*BACTERIAL CONJUGATION: ELECTRON MICROSCOPE OBSERVATIONS ON THIN SECTIONS**

By Werner-Hans Schreil and Roger James Christensen

INTERNATIONAL LABORATORY OF GENETICS AND BIOPHYSICS, NAPLES, ITALY, AND DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF ROCHESTER, ROCHESTER, NEW YORK

Communicated by Jean Brachet, January 29, 1968

Zygote formation during bacterial conjugation can be considered as consisting of three successive steps:[1] random collision, the formation of specific unions, and chromosome transfer. The latter two steps have been studied primarily by genetic experiments. For morphological studies of conjugation, especially for chromosome transfer, the technique of choice is thin-section electron microscopy. We report here some preliminary results of the application of this technique to conjugating mixtures of Hfr (donor) and F^- (recipient) strains of *Escherichia coli*.

Crosses were done between strain CS 100 (Hfr, lac^+, str^s) and an F^-, streptomycin-resistant derivative of strain CS 100 (F^-, lac^-, $T6^r$). To ensure that satisfactory levels of recombination were occurring in each experiment, aliquots of the mating mixture were allowed to shake slowly at 37° for 60 minutes, after which samples were plated on eosin methylene blue (EMB) or MacConkey's agar plates to which streptomycin had been added to select against the Hfr parent; lactose-fermenting colonies were scored as recombinants.

Log phase cultures of the parental strains were grown with slow shaking in nutrient broth at 37°, and then mixed to give approximately two F^- cells per Hfr; slow shaking then continued for 15–30 minutes. Buffered OsO_4 was then added to give a final concentration of 0.1 per cent, and the "prefixed" cells were either gently centrifuged or collected on a membrane filter. The collected cells were carefully mixed into warm 2 per cent agar dissolved in buffer, and the agar was promptly drawn up into Pasteur pipettes. After solidification, the agar was extruded from the pipettes, cut into small blocks, and fixed overnight either according to Ryter and Kellenberger[2] or with a buffered mixture of 1 per cent OsO_4 and 0.5 per cent uranyl acetate. Although the latter fixation gives rather poor preservation of cytoplasmic structures, such as ribosomes, it ensures rapid gelation of DNA plasms.[3] This minimizes the possibility that the empty-appearing "bubbles" (see below) had originally contained DNA which was lost during fixation and dehydration. The blocks were dehydrated, embedded in Vestopal, sectioned, and stained with Karnovsky's strain[4] method B. Sections were observed in the Siemens Elmiskop 1A (Armbruster transformation) at 80 kv, using condenser 1, a 5-μ spot, heated aperture, and decontamination device.

Structures seen frequently in these experiments were empty-appearing "bubbles"; these varied in size, and frequently seemed to form a bridge between two cells (Fig. 1). Examination of hundreds of bubbles showed that their formation involved only the outer two layers of the cell wall, and never the innermost mucopeptide layer. Bubbles also appeared in control preparations of Hfr cells alone, but seemed to be less frequent than in mating mixtures. They were not observed in F^- cultures unless these F^- cells were incubated for several minutes

in supernatant fluids from Hfr cultures, in which case bubbles were very rare and very small compared to typical Hfr bubbles. The F^- cell walls appeared to be "wavy." Where bubbles appeared as bridges between two cells, there was no striking tendency for the nucleoplasms of the cells involved to have any particular relationship to the bubbles.

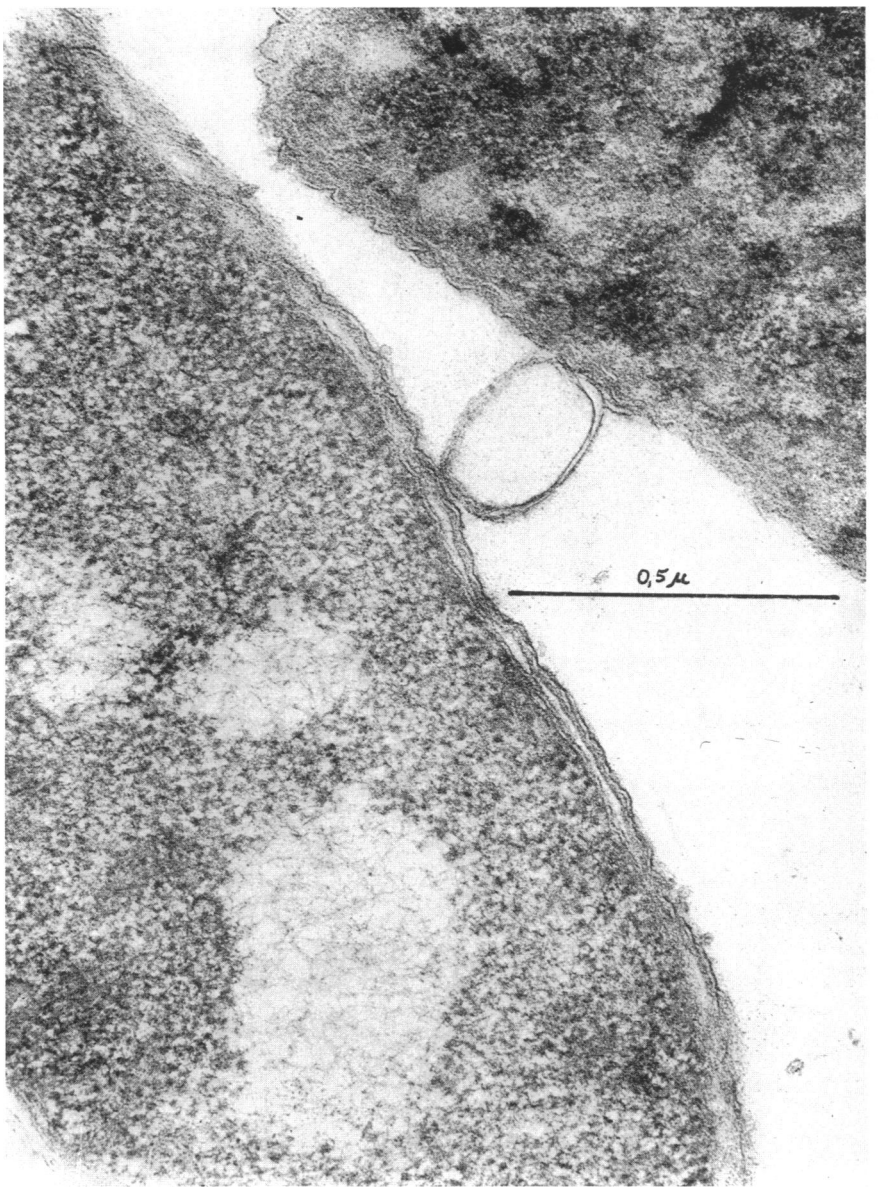

FIG. 1.—Cells collected by centrifugation after 30 min conjugation. Ryter-Kellenberger fixation. The wall of the bubble has two dense layers, while the wall of the leaky cell to the left clearly has three.

In almost all sections of bacterial pairs with close contact, one of the cells, like the left cell in Figure 1, appeared to be "leaky," as described by one of us,[5] i.e., the cytoplasm was less dense, the deoxyribonucleic acid (DNA) in the nuclear region appeared to be less closely packed, and there was a distinct separation of the cytoplasmic membrane from the cell wall. If phage T6 were added to the mating mixture shortly before prefixation, it was observed that it was only the leaky

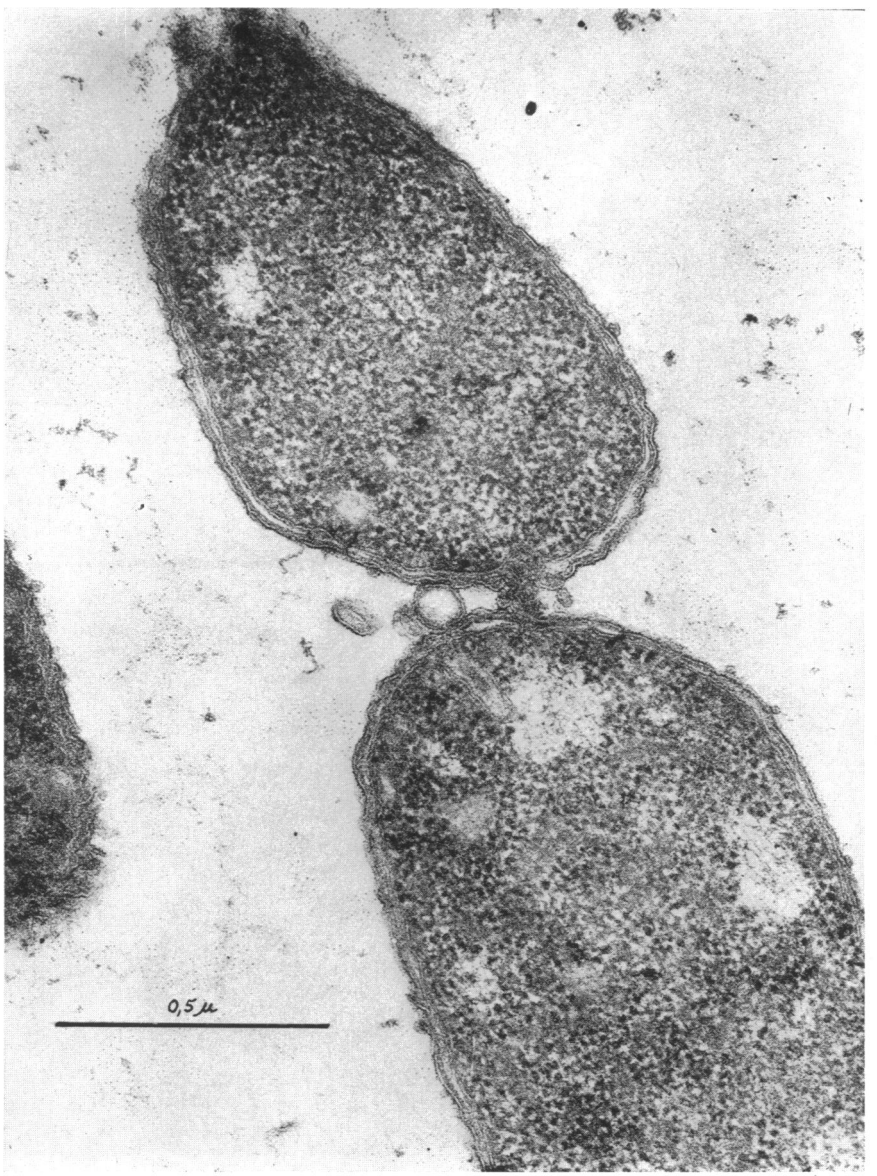

FIG. 2.—Bacteria and fixation as in Fig. 1. The round bubble connecting the cells seems to open toward the lower cell. Mesosome nearby in contact with DNA.

member of a pair that was ever labeled with phage. Since the recipient bacteria are resistant to T6, it is concluded that it was the donors that were leaky. It must be emphasized that the addition of phage was not necessary in order to regularly find leaky cells. Although leaky cells can be observed occasionally in many strains of *E. coli*, the combined appearance of one leaky with one nonleaky cell in pair formation seemed noteworthy.

A second morphological feature which was observed fairly frequently is shown in Figure 2. Here, near the point of contact of two bacteria, and in apparent contact with the nucleoplasm of the lower cell, there is an internal membranous element, deriving from an infolding of the cytoplasmic membrane. These structures, which can be considered as examples of a simple type of mesosome, usually show a roughly rectangular or flattened oval cross section. In any case in which these mesosomal structures were observed, they always appeared near the point of presumed conjugational contact and always seemed to be in contact with the nucleoplasm, which also appeared near the cell-to-cell contact point. Since no serial sectioning was done for these observations, the frequency of this appearance seemed to be rather remarkable. It is not known whether these mesosomes play any role in conjugation, but they could be related to the hypothetical attachment of the sex factor to the membrane,[6] by way of the "complex of replication." Although mesosomes are not often seen in Gram-negative bacteria, Steed and Murray[7] have recently shown that proper techniques will reveal mesosomes in dividing *E. coli* cells growing at 45°. Ryter and Jacob[8] have shown the relationship between nucleoplasm, cytoplasmic membrane, and infolding mesosomal structures. (We have found that addition of chloramphenicol to rapidly dividing cell cultures of *E. coli* and subsequent conventional fixation (15–45 min later) increases the number of mesosomes observed in the electron microscope. It may be that CAP stops degradation of mesosomal structures prior to the arrival of the fixing fluids in the cells.)

A third finding in these experiments, seen several times but less often than the structures noted above, is shown in Figure 3. Here there seems to be an orientation of DNA fibrils, in both cells, perpendicular to the cell walls at a point of contact, as though DNA were passing from one cell to the other. Unfortunately, the point of transfer, assuming that one exists, is out of the plane of the section.

Such appearances may be relatively infrequent for two reasons: (*a*) conjugating pairs would be easily separated during the preparative procedures, and (*b*) a random section through a conjugating pair would be less likely to include a small region of cell contact than, for instance, a large bubble.

While these observations are preliminary, we wish to make a few tentative comments. The only evidence in our pictures for transfer of DNA from cell to cell shows a close contact between the cells, rather than any elongated "conjugation tube." Metal-shadowed whole mounts of conjugating cells have been interpreted as showing such a tube,[9] but it is not difficult to imagine that surface forces occurring within a small droplet drying on the microscope grid might possibly produce such an appearance by dragging apart two cells that had originally been in close contact.

Brinton[10] has shown that there is a class of male-specific "pili" which are neces-

sary for conjugation, and has given indirect evidence that these pili serve as conjugation tubes. While this is a most interesting idea, we feel that our evidence for close conjugal contact deserves consideration as the mechanism of DNA transfer. Pili could still play an essential role in specific pair formation. Unfortunately, pili would rarely be seen in thin sections, although our donors are piliated, as seen in shadowed preparations.

The leakiness of the donor member in our pairs remains to be explained. We

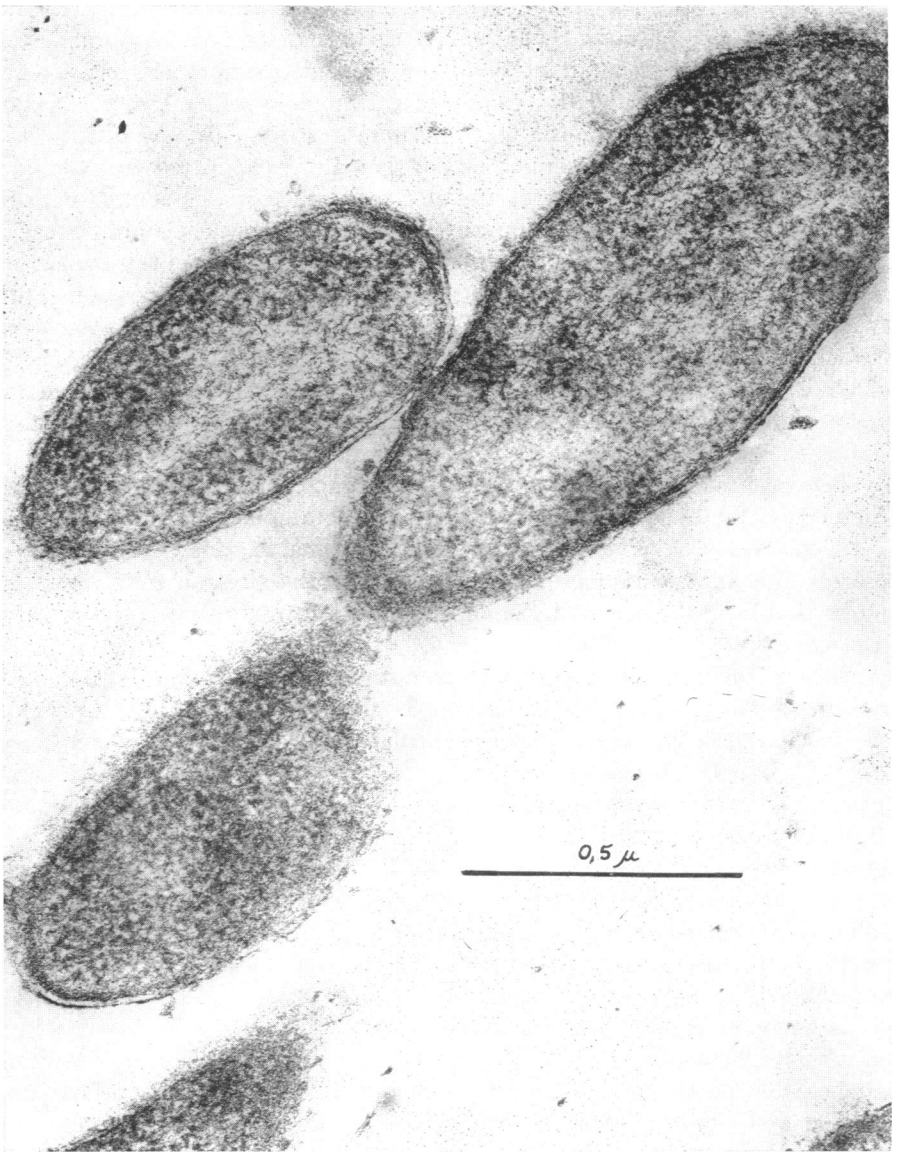

FIG. 3.—Cells collected on membrane filter. Fixation by OsO_4 and uranyl acetate. Note orientation of DNA.

can only add that if the donor culture is labeled with H^3-thymidine before mating, there is no evidence for the leakage of donor DNA into the medium.

We are continuing our study, using a thymidine-requiring donor strain labeled with H^3-thymidine, and the techniques of autoradiography and serial thin-sectioning.

Summary.—Empty-appearing bubbles are formed by Hfr strain of *Escherichia coli*. In conjugating mixtures, these often connect two cells, one of which often appears to be leaky. It is inferred from labeling experiments with bacteriophage T6 that the leaky-appearing cell in conjugating pairs represents the donor cell (Hfr). Simple mesosomes sometimes connect the cell membrane to the nucleoplasm. Deoxyribonucleic acid seems to pass directly from cell to cell in some cases.

* Supported by research grants GM 11759 and AI 02781 from the National Institutes of Health, Bethesda, Maryland.

[1] Hayes, W., *The Genetics of Bacteria and Their Viruses* (New York: Wiley, 1964), pp. 577–580.

[2] Ryter, A., and E. Kellenberger, *Z. Naturforsch.*, **13b**, 597 (1958).

[3] Schreil, W. H., *J. Cell Biol.*, **22**, 1 (1964).

[4] Karnovsky, M., *J. Biophys. Biochem. Cytol.*, **11**, 729 (1961).

[5] Schreil, W. H., *Proceedings of the Third European Regional Conference on Electron Microscope* (Prague: Czechoslovak Acad. Science, 1964), vol. B, p. 47.

[6] Jacob, F., S. Brenner, and F. Cuzin, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 329, N.B. Fig. 9.

[7] Steed, P., and R. Murray, *Can. J. Microbiol.*, **12**, 263 (1966).

[8] Ryter, A., and F. Jacob, *Ann. Inst. Pasteur*, **110**, 801 (1966).

[9] Anderson, T., E. Wollman, and F. Jacob, *Ann. Inst. Pasteur*, **93**, 450 (1957).

[10] Brinton, C., *Trans. N.Y. Acad. Sci.*, **27**, 1003 (1965).