J. gen. Microbiol. (1966), 45, 365–376
With 4 plates
Printed in Great Britain

The Relationship of F type Piliation and F phage Sensitivity to Drug Resistance Transfer in R^+F^- *Escherichia coli* K12

BY NAOMI DATTA

Department of Bacteriology, Postgraduate Medical School, Ducane Road, London W.12

A. M. LAWN

Lister Institute of Preventive Medicine, Chelsea Bridge Road, London S.W.1

AND ELINOR MEYNELL

M.R.C. Microbial Genetics Research Unit, Hammersmith Hospital, Ducane Road, London W.12

(*Received* 21 *April* 1966)

SUMMARY

Resistance factors (R factors), of the kind which confer sensitivity to F specific phage as well as promoting conjugation in enterobacteria determine the production of a pilus similar to the specific pilus seen on F^+ bacteria. R factors, however, unlike F, also determine the production of a repressor of function: this means that in an established R^+ culture, only a small proportion of the bacteria can conjugate, be infected with F phage, or produce the pilus. Under conditions where repression is lifted, the three characters, conjugation, F phage sensitivity and production of the pilus, are coordinately de-repressed.

INTRODUCTION

The presence of the sex factor, F, in a bacterial strain can be recognized by three effects: the bacteria are able to conjugate, they are sensitive to certain phages, and they produce a special type of fimbria (Crawford & Gesteland, 1964), or pilus (Brinton, Gemski & Carnahan, 1964), which can be seen to act as the receptor for one group, at least, of F specific phages. The evidence suggests that the particular action of the F factor in this connexion is to determine the production of the pilus, which in addition to acting as phage receptor (Edgell & Ginoza, 1965), is involved in conjugation (see Brinton, 1965).

Resistance factors (or R factors) resemble F in promoting cell unions which are able to lead to their own transfer from one bacterium to another (see Watanabe, 1963*a*). However, while virtually every bacterium in an F^+ culture may be able to conjugate, R factors determine the synthesis of a cytoplasmic repressor which limits their own function, so that once the R factor is established in a strain, the ability to conjugate is expressed in only a minority of the bacteria (Meynell & Datta, 1965). If the R factor is transferred to recipient R^- bacteria which have no preformed repressor in their cytoplasm, a period follows during which its conjugating function is uninhibited, before its own repressor gene has had time to restore the repression. High frequency transfer (HFT) systems can therefore be

produced by initiating the rapid spread of an R factor through a large R⁻ recipient population from a small number of R⁺ bacteria (Watanabe, 1963*b*).

One large class of R factors is closely related to F (Meynell & Datta, 1966*a*). These have been named *i*⁺ (Egawa & Hirota, 1962), or *fi*⁺ (for 'fertility inhibition'; Watanabe *et al.* 1964), because they inhibit the expression of F; so that after acquiring an R factor, an F⁺ culture is no longer able to conjugate with its accustomed frequency nor visibly lysed by F specific phage. R factors belonging to this class are not only conjugation factors, but actually confer sensitivity to F phage (Meynell & Datta, 1966*a*); they must therefore, like F, determine the production of the specific F phage receptor, since it is lack of this receptor which is responsible for the insensitivity of F⁻ bacteria* (Engelhardt & Zinder, 1964). Evidently the gene determining synthesis of the specific phage receptor is present in *fi*⁺ R factors as well as in F. Phage sensitivity in R⁺ cultures is repressed in the same way as conjugating ability, so that the number of bacteria which can be infected with F phage is very small in established R⁺ cultures and greatly increased in HFT systems with bacteria which have newly acquired the factor (Meynell & Datta, 1965). Since the production of the phage receptor by R or by F is subject to repression by R, it is probable that the *fi*⁺ character results from an extension to F of the repression which the R factor ordinarily exerts on itself. In this way, F and *fi*⁺ R factors appear as conjugation factors determining synthesis of the same surface structure and subject to the same regulatory mechanism.

The specific phage receptor on F⁺ bacteria is a particular kind of pilus present in small numbers on the bacterium, slightly broader and often very much longer than the common pili (Type I: Brinton, 1965) which closely cover the surface of either an F⁺ or an F⁻ bacterium. It was therefore important to see whether the same morphological structure acted as phage receptor on R⁺F⁻ bacteria. Bacteria with attached phage particles could not be seen in established R⁺ cultures, which was not surprising since these cultures contain so few phage-sensitive bacteria. However, in HFT systems where conjugation and phage infection were greatly increased, bacteria were easily found which showed pili similar to those on F⁺ bacteria and to which phage particles attached all along their length. The same thing was seen with each of three different R factors. In the present paper, we report the results of tests designed to compare the proportions of bacteria able to conjugate, to be infected by F phage and to produce the specific pilus, in R⁺F⁻ cultures grown under various conditions and de-repressed to different extents.

METHODS

Bacteria. These were all derivatives of *Escherichia coli* strain K12, and are listed in Table 1. The R factors were examined in the F⁻ strains RC709, RC711, RC12, RC24; and strains HfrH and HfrC were used as control bacteria carrying the F factor, and for assay of the phage.

R factors. These were R1, R124 (Meynell & Datta, 1966*a*) and R237, which confers resistance to ampicillin, streptomycin, chloramphenicol and sulphonamide and which was received by courtesy of Dr E. S. Anderson (Enteric Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W. 9). All were shown to be *fi*⁺.

F specific phage. The phage used was MS2 (Davis, Strauss & Sinsheimer, 1961), one of the group of spherical RNA phages. Preparations grown on strain Hfr H and passed through a membrane filter (Millipore, Type HA, pore size 0·45 μ) after preliminary removal of the bacteria by centrifugation showed titres of over 5×10^{11} plaque-forming particles (p.f.p.)/ml.

Table 1. *Escherichia coli K12 derivatives used*

Strain	Synonym and Characters	Reference
RC 709	J5-3 (*pro*$_1^-$.*met*$_2^-$.*lac*$^+$.*S*^{s}) F$^-$ (acridine cured)	Clowes & Rowley (1954)
RC 711	J6-2 (*pro*$_2^-$.*his*$^-$.*try*$^-$.*lac*$^-$.*S*^{s}) F$^-$ (acridine cured)	Clowes & Rowley (1954)
RC 12	W677 (*thr*$^-$.*leu*$^-$.*B1*$^-$: F$^-$) *S*^{r}	Lederberg (1950)
RC 24	129 (W677; *S*^{r}) *fim*$^-$	Maccacaro, Colombo & Nardo (1959)
Hfr H	From 58.161 (*met*$^-$: F$^+$) with F integrated near the *thr* locus.	Hayes (1953)
Hfr C	From 58.161 (*met*$^-$: F$^+$) with F integrated near the *pro* locus	Cavalli-Sforza (1950)

Antiphage serum. This was prepared against another F specific phage μ2 (Dettori, Maccacaro & Piccinin, 1961), but neutralized phage MS2 at the same rate, with a K value of about 2500. Antibacterial antibodies were absorbed from the serum to prevent agglutination during tests of the bacterial suspensions for phage sensitivity; the antiphage activity of the serum remained unaltered.

Media. Nutrient broth was either Oxoid Nutrient Broth no. 2 or a tryptic digest of beef.

TYECa broth consisted of: Oxoid Tryptone, 10 g.; yeast extract, 1 g.; NaCl, 8 g.; dissolved in 1 l. distilled water and adjusted to pH 7·2. After sterilization by autoclaving, glucose was added to 0·15 % (w/v) and $CaCl_2$ to 0·002 M, for Ca^{2+} or other divalent cations are required for phage penetration (Paranchych, 1966), and possibly also for attachment (Valentine & Strand, 1965).

Nutrient agar consisted of Oxoid Nutrient Broth no. 2, solidified with 1·25 % (w/v) Davis N.Z. agar.

Tryptone Difco agar contained: Oxoid Tryptone, 10 g.; NaCl, 8 g.; glucose, 1 g.; Difco Bacto agar, 10 g.; dissolved in 1 l. distilled water and adjusted to pH 7·2.

The mineral salts medium was that of Tatum & Lederberg (1947), used with the omission of asparagine, and solidified with 1·5 % (w/v) Davis N.Z. agar.

Experimental procedure

HFT systems were prepared by using donor and intermediate strains nutritionally distinguishable from the strain chosen to act as recipient. In some cases, a donor and intermediate were used which could also be distinguished from one another. The donor (R^+) and intermediate (R^-) bacteria, taken from nutrient agar plates (which for the R^+ strain contained antibiotic), were grown for about 6 hr in broth. The R^+ culture was then mixed with the R^- culture in the proportions given in Table 2, column 5; 1 ml. of the mixture was then added to 9 ml. fresh broth and incubated overnight. Cultures with various degrees of HFT were made by altering the ratio of donor to intermediate, the broth, and the temperature of incubation; and by using different bacterial strains. Spread of the R factor throughout the intermediate

strain sometimes yielded a culture uniformly drug-resistant and largely released from repression. On the other hand, it should be noted that where the spread was poor, many of the bacteria examined were in fact R^-. While it was possible to get reproducible results by exactly repeating a given experiment, the conditions determining the extent to which the R factor was disseminated through the intermediate are not understood.

For tests of established R^+ cultures, pure cultures of the R^+ strain were incubated overnight.

Next morning, each culture to be tested was diluted 1/20 in TYECa broth and incubated in a water bath at 36·5° (unless otherwise stated). At the end of 2 hr, when the bacterial concentration was about 4×10^8/ml., the following procedures were carried out.

(*a*) Electron microscopy for piliation. A sample of 0·5 ml. of culture for electron microscopy was mixed with 0·5 ml. of phage suspension at 2×10^{11} p.f.p./ml. and incubation was continued for 20 min., when formalin was added to a final concentration of 0·25% (v/v). A second sample was formalinized without the addition of phage. Material was prepared for electron microscopy by modifications of the negative contrast method of Brenner & Horne (1959). In almost all the preparations used for determining the proportion of piliated bacteria, a drop of the culture was dried on a carbon-backed formvar-coated grid. The grid was then stained with uranyl acetate. In a few cultures, this 'dry' method retained so much debris that pili could not be satisfactorily identified. In these cases, a 'wet' method was used; the only difference was that the drop of culture was allowed to remain on the grid for 2–3 min., then stained before finally drying. This gave cleaner grids but fewer bacteria. In both methods, only a fraction remained of the bacteria in the initial drop of culture. 'Wet' and 'dry' preparations of the same culture were compared on two occasions. There were no significant differences in the proportions of piliated bacteria. The material was examined in a Philips EM 200 microscope (80 kV, 25 μ objective aperture). It is not certain that the method of preparation of the specimens produced representative results. Common (type I) pili are known to promote adhesiveness (see Duguid & Wilkinson, 1961). If bacteria with F type pili are either more or less likely to adhere to the grid than those without, sampling would be biased; however, the fact that the proportions were the same whether the grids were prepared by the 'wet' or the 'dry' technique makes such a bias less probable. Some loss of F type pili is likely to occur during preparation, since they are long and detached fragments are not uncommonly seen. Short pili will not be recognized unless they project sideways, otherwise they are obscured by the cell itself. It is not always easy to attribute pili to individual bacteria when these occur in clumps.

(*b*) Assessment of conjugation by resistance transfer. A further sample of 0·5 ml. of the culture under test was added to 4·5 ml. of a 22 hr broth culture of the R^- strain which was to act as recipient. The mixture was incubated for 40 min. and then serial ten-fold dilutions were spread on a set of plates of mineral salts agar appropriately supplemented with nutrients and antibiotics to allow growth of: (i) the donor R^+ strain, (ii) the intermediate strain, (iii) R^+ members of the intermediate strain, (iv) the recipient strain, (v) members of the recipient strain which had received the R factor. Only where donor and intermediate strains were nutritionally

different, could (iii) be distinguished from (i). The frequency of drug-resistance transfer was calculated from the numbers of colonies on these plates, and was expressed either as % resistant bacteria (Table 2, column 8) or % total bacteria (column 9) in the donor culture which transferred resistance. To arrive at these figures, the number of resistant colonies produced by the recipient strain was divided by the number of either resistant colonies or total colonies, produced by bacteria of the donor culture.

Forty minutes was chosen as a suitable time to incubate the mixtures for the following reason. When mixtures of R^+ bacteria just entering the stationary phase and stationary phase R^- bacteria were sampled at intervals and the transfer of

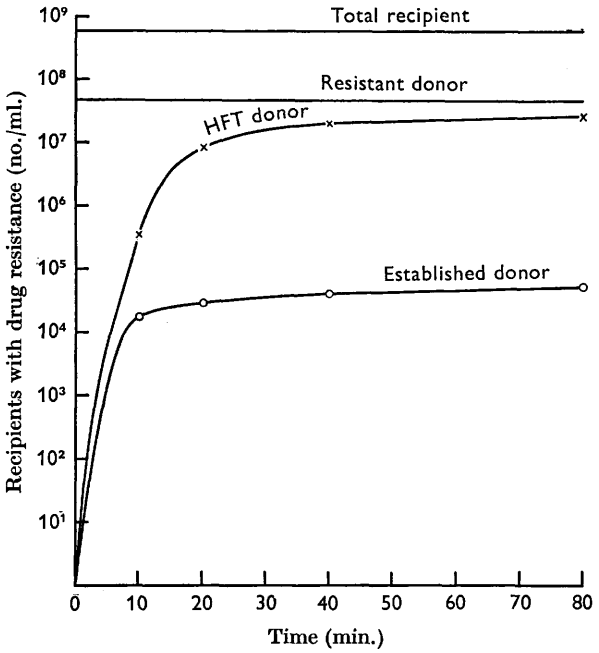


Fig. 1. Resistance transfer. A mixture of one volume of donor culture and nine volumes of recipient culture (see Methods) was incubated at 37°. Samples taken at intervals were assayed for number of bacteria of the recipient culture which had received drug resistance. The two curves show, respectively, the numbers which received resistance from an HFT donor culture and from an established R^+ culture.

resistance plotted graphically, the curve obtained showed an initial rise followed by a levelling off (Fig. 1). While the level of the plateau varied with different R factors, and also with the same factor in different conditions, it was reached by 40 min. in all cases. This suggests that each bacterium able to transmit an R factor does so within a short time of mixing, after which there is no further transfer. The number of transfers sometimes reached, but never exceeded, the number of R^+ bacteria of the donor strain. In these experimental conditions, it appears that each conjugating R^+ bacterium donates R only once, and that there is no spread of the R factor in the stationary phase recipient population.

(*c*) Measurement of proportion of phage-sensitive bacteria. Another sample (2·25 ml.) of the culture under test received 0·25 ml. of phage MS 2 diluted in TYECa

broth to a titre of 3–6 × 10^{10} p.f.p./ml., the ratio of phage to bacteria being thus between 10:1 and 20:1. Incubation was continued for 8 min. to allow adsorption, and 0·25 ml. of the mixture was then transferred to 2·25 ml. of a dilution of antiserum which neutralized about 99·99 % of the phage in 10 min. After a further 10 min., about 10 ml. of TYECa broth were added and the whole amount was poured on a Millipore filter which was sucked almost to dryness before being washed through with another 10 ml. of TYECa broth. The filter was then transferred to 10 ml. TYECa broth in a 250 ml. flask, rinsed well to release the bacteria and discarded. In this way, residual free phage and antibody could be largely eliminated while the bacteria remained relatively concentrated. The released bacterial suspension was immediately assayed for phage to measure the number of bacteria that had become infected; the agar layer method was used with strain Hfr H as indicator on plates of Tryptone Difco agar. The plates were incubated at 42°, since plaques of F specific phage are better developed at this temperature than at 37° (Dettori, Maccacaro & Turri, 1963). Plating was completed by 25 min., for in one-step growth experiments with strain Hfr H, the minimum latent period of the phage was between 30 and 35 min. The infected bacterial suspension was again assayed to observe any increase in the titre of phage after 1·5 hr incubation at 37°. As control on the recovery of infected bacteria from the filter, the number of plaques produced initially by the released bacterial suspension was compared with the number given by simple dilution of the antiserum mixture, using either strain Hfr H or HFT R^+ cultures with a large proportion of phage-sensitive bacteria. These control tests showed that 70–100 % of the infected bacteria (plaque-formers) were recovered from the filter.

Background of non-specific plaques

When the proportion of phage-sensitive bacteria was very low, as in the established R^+ cultures, measurements became inaccurate because of the background phage contaminating the preparations. Attempts to lessen this background of non-specific plaques failed. It was not due to retention of free phage particles by the filter, for although it is reported (Lodish & Zinder, 1965) that in certain media such as 0·15 M-NaCl, over 80 % of phage f2 attached to Millipore filters, only about 1 % is retained from broth. In the present experiments with phage MS2 in TYECa broth, there was no loss of phage in filtrates, and only 0·5–1·5 % was recovered on rinsing the filters in fresh medium. The same held true for antibody treated phage suspensions in which only 0·01 % remained unneutralised, provided the phage stock had previously been filtered. When, however, the phage preparation had simply been treated with chloroform and low-speed centrifugation to remove bacteria, about 20 % of the antibody-surviving plaque-forming particles could be recovered which may have represented phage particles either in clumps or attached to F type pili which are reported to adhere to membrane filters (Valentine & Strand, 1965). The background of plaques in the present experiments appeared to be due to some effect of the presence of bacteria, since the plaque count obtained with a suspension of phage alone was usually lower. The effect was non-specific, however, for the count was just as high with *Bacillus subtilis*, *Staphylococcus aureus* or an aerial micrococcus as it was with *Escherichia coli* K12 F^-R^-; and the number of plaques with *E. coli* K12 F^-R^- was the same whether the bacteria were used live or

after boiling for 30 min., a treatment reported to destroy the ability of F pili to take up the F specific phage f2 (Valentine & Strand, 1965).

RESULTS

Pili similar to those found on F^+ bacteria were seen in HFT preparations of R^+F^- bacteria, including the *fim*$^-$ strain RC 24 which produces no common pili. Plates 1–4 show F^- bacteria carrying R factors, R1, R124 and R237, with strain Hfr H for comparison. F type pili were easily identified for enumeration by their adsorption of F specific phage at high multiplicity, but even in phage-free preparations, the specific pili could be distinguished from common (type I) pili (see Brinton, 1965) by their greater length and diameter or by the presence of end knobs (Lawn, 1966).

Table 2 shows the results of experiments made to examine quantitatively the relationship between F type piliation, as observed directly in the electron microscope; F phage sensitivity, as measured by the proportion of bacteria which could be infected; and the ability to conjugate, as measured by the proportion of bacteria which transferred drug resistance to a recipient within 40 min. Table 2, columns 9–11, shows the % bacteria with the three characters in the different cultures, arranged in ascending order of % bacteria showing F type pili. Low figures are not due only to repression in R^+ bacteria, but in part to poor spread of the R factor through the intermediate strain in the donor culture, so that a considerable proportion of the bacteria examined were still R^-. The distinction can be made from Table 2, column 8, which shows the % of resistant (R^+) bacteria which expressed their conjugating function. Although the R factor did not always spread through the culture, nevertheless those bacteria which were R^+ in mixtures of donor and intermediate were clearly less repressed than bacteria in established R^+ cultures, where no intermediate was present, as can be seen by comparing their respective transfer frequencies given in Table 2, column 8.

The cultures in Expt. 6 were incubated at 39·5°; as the temperature of incubation is increased above 37°, cultures of $R1^+$ bacteria contain progressively fewer bacteria with sensitivity to the phage or ability to transfer R. This is due to failure of function at high temperature, not a failure of the R factor to replicate, as with some temperature-sensitive F-*lac* (Jacob, Brenner & Cuzin, 1963). The effect was much less marked with R124.

The number of pili seen on individual bacteria was very variable. In preparations where many bacteria were piliated, the numbers ranged from one to twelve. However, when only an occasional piliated bacterium was present, it almost never showed more than one or two. This suggested that there were more pili per piliated bacterium in cultures with a high proportion of piliated bacteria than in cultures with a smaller proportion; and also more than the numbers usually reported for F^+ or Hfr cultures (see Brinton, 1965). The impression was supported by the results of an experiment, shown in Fig. 2, in which the numbers of pili on individual bacteria were counted in two HFT R^+ preparations, one showing 96% and the other 6% piliation. Data of Brinton (1965) for an F^+ culture and an Hfr culture containing respectively 50% and 43% piliated bacteria are included for comparison.

No piliated cells were seen in established R^+ cultures, and any estimate of the proportion present would have required the examination of an inordinate number

Table 2. *Capacity of R⁺F⁻ cultures of Escherichia coli K12 to produce F type pili, transfer drug resistance and support growth of F specific phage*

Expt. (1)	R factor (2)	Donor culture: R⁺ donor (3)	Donor culture: R⁻ intermediate (4)	Ratio (5)	Recipient (6)	No. of bacteria screened for piliation (7)	Resistance transfer: % resistant bacteria transferring R (8)	Resistance transfer: total % bacteria transferring R (9)	Phage infection. % total bacteria infected (10)	Specific pilation. % total bacteria with F type pili (11)
1	None	. HfrH		.	.	100	.	.	68	61
1	None	. Hfr C		.	.	100	.	.	50	46
5	None	. HfrH		.	.	50	.	.	44	86
1	1	RC 711	None	.	RC 709	None	0·009	0·009	0·006	N.E.
1	1	RC 24	None	.	RC 709	None	0·004	0·004	0·005	N.E.
3	1	RC 709	None	.	RC 711	None	0·03	0·03	0·09	N.E.
6	1	RC 709	RC 709	1:50	RC 711	None	< 0·005	< 0·0001	< 0·005	N.E.
6	124	RC 709	RC 709	1:50	RC 711	None	0·29	0·011	0·12	N.E
5	1	RC 711	RC 711	1:50	RC 12	500	3·3	0·08	0·06	< 0·2
5	1	RC 709	RC 711	1:50	RC 12	500	0·7	0·08	0·13	< 0·2
3	1	RC 24	RC 24	1:200	RC 711	300	2·7	0·036	0·17	< 0·3
4	1	RC 711	RC 711	1:50	RC 12	500	0·24	0·009	0·34	< 0·2
4	124	RC 709	RC 711	1:50	RC 12	500	0·49	0·09	1·2	< 0·2*
3	124	RC 709	RC 709	1:100	RC 711	300	11	1·8	N.E.	< 0·3
4	1	RC 709	RC 711	1:50	RC 12	500	0·33	0·057	0·2	0·2
5	124	RC 709	RC 711	1:50	RC 12	500	0·67	0·12	0·49	0·2
3	1	RC 709	RC 709	1:200	RC 711	None	5·6	0·6	1·7	N.E.
4	237	RC 709	RC 711	1:50	RC 12	515	1·7	0·49	4·0	1·1
3	1	RC 709	RC 709	1:5	RC 711	500	4·2	0·9	2·1	2·2
5	237	RC 709	RC 711	1:5	RC 12	600	7·7	2·8	5·0	6
3	237	RC 709	RC 709	1:100	RC 711	100	7·2	2·3	10	14
1	1	RC 709	RC 709	1:10	None	100	N.E.	N.E.	9·6	17
1	1	RC 24	RC 24	1:10	RC 709	100	7·9	5·8	2·2	18
4	124	RC 709	RC 709	1:50	RC 711	100	61	31	18	22
5	124	RC 09	RC 709	1:50	RC 711	100	33	11	9	22
1	1	RC 711	RC 711	1:10	RC 709	100	66	29	5	24
4	237	RC 709	RC 709	1:50	RC 711	100	55	36	15	30
5	1	RC 709	RC 709	1:50	RC 711	50	100	62	22	40
4	1	RC 709	RC 709	1:50	RC 711	100	100	42	13·4	48
5	237	RC 709	RC 709	1:50	RC 711	50	100	50	24	53
2	1	RC 709	RC 709	1:100	RC 711	50	78	77	N.E.	96

* Two loose pili seen.

The experimental technique is described in Methods, The results were obtained in six different experiments, performed on different days, whose numbers are given in column (1). In expt. 6 incubation was at 39·5° instead of the usual 36·5°.

After incubation for 1½ hr the HFT cultures showed an increase in phage titre of between 1000- and 10,000-fold, except in the case of expt. 6, where there was no increase with R1 and only a 250-fold increase with R124.

N.E. = not examined.

of bacteria. Thus, in established cultures, the only comparison possible was between transfer of the R factor and phage infection; and measurements of the proportion of phage-sensitive bacteria become inaccurate as the plaque count decreases to the value of the non-specific background observed in control preparations without any sensitive bacteria.

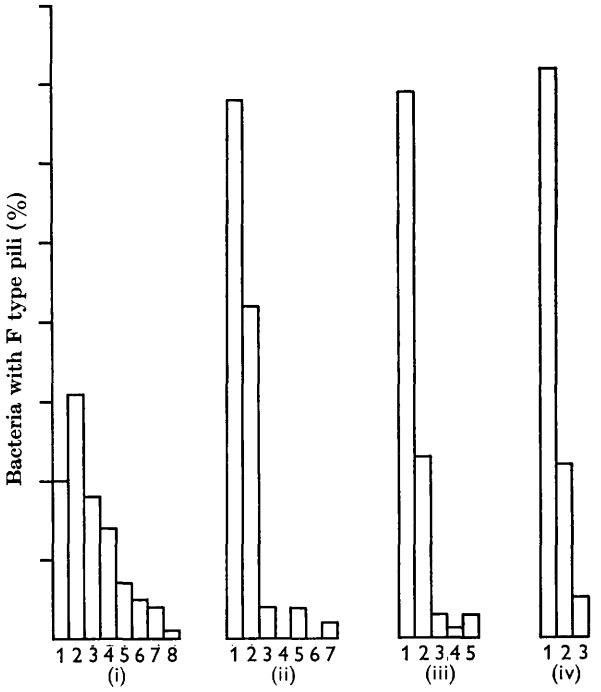


Fig. 2. Numbers of pili on individual piliated bacteria. (i) HFT culture of RC 709 R1$^+$ with 96 % piliated bacteria : 100 bacteria examined. (ii) HFT culture of RC 709 R237$^+$ with 6 % piliated bacteria: 50 bacteria examined. (iii) Data of Brinton (1965) for F$^+$ culture with 50 % piliated bacteria: 61 bacteria examined. (iv) Data of Brinton (1965) for Hfr culture with 43 % piliated bacteria: 72 bacteria examined.

DISCUSSION

The results of the present experiments, made to compare the frequencies with which F type piliation, F phage sensitivity and conjugating ability were expressed in R$^+$F$^-$ cultures, have shown a high degree of correlation between the three characters. Thus, de-repression for one character was accompanied by de-repression for the other two. The different cultures showed a wide range of expression for all three characters, but the trend was the same for each. In Table 2, the cultures are arranged in ascending order of % of bacteria showing F type pili, but they would have been arranged in much the same order had either of the other two characters been chosen.

It may be seen, nevertheless, that where there were few piliated bacteria (i.e. < 15 %), their numbers agreed with the numbers of plaques, whereas, where there

were many (i.e. > 15%), their numbers were closer to the figures for transfer. Correlation between the three characters, as they were examined, might be expected to be imperfect for various reasons. The only one which was directly measured was the F piliation, and even here, complete accuracy cannot be assumed (see Methods). In the second half of Table 2, where the level of piliation is above 15%, the incomplete agreement between the numbers of plaques, on the one hand, and the numbers of bacteria showing F type pili and transferring resistance, on the other, might be attributed to failure of the phage to infect all the bacteria to which it adsorbed. Against this interpretation is the good agreement between plaque counts and the numbers of piliated bacteria in the cultures where few bacteria showed pili. Another explanation might be that, since production of the pilus tends to cause aggregation of the bacteria, the number of plaques underestimates the number of infected bacteria; for some plaques will be produced, not by single infected bacteria, but by clumps of several. Total numbers of bacteria were counted after dilution in the recipient culture, and by spreading the samples on the agar surface, which might be expected to give better dispersion of the bacteria than inoculation in an agar overlay. Marked clumping is observed in HFT systems with *col* I (Stocker, Smith & Ozeki, 1963) where it is attributed to the formation of mating pairs, and lattice formation is well known to occur with F^+ cultures under appropriate conditions. Microscope and electron microscope observations have also confirmed a tendency to clump in HFT R^+ cultures with many piliated bacteria.

When the proportions of bacteria expressing the three characters were low, as in the cultures in the first part of Table 2, the numbers of plaques as well as of piliated bacteria were slightly in excess of the numbers of bacteria apparently able to conjugate. Here the explanation may perhaps lie in the fact that resistance transfer was used as the index of conjugating ability and conjugation may not always inevitably result in actual transfer of the factor. It is conceivable that de-repression may lead not only to the free expression of conjugating ability, but also to a greater probability that the plasmid will be transferred at conjugation.

It seems likely that the specific pilus is involved in conjugation as well as in sensitivity to F phage. This can be tested by using mutants of the F factor defective in either conjugation or phage sensitivity to see whether the two can be dissociated from one another, or whether a decrease in phage-sensitive bacteria is necessarily correlated with a decrease in the frequency of conjugation. In experiments of this kind made by Cuzin (1962), Galucci & Sironi (1964) and Meynell & Datta (1966*b*), no separation was observed and in this way phage sensitivity and the capacity to conjugate appeared to be two different manifestations of the activity of one gene. This may be the gene which determines the synthesis of the pilus. R factors, like F, confer ability to conjugate, sensitivity to F phage and synthesis of the pilus. But they also determine the synthesis of a repressor of function, and, by using R factors instead of F, it is thus possible to demonstrate that the three characters, conjugation, phage sensitivity and production of the pilus, are coordinately de-repressed.

The authors are grateful to the Fleming Memorial Fund for Medical Research for providing the electron microscope, and for support for one of us (A.M.L.); and also to the Medical Research Council for a grant to one of us (N.D.).

REFERENCES

BRENNER, S. & HORNE, R. W. (1959). A negative staining method for high resolution electron microscopy of viruses. *Biochim. biophys. Acta* **34**, 471.

BRINTON, C. C. (1965). The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. *Trans. N.Y. Acad. Sci.* **27**, 1003.

BRINTON, C. C., GEMSKI, P. & CARNAHAN, J. (1964). A new type of bacterial pilus genetically controlled by the fertility factor of *E. coli* K12 and its role in chromosome transfer. *Proc. natn. Acad. Sci., U.S.A.* **52**, 776.

CAVALLI-SFORZA, L. L. (1950). La sessualita nei batteri. *Boll. Ist. sieroter. Milan.* **29**, 281.

CLOWES, R. C. & ROWLEY, D. (1954). Some observations on linkage effects in genetic recombination in *Escherichia coli* K12. *J. gen. Microbiol.* **6**, 250.

CRAWFORD, E. M. & GESTELAND, R. F. (1964). The absorption of bacteriophage—R17. *Virology* **22**, 165.

CUZIN, F. (1962). Mutants défectifs de l'episome sexuel chez *Escherichia coli* K12. *C.r. hebd. Séanc. Acad. Sci., Paris* **255**, 1149.

DAVIS, J. E., STRAUSS, J. H., JR. & SINSHEIMER, R. L. (1961). Bacteriophage MS2: another RNA phage. *Science* **134**, 1427.

DETTORI, R., MACCACARO, G. A. & PICCININ, G. L. (1961). Sex-specific bacteriophages of *Escherichia coli* K12. *G. microbiol.* **9**, 141.

DETTORI, R., MACCACARO, G. A. & TURRI, M. (1963). Sex-specific bacteriophages of *E. coli* K12. IV. Host specificity, pattern of lysis and lethality of phage $\mu 2$. *G. Microbiol.* **11**, 15.

DUGUID, J. P. & WILKINSON, J. F. (1961). Environmentally induced changes in bacterial morphology. *Symp. Soc. gen. Microbiol.* **11**, 69.

EDGELL, M. H. & GINOZA, W. (1965). The fate during infection of the coat protein of the spherical bacteriophage R17. *Virology* **27**, 23.

EGAWA, R. & HIROTA, Y. (1962). Inhibition of fertility by multiple drug-resistance factor in *Escherichia coli* K12. *Jap. J. Genet.* **37**, 66.

ENGELHARDT, D. L. & ZINDER, N. D. (1964). Host-dependent mutants of the bacteriophage f2. 3. Infective RNA. *Virology* **23**, 582.

GALLUCCI, E. & SIRONI, G. (1964). A defective sex factor in *E. coli* K12. *G. Microbiol.* **12**, 175.

HAYES, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 75.

JACOB, F., BRENNER, S. & CUZIN, F. (1963). On the regulation of DNA replication in bacteria. *Cold Spr. Harb. Symp. quant. Biol.* **28**, 329.

LAWN, A. M. (1966). Morphological features of the pili associated with R^+F^- and R^-F^+ bacteria. *J. gen. Microbiol.* **45**, 377.

LEDERBERG, J. (1950). The selection of genetic recombinations with bacterial growth inhibitors. *J. Bact.* **59**, 211.

LODISH, H. F., & ZINDER, N. D. (1965). Attachment of f2 bacteriophage to cellulose nitrate filters. *Biochem. biophys. Res. Comm.* **19**, 269.

MACCACARO, G. A., COLOMBO, C. & NARDO, A. di. (1959). Studi sulle fimbrie batteriche. I. Lo studio genetico delle fimbrie. *G. Microbiol.* **7**, 1.

MEYNELL, E. & DATTA, N. (1965). Functional homology of the sex-factor and resistance transfer factors. *Nature, Lond.* **207**, 884.

MEYNELL, E. & DATTA, N. (1966*a*). The relation of resistance transfer factors to the F factor of *Escherichia coli* K12. *Genet. Res., Camb.* **7**, 134.

MEYNELL, E. & DATTA, N. (1966*b*). The nature and incidence of conjugation factors in *Escherichia coli*. *Genet. Res., Camb.* **7**, 141.

PARANCHYCH, W. (1966). Stages in phage R17 infection: the role of divalent cations. *Virology* **28**, 90.

STOCKER, B. A. D., SMITH, S. M. & OZEKI, H. (1963). High infectivity of *Salmonella typhimurium* newly infected by the *col* I factor. *J. gen. Microbiol.* **30**, 201.

TATUM, E. L. & LEDERBERG, J. (1947). Gene recombination in the bacterium. *J. Bact.* **53**, 673.

VALENTINE, R. C. & STRAND, M. (1965). Complexes of F-pili and RNA bacteriophage. *Science* **148**, 511.

WATANABE, T. (1963*a*). Infective heredity of multiple drug resistance in bacteria. *Bact. Rev.* **27**, 87.

WATANABE, T. (1963*b*). Episome-mediated transfer of drug resistance in Enterobacteriaceae. VI. High-frequency resistance transfer system in *Escherichia coli*. *J. Bact.* **85**, 788.

WATANABE, T., NISHIDA, H., OGATA, C., ARAI, T. & SATO, S. (1964). Episome-mediated transfer of drug resistance in Enterobacteriaceae. VII. Two types of naturally occurring R factors. *J. Bact.* **88**, 716.

EXPLANATION OF PLATES

Plates 1–4 are electron micrographs of *Escherichia coli* K12 carrying different conjugation factors and mixed with F. specific phage MS2. Three kinds of appendage can be differentiated: the specific pili are coated with round particles of phage MS2; common (Type I) pili have no adsorbed particles; flagella can be recognised by their sinuous shape, greater width and characteristic ultrastructure. All the preparations were negatively stained with uranyl acetate. The calibration bar in the full plate micrograph represents 1 micron, that in the inset micrograph represents 2000 Å.

PLATE 1

Escherichia coli K12 F⁻R1⁺

PLATE 2

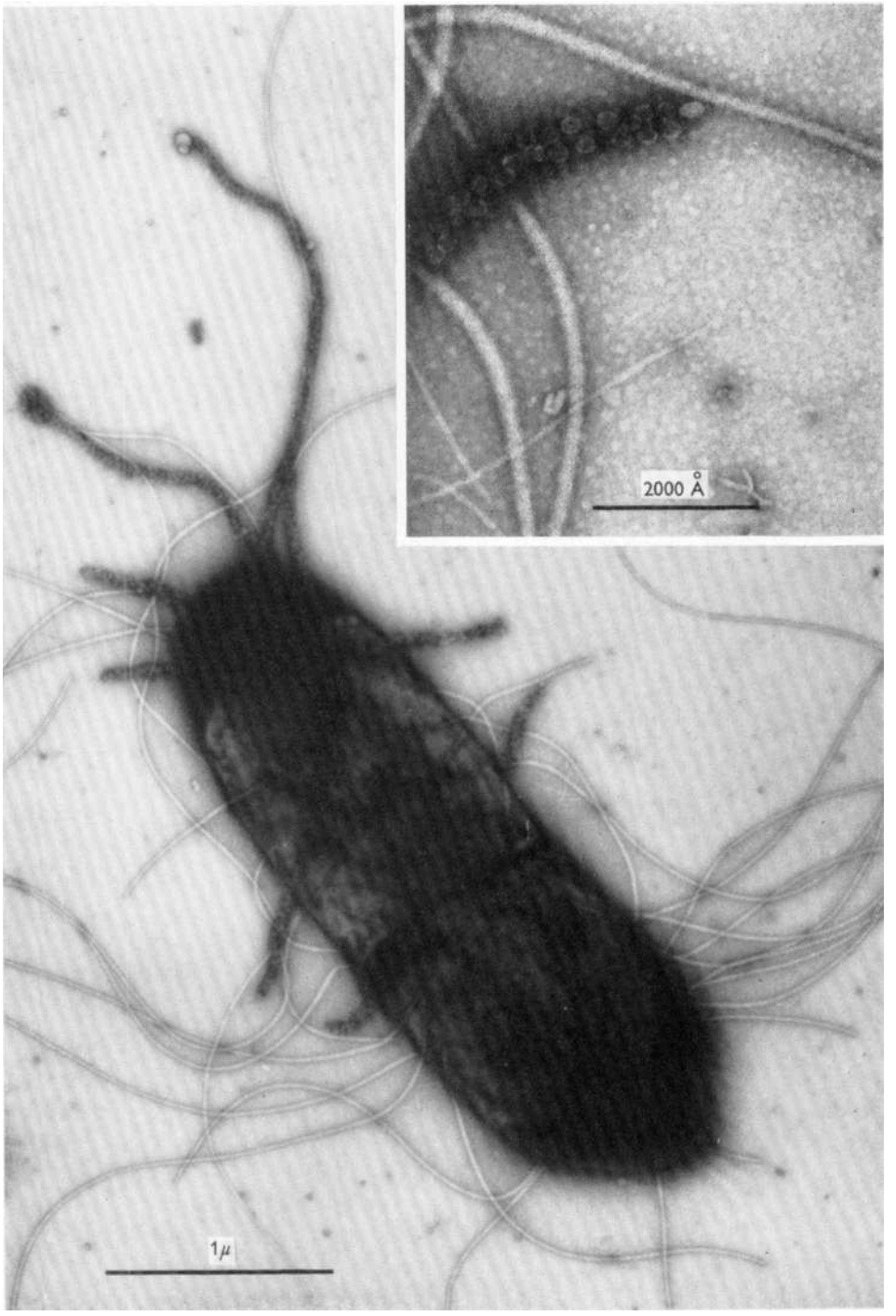
Escherichia coli K12 F⁻R124⁺

PLATE 3

Escherichia coli K12 F⁻R237⁺

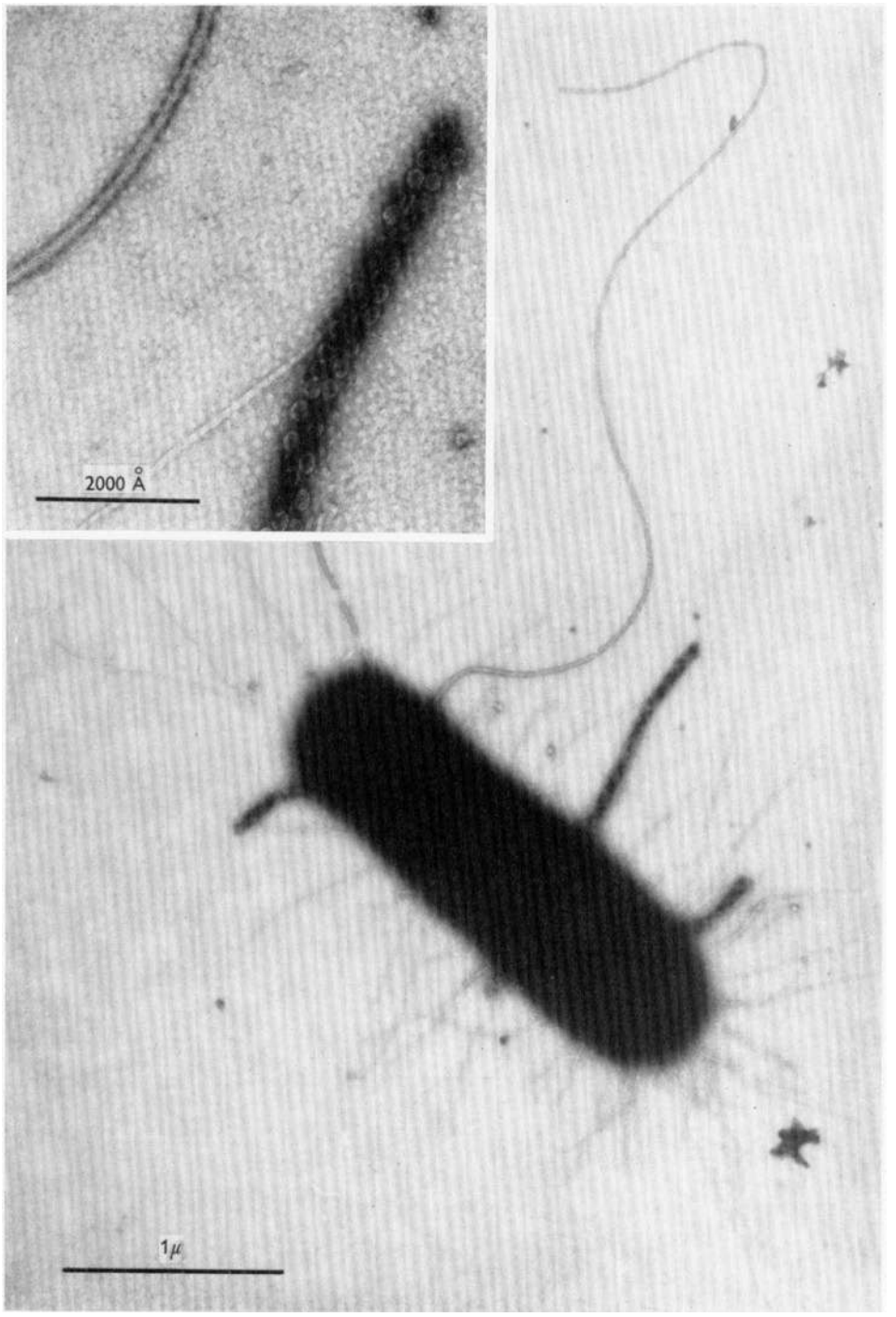
PLATE 4

Escherichia coli K12 Hfr H

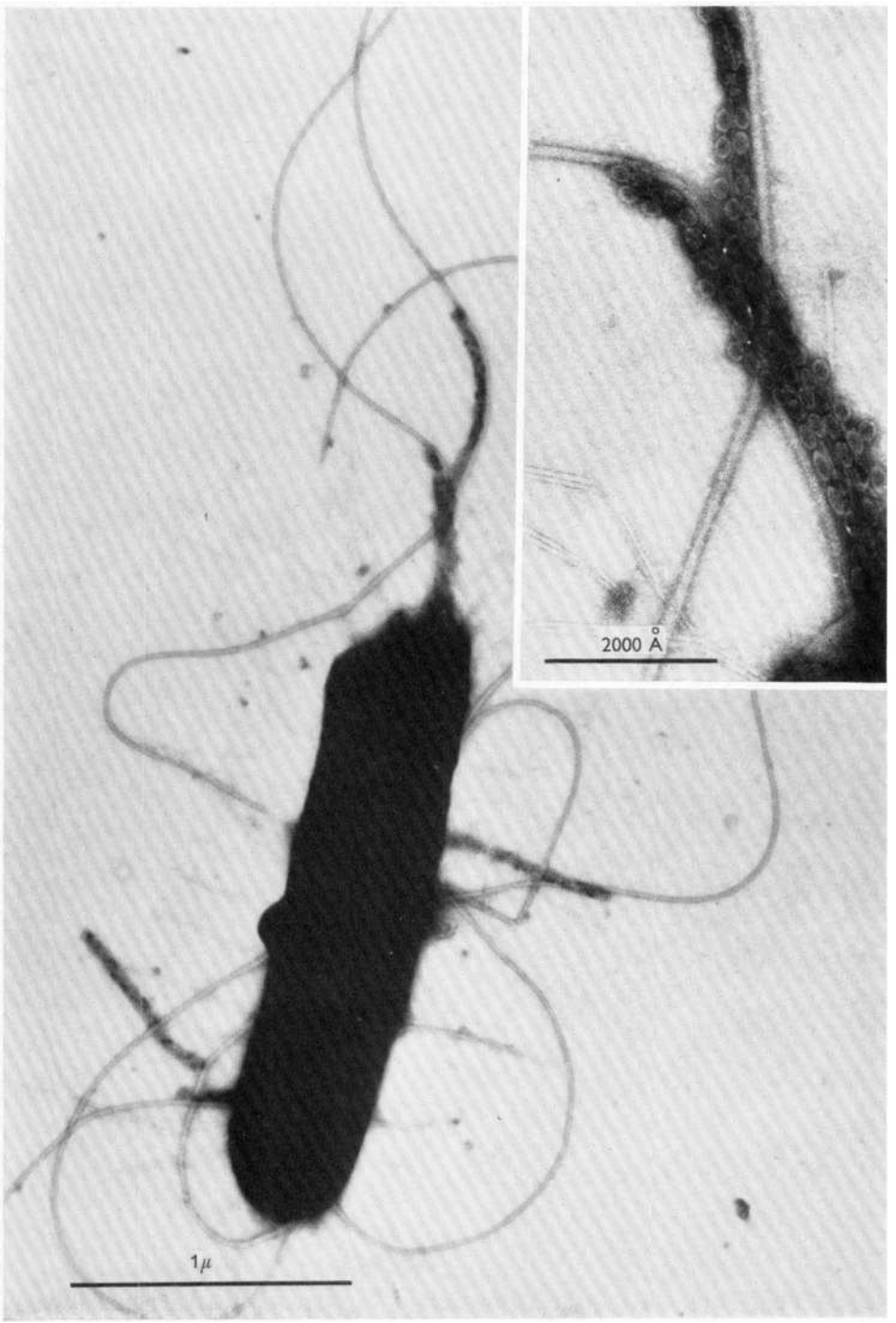


N. DATTA, A. M. LAWN AND E. MEYNELL

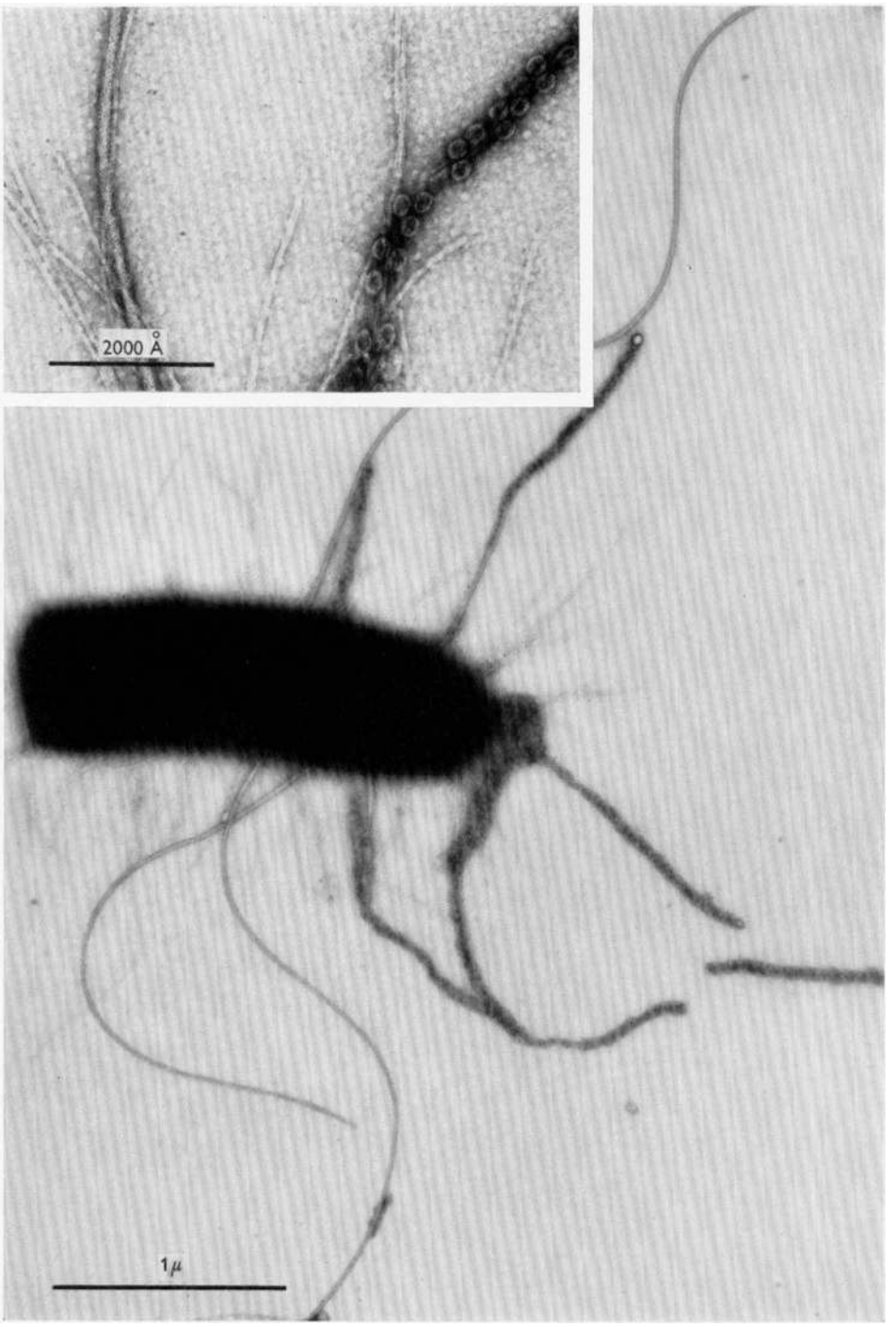
(*Facing p.* 376)



N. DATTA, A. M. LAWN AND E. MEYNELL



N. DATTA, A. M. LAWN AND E. MEYNELL



N. DATTA, A. M. LAWN AND E. MEYNELL