	+	+
L-arabinose	+	—	—	+	—	+
D-xylose	+	—	—	+	+	+
L-rhamnose	+	—	—	+	+	+
L-fucose	+	—	—	—	—	—
D-arabinose	+	—	—	—	—	—
L-cystine	+	—	—	—	—	—
L-tryptophan	+	—	—	—	—	—
Methionine	—	+	+	+	—	—
Indol	+	—	—	+	+	+
Vi antigen	—	+	+	+	+	—
O antigen 9, 12	—	+	+	+	+	+
H antigen d:-	—	+	+	+	+	+
Electron microscopy	Piliated	Non-piliated	Non-piliated	Piliated	Non-piliated	Piliated
Agglutination with anti-pili serum from *E. coli* B-L(E)	+	—	—	+	—	+
Agglutination with antiserum against live *E. coli* K-12, Hfr, W1895	+	—	—	+	—	+
Hemagglutination	+	—	—	+	—	+
Colonies	Large rough and smooth	Large, smooth translucent	Large, smooth translucent	Medium, dense	Large, smooth translucent	Small, dense
Agglutination with anti-serum against hybrid X30D	+	+	+	+	+	+



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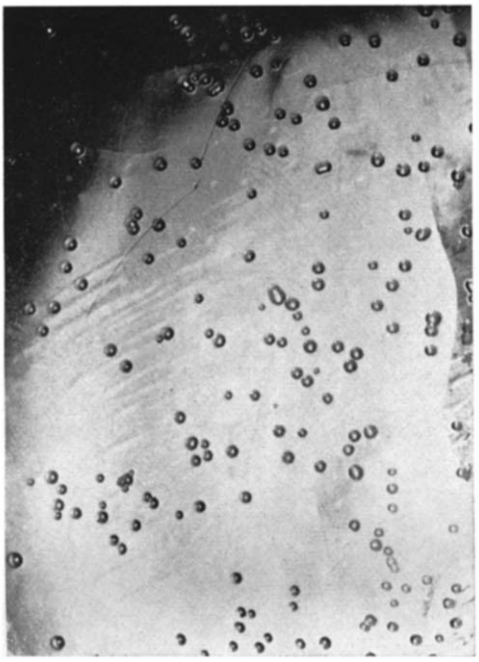

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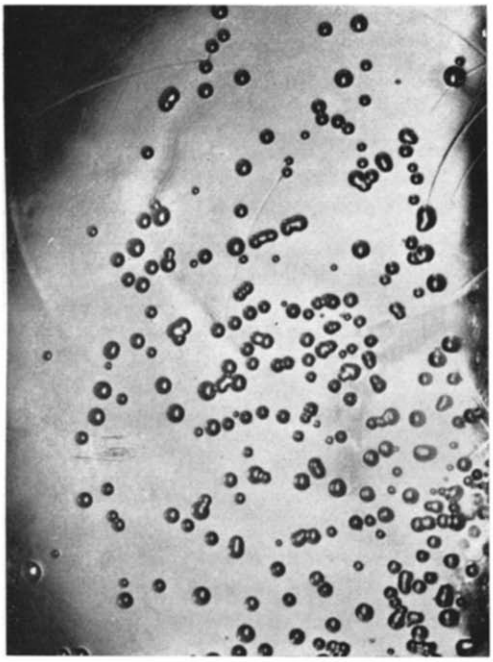

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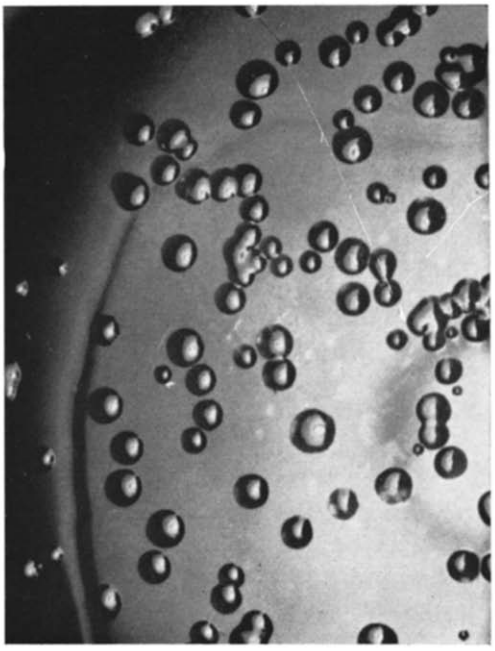

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Fig. 1. Colonial morphology. Single colonies of each strain were inoculated into broth and grown to about 10^8 cells/ml. These cultures were diluted, spread on nutrient agar plates, and incubated overnight. a, male parent *E. coli* K-12 Hfr W1895 "rough"; b, male parent *E. coli* K-12 Hfr W1895 "smooth"; c, original *S. typhosa* 643; d, female parent *S. typhosa* 643L+; e, hybrid X30D; f, hybrid X30P (segregant X30D); g, hybrid X30T (segregant X30D).

under reflected light (Fig. 1). However, both 643 and 643L+ when subcultured can spontaneously produce a colonial variant which has lost the Vi antigen. The Vi+ form of 643L+ was used as the parent in the cross which produced the hybrid X30D.

BARON *et al.*[8] had observed a cross-reaction between X30D or X30P with antiserum prepared against living cells of *E. coli* K-12, by slide and tube agglutination procedures. X30T did not show this cross-reaction. An antiserum prepared against cells of *E. coli* K-12 boiled for 1 h did not agglutinate either X30D, X30P, or X30T, showing that the cross-reaction was not due to the transfer of a heat-stable somatic antigen. Other results in this study, using serum immobilization procedures, had served to eliminate the possibility of a transfer of flagellar antigen from *E. coli* to these *Salmonella* hybrids. The likelihood that a transfer of pili was involved was considered, because of the known antigenicity of pili and the remarkable resemblance of the colonial variation of the cross-reacting *Salmonella* hybrids to that of the piliated form of *E. coli* B–L(E) (see ref. 3). Both *E. coli* B–L (E) P+ and the hybrids X30D and X30P have small, dense colonies which upon sub-culture at 37° rapidly break down to larger, more translucent colonies.

Electron microscopy

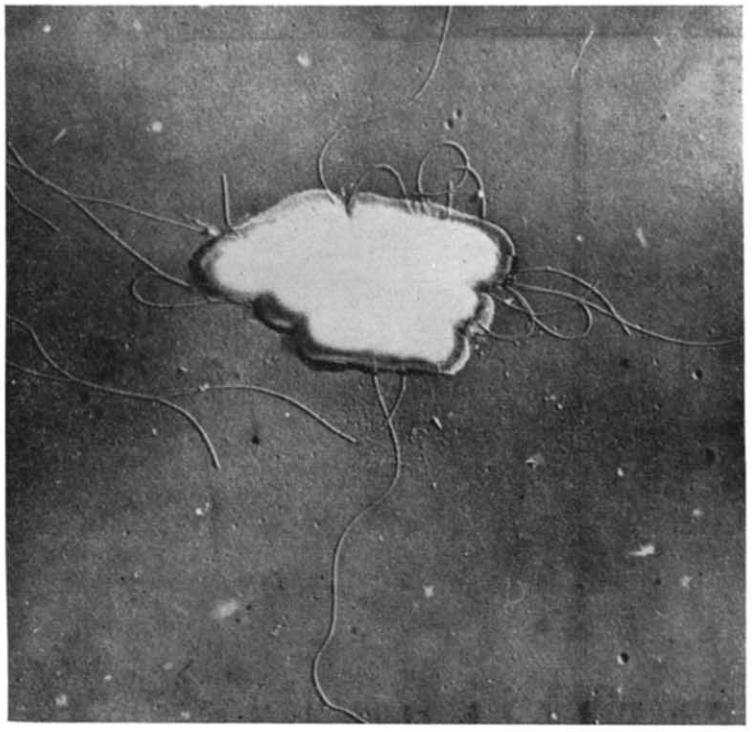
These studies were undertaken in order to detect visually and in a definitive manner the presence or absence of pili. Cells from a broth culture of the *E. coli* K-12 Hfr parent, examined in the electron microscope, were almost all piliated. Several hundred individual cells of the *S. typhosa* 643L+ parent were examined and none were found to be piliated, nor were any fragments of pili ever seen on the supporting collodion film (Fig. 2). Over a hundred cells of the original strain, *S. typhosa* 643, were examined and pili were completely absent in this strain also.

Practically all cells of the hybrid X30D, however, were found to be richly piliated as were cells of X30P, while the X30T segregant, which fails to cross-react with *E. coli* showed a complete absence of pili (Fig. 2). Thus, the serological cross-reaction of the hybrid X30D and its segregant X30P with the *E. coli* K-12 Hfr parent, as well as the lack of cross-reaction of the hybrid segregant X30T with the *E. coli* parent, can be explained by the observation that pili are common to the cross-reacting strains.

The pili of these strains were very similar in size, shape, and distribution to each other and to the pili of many other strains. They were of a uniform diameter of about 100 Å, with lengths from less than 0.1 μ to about 3.0 μ, and were distributed uniformly. They were fairly straight and did not exhibit the regular wave-like structure of *flagella*. All of the strains, both parents and hybrids were flagellated.

Serological studies

The results obtained with antisera to the W1895 strain of *E. coli* have been described briefly above and are covered in detail by BARON *et al.*[8]. Further confirmation of the distribution of pilial antigens among the six strains examined, however, was achieved by means of agglutination tests of each strain against antiserum made with purified pili from *E. coli* B–L(E). This rabbit antiserum was prepared using pili which had been mechanically detached from the bacteria and purified by electrophoresis[3]. It agglutinated the piliated phase of *E. coli* B–L(E) to a titer of 1:2560, but did not agglutinate the non-piliated phase. The results of a tube agglutination



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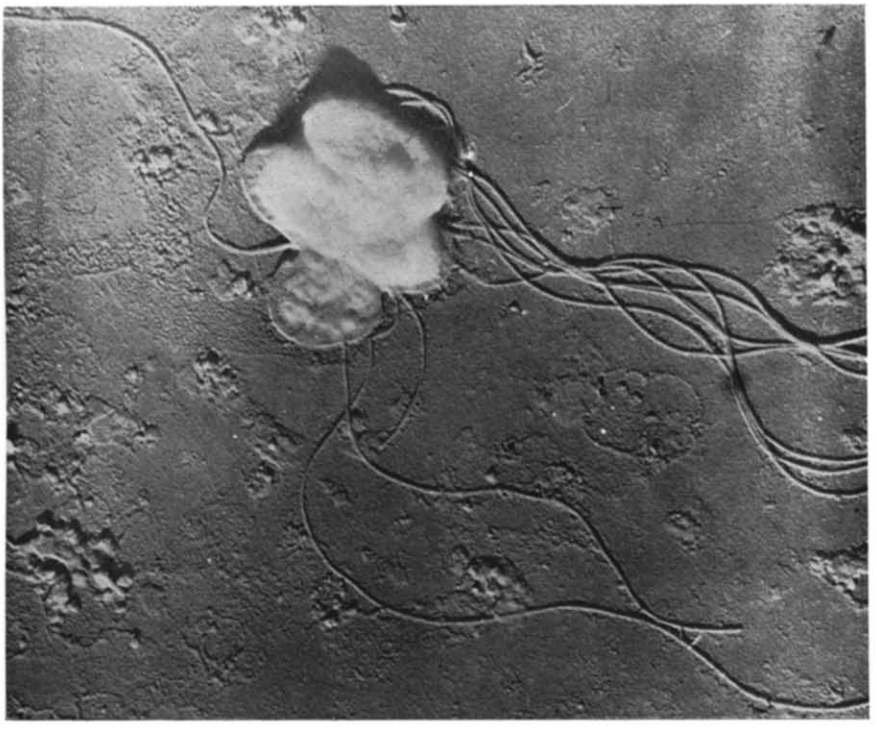

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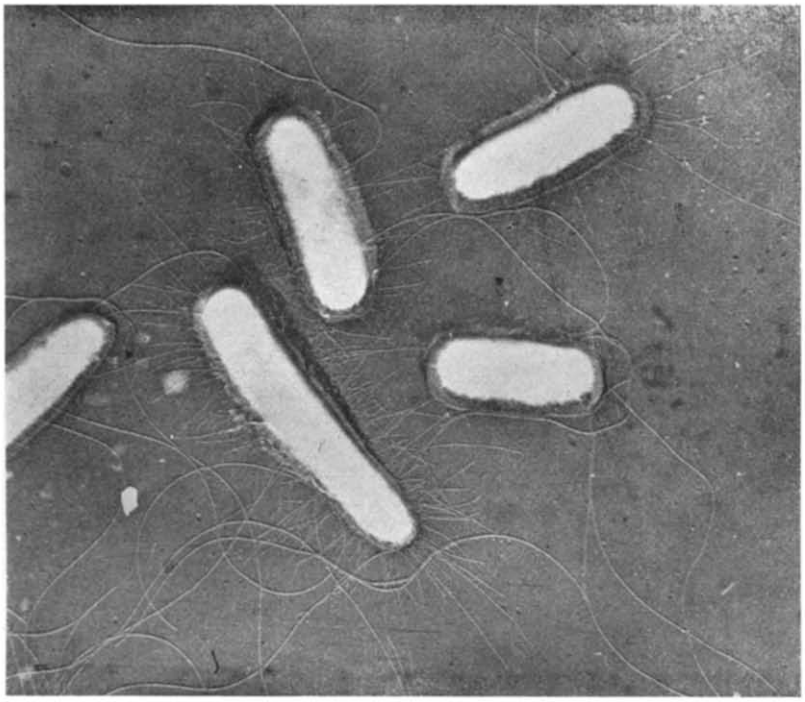

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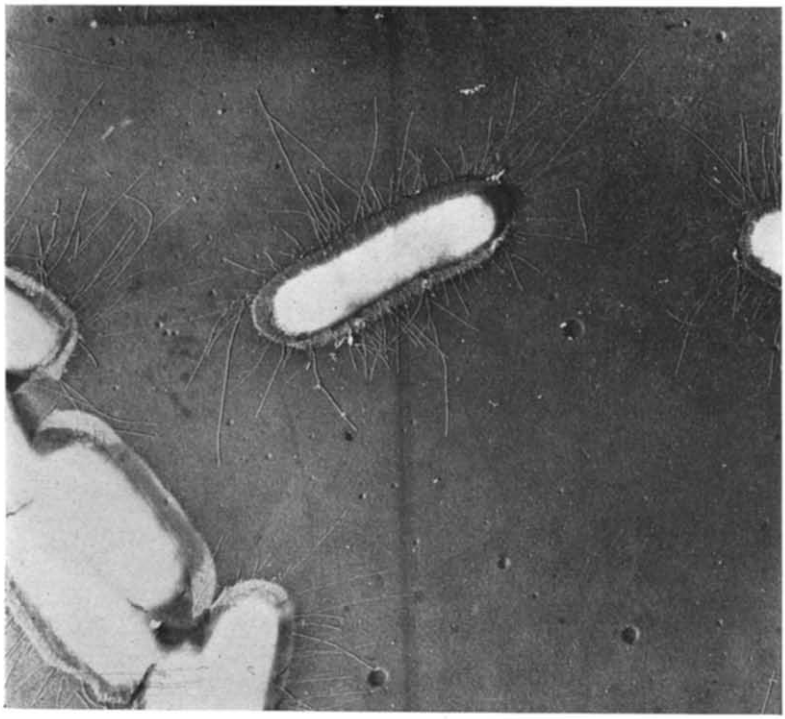

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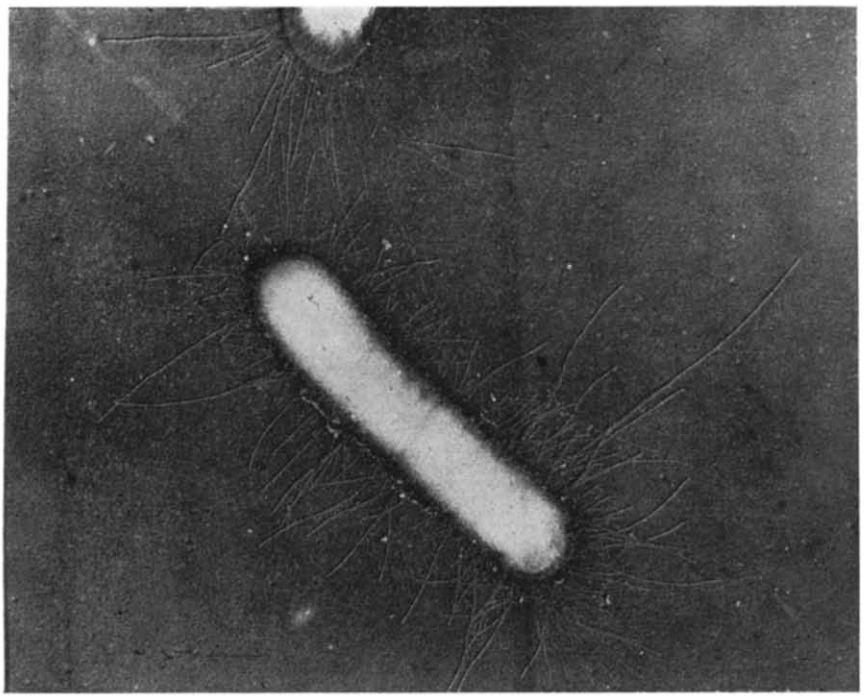

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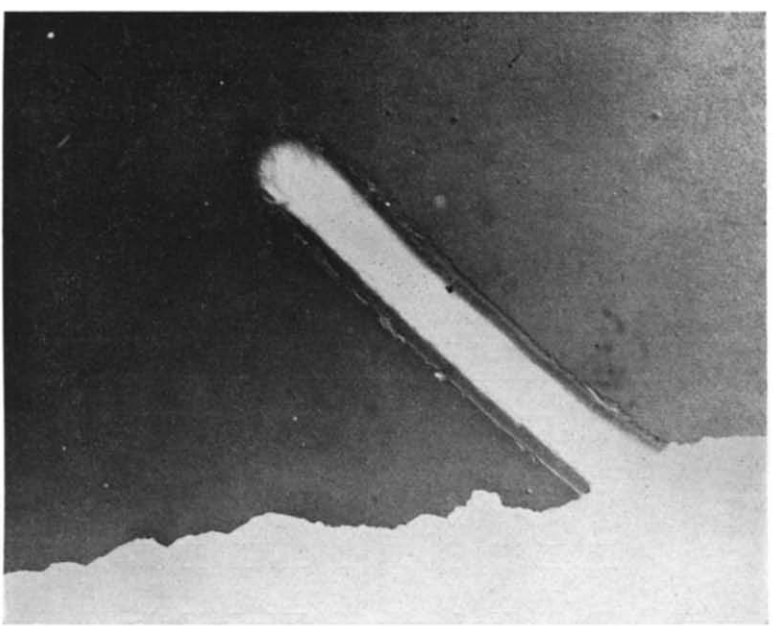

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Fig. 2. Electron micrographs. The organisms in Fig. 2b, *S. typhosa* 643L+, were fixed in broth culture and dialyzed against distilled water. The other specimens were washed with distilled water before fixation. a, original *S. typhosa* 643; b, female parent, *S. typhosa* 643L+; c, male parent, *E. coli* K-12, Hfr, W1895; d, hybrid X30D; e, hybrid X30P (segregant from X30D); f, hybrid X30T (segregant from X30D).

test are summarized in Table II, showing that P+ strains, as established in the electron microscope, are agglutinated while P— strains are not agglutinated.

TABLE II

AGGLUTINATION OF PARENT AND HYBRID STRAINS WITH ANTISERUM PREPARED AGAINST PURIFIED PILI FROM *E. coli* B–L(E) P+

Strains	*Antiserum dilutions*				
	4	*16*	*64*	*256*	*1024*
E. coli B–L(E) P+	+	+	+	+	+
E. coli B–L(E) P—	—	—	—	—	—
E. coli K12 Hfr W1895	+	+	+	±	±
Salmonella typhosa 643	—	—	—	—	—
Salmonella typhosa 643L+	—	—	—	—	—
Salmonella X30D (P+)	±	+	+	+	+
Salmonella X30T (P—)	—	—	—	—	—
Salmonella X30P (P+)	±	+	+	+	+

Hemagglutination studies

The phenomenon of agglutination of red blood cells by piliated strains of bacteria[10] provides additional confirmation of the distribution of pili among the parents and hybrids of this cross. Agglutination of chicken red blood cells by cultures of the various strains is indicated in Table III. The *E. coli* parent and the two piliated hybrid forms, X30D and its segregant, X30P, show strong hemagglutination, while the *Salmonella* parents, 643 and 643L+, and the non-piliated hybrid, X30T do not cause hemagglutination even at high ratios of bacterial cells to blood cells. Since hemagglutination has been shown to be due to the presence of the pili themselves, rather than some other material produced by piliated bacteria[3], the results of this test can be taken to indicate clearly the presence or absence of piliation in the various strains.

TABLE III

HEMAGGLUTINABILITY* OF PARENT AND HYBRID STRAINS

Strains	*Dilutions***													*Hemagglutinability (bacterial cells/ blood cells)*
	1	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*512*	*1024*	*2048*	*4096*	
E. coli K12 Hfr W1895 "R"	+	+	+	+	+	+	+	+	±	—	—	—		12
E. coli K12 Hfr W1895 "S"	+	+	+	±	+	+	+	+	±	—	—	—		12
S. typhosa 643	—	—	—	—	—	—	—	—	—	—	—	—		>1540
S. typhosa 643L+		—	—	—	—	—	—	—	—	—	—	—	—	> 770
Salmonella X30D (P+)		+	+	+	+	+	+	+	+	+	+	±	±	1.5
Salmonella X30T (P—)		—	—	—	—	—	—	—	—	—	—	—	—	> 770
Salmonella X30P (P+)			+	+	+	+	+	+	+	—	—	—	—	6.0

* Bacteria per blood cell just needed to give complete agglutination expressed as the ratio of bacterial cells to blood cells.

** Bacterial dilution of 1 corresponds to $2 \cdot 10^{10}$ bacteria/ml.

Biochemical characteristics

Table I also shows the biochemical characteristics of the parent strains and indicates that the hybrids have retained the nutritional requirements typical of the recipient parent strain. Also evident are certain differences between the hybrid X30D, and the segregant forms which arise from it, X30T and X30P. These changes are as follows: X30T is P— and Ara—, while X30P, still P+ and Ara+, differs only in the loss of the Vi antigen. If the loss of pili and the inability to utilize L-arabinose were merely a fortuitous association, linkage of these determinants would not be indicated. That P and Ara are probably closely linked, however, is demonstrated by the fact that more than one hundred separately isolated X30T segregants were both P— and Ara—. Also, a number of Ara+ reversions obtained by plating X30T on EMB arabinose plates were tested. These were all still P— indicating that pili production and arabinose utilization need not always be associated, as would be expected in the case of pleiotropy.

Mutation rate studies

To demonstrate conclusively that the ability of hybrids X30D and X30P to produce *E. coli* cross-reacting pili has been acquired by recombination, it must be shown that the presumably non-piliated *Salmonella* parent could not have mutated to a form with *coli* cross-reacting pili before recombination occurred. Such a mutation is, *a priori*, extremely unlikely, since strains of *Salmonella* do not cross-react with *E. coli*. Even if *Salmonella* 643L+ mutated to a piliated form, the probability of these pili also being *E. coli*-specific is extremely low. GILLIES AND DUGUID[2] have reported that antiserum against the pili of a strain of *Shigella flexneri* strongly agglutinated a series of piliated strains of *Escherichia coli* but did not agglutinate piliated cultures of *Salmonella typhi*, *S. paratyphi* B, *S. enteriditis*, *S. typhimurium*, and *S. thompson*.

A large number of cells of the parent 643L+ culture were examined for the possible presence of pili. Out of 300 individual cells directly examined by electron microscopy, none were piliated. However, this method of examination is very time consuming and other, more efficient methods were employed. Since many strains of bacteria existing as both P+ and P— forms show a parallel colony difference, one can often detect piliated variants among large numbers of non-piliated colonial types[3]. However, out of a total of about 6000 colonies examined by reflected light, only three were clearly different from the typical 643L+ colonies. Two were somewhat smaller with the same surface texture as the original form, and one was about the same size with a rougher surface texture. None of these three colonies were hemagglutinating at a concentration of about 10^{10} cells per ml in saline, nor were the typical 643L+ colonies hemagglutinating. Furthermore, neither the mutant colonial forms nor the normal 643L+ colonial forms showed the rapid colonial breakdown upon subculture as did the hybrid forms. In a third kind of examination, 871 tubes containing 0.5 ml of broth were inoculated with an average of 10 643L+ bacteria each and were grown up to a concentration of about 10^9 organisms per tube. Saline washed chicken red blood cells were added to a concentration of $5 \cdot 10^6$ per tube. No hemagglutination was observed in any of the tubes.

Pooling the above data, out of about 7000 individual cells, colonies, or clones of parent strain 643L+ examined, none showed any evidence of piliation. Thus, the

presumably non-piliated parent culture could not have contained more than about 10^{-4} piliated cells per non-piliated cell. However, nearly all the colonies selected on xylose and rhamnose were hybrids with *coli*-specific pili. Since xylose and rhamnose utilization are characters independent from pili production, these hybrids must have acquired their ability to produce pili by transfer of genetic material.

DISCUSSION

The results of the preceding experiments have demonstrated that the ability to produce pili can be transferred from a donor cell to a recipient by means of sexual recombination. In addition, the pili production character is linked to at least one other genetic marker, arabinose utilization. Thus, the production of pili can be under nuclear genetic control, as opposed to an inheritance deriving exclusively from the cytoplasm. It may now be possible to analyze what type of mechanism, whether nuclear, cytoplasmic, or both, has determined the presence or absence of pili in strains of *E. coli* which show a high rate of variation from P+ to P—. Previous studies[3] have shown that other strains can exist in these two alternative forms, and that the rate of change from one form to the other can be high (10^{-4}—10^{-2} per bacterium per division) compared to ordinary mutation rates. In one strain, *E. coli* B–L(E), the P+ to P— rate of spontaneous change is 10^{-3} per bacterium per division at 32° and decreases by a factor of 10 for 10° rise in temperature.

These results seem to suggest a pattern of inheritance of pili possibly more complex than simple mutation and reversion at a single gene locus on the genetic map of this organism. The term "mutation" is used here in the formal sense, and refers to a sudden, random, inheritable change having a constant probability of occurrence per bacterium per generation. Changes of this nature, however, can occur by mechanisms other than that of ordinary spontaneous mutation. Some of these possibilities would be the segregation of diploid heterozygotes after recombination[11], the segregation of heterogenotes after transduction by phage[12], variation in the "local state" of a gene locus as in phase variation in *Salmonella*[13], and ordinary mutation. All of these mechanisms of cellular variation produce clonally stable variants in a stochastic manner. A mathematical analysis of the "mutation" rate and a study of the distribution of "mutants" by the fluctuation test of LURIA AND DELBRUCK[14] would not suffice to distinguish among the above possibilities. Recombinational analysis, however, can provide the necessary tool for this purpose, and investigations along these lines are now in progress.

The frequencies with which P+ recombinants occur when hybrids from a mating between P+ and P— parents are selected for various other traits has not yet been determined with sufficient accuracy to justify assigning the P+ character to a definite locus on the bacterial linkage group. Previous results by BARON *et al.*[8] with strain X30D, however, have dealt with the "diploid" nature of this culture and its resemblance to the heterozygotes reported in *E. coli* by LEDERBERG[11]. The segregant, X30T, a stable haploid form of X30D which is P—, is also Ara—; this is not fortuitous since all other segregants of the X30T variety were found to be invariably Ara— as well as P—. The P+ stable haploid segregant, X30P, derived from the unreduced diploid X30D is an Ara+ strain, which has lost the Vi antigen. During the reduction of this partial diploid (X30D), cross-overs presumably have occurred which, in the

first instance (X30T) have resulted in the elimination of the P+ character as well as the Ara+ marker, probably indicative of the close linkage of these two markers. That this may explain the situation entirely can be surmised from previous findings which eliminate the possibility of pleiotropism, since the majority of hybrids selected for Ara+ still remained P—. Thus, recombinants from a mating of *S. typhosa* hybrid 643L+ with *E. coli* Hfr, selected for Ara+ were never found to be P+ unless they possessed as well the identical biochemical characteristics observed in the X30D form or the other more unstable types (Lac+, Ara+, Xyl+, Rha+, Indol+). These were diploid forms which showed antigenic cross-reactivity with *E. coli* and were readily identifiable. In addition, strains of *S. typhimurium* which are P+ have been isolated as a consequence of *in vivo* matings of *E. coli* and *S. typhimurium* in mice[15]. Such P+ forms of *S. typhimurium* were invariably able to produce indol, indicating extensive transfer of genetic material from *E. coli*, since the linkage relationship sequence as studied by BARON *et al.*[9] seems to be Lac–Ara–Inos–Rha–Indol. Indol producing hybrids are obtained following selection for Rha+ hybrids, but have never been detected after Lac+ or Ara+ selections.

Before the production of pili can occur, the results would seem to indicate a necessity for more extensive transfer of genetic material than usually takes place in selection of Ara+ hybrids, although loss of pili can be correlated with a locus linked to arabinose utilization. The reversion of Ara— P— strains (X30T) to Ara+ strains which still remain P— also eliminates the possibility of a pleiotropic effect.

It is possible that a further approach to the genetics of pili can be made by means of transduction studies using the Vi typing phages. Vi phage C_2 has been propagated on hybrid X30D (see ref. 16), and could be used to transduce various markers (Lac, Ara, Xyl, Rha) in an attempt to determine possible linkage relationships of loci controlling pili production. Studies of this nature and further work with the transfer of pili by genetic recombination procedures are in progress.

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