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A Comparison of the Adherence of Fimbriated and Nonfimbriated *Haemophilus influenzae* Type b to Human Adenoids in Organ Culture

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Adherence of fimbriated and nonfimbriated variants of a single strain of *Haemophilus influenzae* type b to organ cultures of human adenoidal tissue was measured by three assays, two of which were quantitative. In one assay, the adherence of radioactively labeled bacteria was measured; the numbers of CFU of bacteria per gram of adenoidal tissue were 16.0 ± 6.7 for fimbriated bacteria and 10.2 ± 4.0 for nonfimbriated bacteria ($P < 0.05$). In the second assay, adherent CFU were determined directly; the results were 23.4 ± 17.2 CFU/g of tissue for fimbriated bacteria and 5.1 ± 2.2 CFU/g for the nonfimbriated variant ($P < 0.02$). By combining data from the two assays it appears that fimbriated and nonfimbriated bacteria do not compete for the same site on the tissue, and that the adherent bacteria do not change their state of fimbriation under the assay conditions used. In contrast, the third assay, scanning electron microscopy, showed very poor adherence of nonfimbriated bacteria. Fimbriated bacteria, on the other hand, adhered in clusters to nonciliated epithelial cells. Overall, the data indicate that fimbriae enhance adherence of *H. influenzae* type b to a type of tissue that is a normal site of human colonization and that nonfimbriated bacteria adhere by a distinctly different mechanism.

Haemophilus influenzae type b is a gram-negative bacterium that is pathogenic for humans, particularly very young children. Based on studies with the infant rat model, systemic infection is believed to occur by colonization of the nasopharynx followed by invasion (14). Indeed both type b and, more frequently, untypable (i.e., unencapsulated) *H. influenzae* are human nasopharyngeal commensal organisms (19).

The cell surface components of *H. influenzae* type b that enable the bacteria to attach to and colonize the nasopharyngeal mucosa in spite of the normal mucosal clearance mechanisms have not been definitively identified. However, *H. influenzae* that are fimbriated, in comparison to nonfimbriated variants, show increased adherence in vitro to isolated human buccal epithelial cells, human nasopharyngeal cells, and human erythrocytes (6, 15), suggesting that the *H. influenzae* fimbriae, like fimbriae of other bacteria, function as adhesins. The recent finding that only human erythrocytes containing the Anton blood group antigen are agglutinated by fimbriated *H. influenzae* type b suggests that this antigen is the host receptor or part of the receptor complex for *H. influenzae* type b fimbriae (20).

There is, however, evidence that fimbriae are not the sole adhesin of *H. influenzae* type b. Nonfimbriated variants adhere better than fimbriated forms to HEp-2 cells, a tissue culture cell line derived from human epithelium (17). Also fresh nonpassaged cultures of human nasopharyngeal isolates from children with systemic *H. influenzae* type b infections consist predominately of nonfimbriated forms (12). These forms do, however, have the ability to switch to fimbriated forms, an event that occurs in vitro at a rate of approximately 10^{-4} bacteria per generation (3). Thus it is possible that, because fimbriae can penetrate the mucus and epithelial glycocalyx (7), fimbriated bacteria may be responsible for the initial adherence to nasopharyngeal tissue but that once this occurs nonfimbriated progeny may be able to establish colonies by a different adherence mechanism. Certainly it is energetically advantageous to the bacterium to be able to switch off the production of fimbriae. It also appears that fimbriated variants may be at a disadvantage in a milieu other than the nasopharynx, since they have never been isolated from systemic sites of infection, such as blood and cerebrospinal fluid (12). Last, it is of interest that in the infant rat, which serves as an animal model for systemic *H. influenzae* type b disease, both fimbriated and nonfimbriated *H. influenzae* type b are able to colonize the nasopharynx and are equally virulent (8). Since the fimbriae of virulent *H. influenzae* type b lack specificity for tissues of rat (and all other animal species examined), adherence in this species must occur by a mechanism not involving fimbriae. (Fimbriated variants of one *H. influenzae* isolate do preferentially colonize the rat nasopharynx [2]. However, this strain is not virulent for the rat [P. Anderson, personal communication].)

In a further attempt to understand the role of fimbriae in adherence, we examined adherence to a tissue that closely resembles the in vivo site of initial colonization in humans, namely, adenoidal tissue obtained from pediatric patients and maintained in organ culture. Three separate adherence assays were employed, two of which were quantitative.

MATERIALS AND METHODS

Bacteria. *H. Influenzae* type b strains were isolated from clinical specimens, serologically typed, and stored as skim milk cultures as previously described (10). The fimbriated (F^+) and nonfimbriated (F^-) forms of a single strain, C54, were used in all experiments. Fimbriated C54 was isolated from the nasopharynx of an infant with a systemic infection (1). This strain is unusual in that it constitutively expresses fimbriae in vitro (15). Isogeneic nonfimbriated C54 was obtained from the blood of an infant rat infected intranasally

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with fimbriated C54 (M. E. Pichichero, E. M. Connor, and P. W. Anderson, Pediatr. Res. 17:279A, 1983). Except for the presence of a 23K protein in the latter (presumably the fimbrial subunit protein), the outer membrane protein profiles of F^+ and F^- strains are identical (9).

Bacteria were grown in brain heart infusion medium (BHI) or on BHI-agar (Difco Laboratories, Detroit, Mich.) supplemented with NAD and hemin (10).

Nasopharyngeal organ cultures. Nasopharyngeal tissue was obtained from children age 19 months to 12 years who were undergoing adenoidectomy for medical indications. Patients were excluded if they had received antibiotics for 24 h before the operation. Tissue was then maintained in organ culture by using a modification of the method described by Stephens et al. (18). Immediately upon removal, a small sample of the adenoidal tissue was rinsed in phosphate-buffered saline (0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2), homogenized, and plated on blood agar, chocolate agar, and MacConkey agar to culture and identify commensal bacteria. The rest of the tissue was placed into 15 ml of organ culture medium (pH 7.4) plus antibiotics (OCM-A), consisting of Eagle minimal essential medium containing Earle salts and L-glutamine, 20 μg of gentamicin per ml, 100 U of penicillin per ml, 0.50 μg of amphotericin B per ml, 5 μg of vancomycin per ml, and transported to the laboratory. After examination and sampling by the pathologist, the tissue was cut into pieces (approximately 3 by 7 mm) and placed into Falcon dishes (60 by 15 mm) containing 10 ml of OCM-A (three pieces per dish) and incubated at 37°C in 5% CO_2 for a total of 20 h, with fresh OCM-A added at 8 h incubation. The tissue pieces were then washed three times with 10 ml of organ culture medium without antibiotics (OCM) and incubated under the same conditions for 5 h.

Adherence assays. (i) Radioactive assay. A sufficient number of 50-ml Erlenmeyer flasks containing either lyophilized [4,5-^{3}H]leucine (1 mCi, 45 Ci/mmol; Amersham Corp.) or lyophilized [2-^{14}C]acetate (50 μCi, 54 mCi/mmol; Amersham) and 3 ml of supplemented BHI were inoculated with 0.06 ml of a suspension of F^+ or F^- bacteria (2×10^7 CFU/ml) obtained from an overnight culture (16 h) on supplemented BHI-agar. After incubation at 37°C with aeration, the bacteria were collected by centrifugation when they reached 10^9 CFU/ml. The F^+ and F^- bacteria were each washed three times with OCM and suspended at approximately equal concentrations as determined by light-scattering measurements in OCM. The actual CFU per milliliter were verified by plating dilutions of the bacteria on solid medium. The counts per minute per bacterium were determined by counting 20-μl samples of each bacterial suspension in 10 ml of Scintiverse II (Fisher Scientific Co.) with a Packard (model 3375; Packard Instrument Co.) scintillation counter. Samples of 5 ml of F^+ bacteria and 5 ml of F^- bacteria were then mixed and added to an organ culture dish containing two pieces of adenoid tissue. The actual total CFU in the dish varied from 6.0×10^8 to 1.2×10^9 CFU/ml, and the ratios of F^+ to F^- varied from 0.7 to 0.9. After incubation at 37°C in 5% CO_2 for 1 to 3 h, the tissues were removed, washed three times in OCM, and then trimmed with a scalpel blade to remove a thin slice of all cut (i.e., nonmucosal) surfaces, thereby eliminating bacteria that might be attached to surfaces not usually exposed in the nasopharynx. Each tissue piece was then weighed and placed in a scintillation vial containing 1 ml of Solusol (National Diagnostics), adjusted to neutral pH with acetic acid, and incubated at 45°C overnight to effect complete solubilization. Fluor (Scintiverse II, 10 ml; Fisher Scientific Co.) was added, and counts per minute were determined. The counts per minute per gram of tissue were then converted to CFU per gram of tissue, based on a knowledge of counts per minute per bacterium. All radioactivity was determined by using optimal settings of the scintillation counter for estimation of samples containing both ^{3}H and ^{14}C. Control experiments indicated that quenching of counts under the conditions used did not occur.

(ii) Viable count assay. The preparation of F^+ and F^- bacteria and incubation with tissue were done essentially as described above, except that radioactivity was omitted. In some experiments tissue pieces were incubated either with 10 ml of F^+ C54 or 10 ml of F^- C54; in other experiments incubation was with combined bacteria as above. Incubation times varied from 1 min to 3 h, and the bacterial concentration varied from 5×10^5 to 1×10^8 CFU/ml. At the end of the incubation the tissues were washed and trimmed as above and then homogenized in 0.5 ml of phosphate-buffered saline. The number of CFU per gram of tissue was determined by plating dilutions of the homogenate on solid medium. In experiments where F^+ and F^- bacteria were added together, the amount of each was determined by measuring the proportion of bacteria that were fimbriated by using the nitrocellulose hemadsorption assay (3). Briefly, bacterial colonies on solid medium were transferred to nitrocellulose, and the nitrocellulose was incubated with a suspension of human erythrocytes. Colonies that turned red consisted of fimbriated bacteria.

(iii) Electron microscopy. Portions of adenoidal organ cultures (24 to 72 h) were compared for analysis by either scanning or transmission electron microscopy. For the former, tissue specimens were prepared in small 2- to 3-mm cubes, with the apical surfaces of the nasoepithelial cells constituting one surface of the specimen. After fixation in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), specimens were dehydrated in increasing concentrations of ethanol, critical point dried with CO_2 as the transition medium, mounted, and sputter coated with gold. Specimens were then observed and photographed with a JEOL JSM-35 CF scanning electron microscope. For transmission electron microscopy, smaller cubed specimens (1 mm), maintaining the nasoepithelium on one surface, were fixed in glutaraldehyde as above, rinsed in buffer overnight, postfixed in 1% OsO_4 in cacodylate buffer, gently dehydrated in increasing concentrations of ethanol, and embedded in epoxy resin. Thick (1.0-μm) sections were cut with an LKB NOVA Ultramicrotome, stained with methylene blue-azure II, and observed with light microscopy. Thin (40- to 80-nm) sections were placed on 300-mesh copper grids, stained with uranyl acetate and lead citrate, and observed and photographed with a Zeiss 10B transmission electron microscope.

Ciliary activity. Ciliary activity was measured by ciliary vigor on a scale of 0 to 4^+ and by determining the percentage of the periphery with ciliary activity as described by McGee et al. (13).

Data analysis. Student's *t* test was used to analyze the statistical significance of the data.

RESULTS

Viability of organ culture. When cultured in the absence of bacteria, the adenoidal tissue showed no deterioration for 24 h, as assayed by measurements of ciliary activity and by observation of transmission electron micrographs (data not shown). By 72 h, a slight deterioration in the tissue was noted, with ciliary vigor and the percentage of periphery

TABLE 1. Radioactivity assay for adherence to organ cultures[a]

Expt	Radiolabel in bacteria		Ratio F^+ to F^- CFU added to organ culture at time zero	Adherence of F^+ and F^- to tissue at 3 h			
				CFU/g tissue (10^6)		F^+/F^- ratio	% of total adherent bacteria that were F^+
	^3H	^{14}C		F^+	F^-		
1	F^+	F^-	0.9	15.5 ± .5	9.5 ± .5	1.6	62
2a	F^+	F^-	0.7	16.5 ± 2.5	8.5 ± 1.5	1.9	66
2b	F^-	F^+	0.9	21 ± 13	11.5 ± 1.5	1.8	64
3	F^+	F^-	0.7	17 ± 1	6.5 ± .5	2.6	72
4	F^-	F^+	0.9	14 ± 0	16.5 ± 1.5	0.9	47

[a] Data on adherence to tissue are the means from two pieces of tissue. Each experiment represents tissue obtained from a different patient. CFU per gram of tissue was calculated from the cpm per bacterium. This varied from 1.0×10^{-3} to 1.8×10^{-3} for the ^3H label and from 0.6×10^{-3} to 1.0×10^{-3} for the ^{14}C label. The tissue pieces at the end of the experiment ranged in weight from 20 to 110 mg and contained 240 to 3,510 cpm of ^3H and 200 to 1,240 cpm of ^{14}C.

with ciliary activity being 85 and 66%, respectively, of the initial values and with electron micrographs showing the beginnings of lysis of cells beneath the epithelial layer (data not shown). In contrast, and as noted by others (4, 5), incubation of the organ cultures with *H. influenzae* resulted in rapid loss of both ciliary vigor and the percentage of cells with ciliary activity. This occurred with either F^+ or F^- bacteria. At 13 and 38 h, ciliary vigor was 69 and 31%, respectively, of initial values ($P < 0.1$ and $P < 0.001$, respectively). The percentage of peripheral cells with ciliary activity similarly declined to 41% at 13 h ($P < 0.05$) and even further by 38 h. Because of the toxic effect of the bacteria in the culture, adherence was assayed after an incubation of 3 h or less.

Relation of fimbriation to adherence. In the radioactivity assay approximately equal amounts of F^+ and F^- bacteria, each with a different radiolabel, were combined and incubated in the organ culture for 3 h (Table 1). At the cell density used, the number of bacteria in the medium increased twofold at most in 3 h; the F^+/F^- ratio of bacteria in the medium as determined by the nitrocellulose hemabsorption assay did not change. The adherence data obtained in the radioactive assay appear in Table 1. When F^+ bacteria labeled with [^{3}H]leucine and F^- bacteria labeled with [^{14}C]acetate were incubated with the organ cultures, the ratio of adherent F^+ bacteria to adherent F^- bacteria on the tissue at 3 h was greater than that at time zero (Table 1, experiments 1, 2a, and 3). When the F^+/F^- ratios were converted to the percentage of total adherent bacteria that were F^+, F^+ bacteria accounted for 66 to 79% of the adherent population (Table 1).

When the radioactive labels were reversed (Table 1, experiments 2b and 4), the data were not as consistent. However, in experiment 4 the adenoidal tissue, as obtained from the patient, was subsequently found to be heavily colonized with type b *H. influenzae*, a unique circumstance for these experiments and one that may have influenced the results. On the other hand, the data obtained in experiment 2b, although they do indicate preferred adherence of F^+ bacteria, varied considerably for adherence of F^+ to the two tissue pieces, as is evident from the large standard deviation (Table 1). Thus, one piece of tissue contained 44% F^+ bacteria, and the other contained 72% F^+ bacteria. It should be noted that experiments 2a and 2b were done at the same time on tissue obtained from the same patient (this was possible due to the large amount of tissue available from this patient); except for the single piece of tissue containing only 47% F^+ bacteria, the other three pieces contained 66, 67, and 72% F^+ bacteria, regardless of how the bacteria were labeled. Therefore, overall the radioactive adherence assay indicates that, whereas both F^+ and F^- bacteria adhere, F^+ bacteria do so in greater numbers than F^- bacteria when tissue is exposed to both of these in approximately equal amounts (see Table 3).

In the second quantitative assay the actual number of CFU in the tissue was determined by grinding the tissue and plating dilutions of the homogenate. Experiments were done either by separate incubation of tissue with F^+ or F^- bacteria or, as in the radioactive assay, with F^+ and F^- bacteria added together to the same piece of tissue. In the latter experiments, the relative proportion of F^+ and F^- bacteria on the tissue was determined by the nitrocellulose hemadsorption assay.

The F^+/F^- ratio of bacteria adherent on the tissue was greater in all five experiments than the input F^+/F^- ratio of bacteria (Table 2). Of the total adherent population, 62 to 89% were F^+. In experiments (no. 3 and 4) in which F^+ and F^- were added either separately to some organ cultures or together to other organ cultures derived from the same patient, the percentage of F^+ adherent bacteria was the same, indicating that adherence of F^+ and F^- is noncompetitive at the bacterial concentrations employed in the assay.

When the absolute numbers of adherent bacteria per gram of tissue as obtained with the radioactive and CFU assays are compared, the data are very similar (Table 3). The relevance of this is considered in the Discussion.

Some other aspects of adherence were also studied with the CFU assay. As shown in a single experiment, most of the adherence by F^+ and F^- bacteria occurred in the first hour and by 2 h, respectively. In an experiment where F^+ and F^- bacteria were added together in equal amounts but in con-

TABLE 2. Adherence of F^+ and F^- bacteria to organ culture as determined by assay of CFU of bacteria associated with the tissue

Expt	Initial culture conditions[a]	Input F^+/F^- ratio	Adherent bacteria after 3 h	
			F^+/F^-	% of total adherent bacteria that were F^+
1	Separately	0.8	8.0	89
2	Separately	1.0	1.6	62
3	Separately	1.1	5.2	84
	Together	1.0	5.6	85
4	Separately	0.6	3.8	79
	Together	0.8	3.7	79
5	Together	1.2	2.1	68

[a] F^+ and F^- bacteria were added separately or together. When they were added separately, one dish containing two pieces of tissue received F^+ bacteria and another dish received F^-. Bacterial concentration varied between 0.7×10^8 and 1.5×10^8 CFU/ml.

TABLE 3. Combined adherence data from the radioactivity and CFU assays

Assay	Adherent CFU/g of tissue (10^6)		
	F^+	F^-	% F^+
Radioactivity	16.0 ± 6.7	10.2 ± 4.0	61 ($P < 0.05$)
CFU			
F^+ and F^- added separately	26.5 ± 23.0	4.9 ± 2.0	84
F^+ and F^- added together	19.3 ± 7.6	5.3 ± 2.9	78
Combined data	23.4 ± 17.2	5.1 ± 2.2	82 ($P < 0.02$)

centrations ranging from 1.7×10^5 to 1.4×10^9 CFU/ml, the adherence of F^+ bacteria relative to F^- bacteria increased as the concentration of input bacteria decreased, a further indication of the greater capacity of F^+ bacteria to adhere. Also, when bacteria were added at the lower concentrations, there was a 24-fold increase in bacterial concentration in the medium, with F^+ and F^- bacteria increasing equally. Actually, in all experiments the ratio of F^+ to F^- in the medium did not change over the 3-h incubation.

In the third assay, employing scanning electron microscopy, F^+ bacteria were seen adhering to some nonciliated epithelial cells (Fig. 1A). Occasionally a cell was fully covered with bacteria (Fig. 1B), and in other cases fimbriae appeared to be attaching the bacteria to the host cell as well as to other bacteria (inset, Fig. 1A). Results of adherence studies with F^- bacteria were quite different, in that it was only with considerable examination of the specimens that adherent F^- bacteria were found; like F^+ bacteria, these also were adherent to nonciliated cells (Fig. 1C). Thus, in contrast to results with the two previous assays, the electron microscopy assay almost exclusively revealed adherence by F^+ bacteria. It is even feasible that the few adherent F^- bacteria represented that small population of F^+ bacteria (about 1 in 1,000) present in F^- cultures (3).

DISCUSSION

With the goal of elucidating the mechanism of adherence of *H. influenzae* type b in the human host, we compared the adherence in vitro of fimbriated and nonfimbriated bacteria to human nasopharyngeal tissue (adenoids) in organ culture. This tissue was selected because, in contrast to other choices, such as human buccal epithelial cells or erythrocytes, it is one of the nasopharyngeal tissues that is the natural site of *H. influenzae* type b colonization in humans, as indeed indicated in one of the tissue samples used in these experiments.

The use of organ cultures to study adherence of bacteria to human nasopharyngeal tissue was first described by Stephens et al. (18), whose organ culture method we adapted for our experiments. Using scanning electron microscopy, they showed that fimbriated *Neisseria meningitidis* adhered to the nonciliated epithelial cells, similar to our results with *H. influenzae* type b; however, adherence of nonfimbriated bacteria was not examined. In a second study, also using scanning electron microscopy, Farley et al. (5) showed that stationary-phase, nonfimbriated, wild-type *H. influenzae* type b, as well as mutants lacking a capsule and/or immunoglobulin A protease, all adhered to the nonciliated cells; however, in these experiments fimbriated *H. influenzae* type b were not studied. Last, in the only other quantitative study of which we are aware, a series of primarily nonfimbriated *H. influenzae* strains in the logarithmic phase of growth, both typable (all six types) and untypable, showed variable adherence (range of 40-fold) to organ cultures of monkey nasal turbinates (16). The actual CFU per gram of tissue was similar to or slightly greater than we found. Although a scanning electron microscopic study was not done, transmission electron microscopy indicated that the bacteria adhered to the cilia themselves, a result quite different from our results and those of others (5). Possible reasons for these differences are the use of a different species (monkey), the use of a different type of nasopharyngeal tissue, and the use of a less definitive detection technique. It would be of interest to know whether fimbriated *H. influenzae* type b have the same specificity for monkey tissues as nonfimbriated forms.

The study reported here is distinguished from other adherence studies with organ cultures in that adherence of fimbriated and nonfimbriated *H. influenzae* type b were directly compared, and three different assays were used, two of which were quantitative. Each of these assays has particular advantages and disadvantages. The radioactivity assay reveals how many of the input F^+ and F^- bacteria adhered, regardless of possible later events, such as increase in the adherent bacterial population by cell division or a switch in fimbrial expression. However, it does not distinguish between adherence of dead or living bacteria or between bacteria and subbacterial components such as shed outer membrane vesicles. The CFU assay, on the other hand, reveals how many living bacteria are on the tissue at the end of the assay and, in conjunction with the hemabsorption assay, what their state of fimbriation is. It does not distinguish between adherence of several bacteria or adherence of a single bacterium that subsequently multiplies. As discussed below, by analyzing the data from both of the assays, some of the questions unanswered by each assay separately can be resolved. Last, the scanning electron microscopy assay, although not readily amenable to yielding quantitative data, can reveal the state of the tissue during incubation with the bacteria as well as the identity of the cell types to which the bacteria adhere.

In the three assays, F^+ C54 was used as a prototype fimbriated strain, and F^- C54 was used as a prototype nonfimbriated strain. All three assays indicated better adherence by fimbriated variants of the bacteria. However, whether these data are applicable to other *H. influenzae* type b strains is not known.

The agreement of actual adherent CFU per gram of tissue within each of the two quantitative assays was unexpected, if only because tissue in each experiment had been obtained from a different patient. That there was also agreement between the two assays suggests the following: (i) the radioactive assay actually does measure adherence of whole bacteria rather than adherence of shed or degraded subbacterial components; (ii) a preponderant proportion of the adherent bacteria are alive; and (iii) under the conditions used, the bacteria do not multiply on the tissue during the 3 h incubation, nor do they change their state of fimbriation. It should be emphasized that none of these conclusions is absolutely assured by the data, but rather that the conclusions are consistent with the data; i.e., the agreement between the two assays could be coincidental.

Another conclusion derived from both assays is that F^+ and F^- bacteria do not compete for receptor sites on the tissue, i.e., the same number of each adhere whether F^+ and F^- bacteria are added together or separately. Since adherence by both F^+ and F^- bacteria is complete by 2 h, with

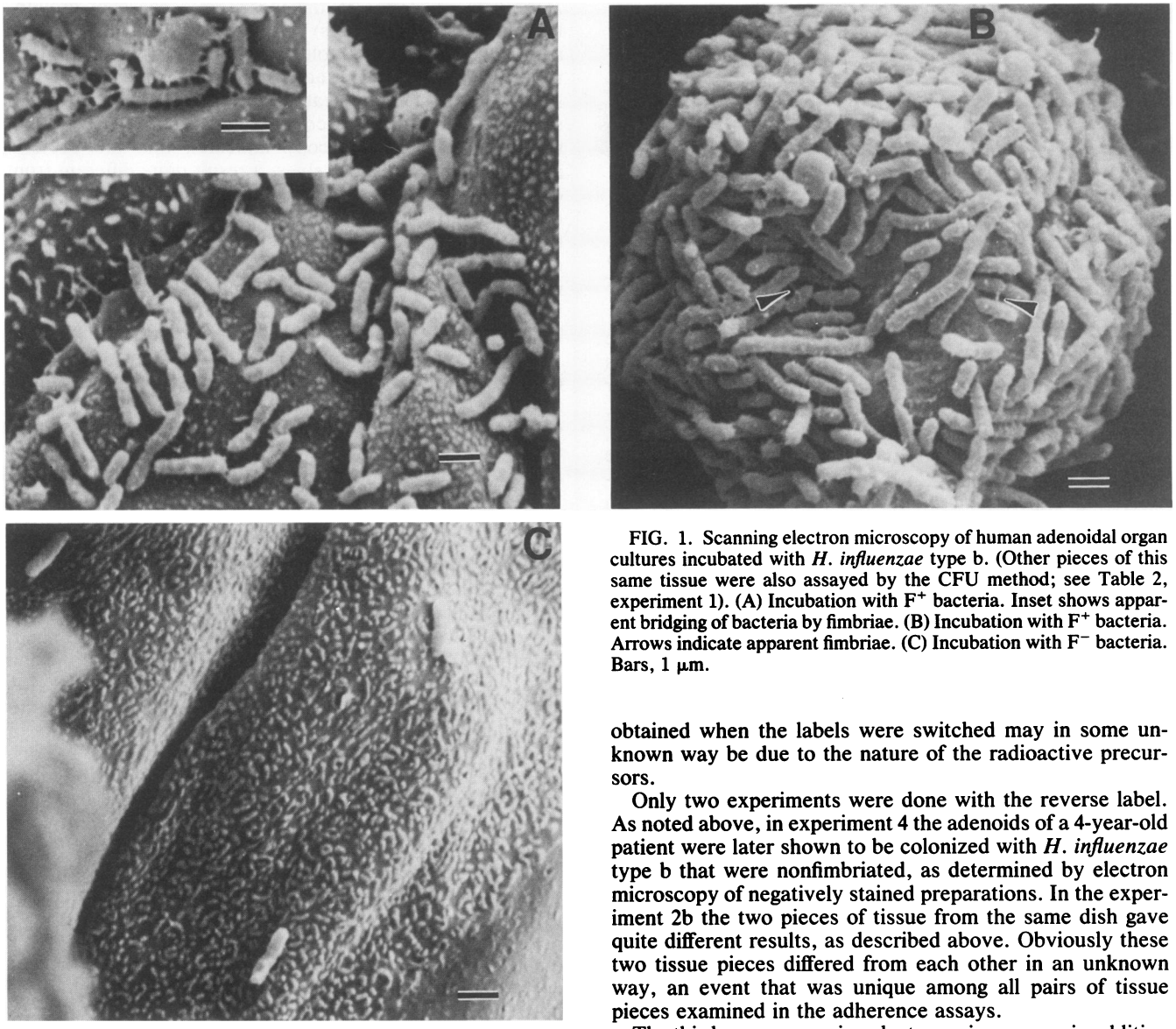

FIG. 1. Scanning electron microscopy of human adenoidal organ cultures incubated with *H. influenzae* type b. (Other pieces of this same tissue were also assayed by the CFU method; see Table 2, experiment 1). (A) Incubation with F^+ bacteria. Inset shows apparent bridging of bacteria by fimbriae. (B) Incubation with F^+ bacteria. Arrows indicate apparent fimbriae. (C) Incubation with F^- bacteria. Bars, 1 μm.

more than 95% of the bacteria remaining in the medium, it is unlikely that this apparent lack of competition is due to a large excess of receptor sites on the tissue. Apparently F^+ and F^- *H. influenzae* each recognize different receptors in the adenoidal tissue, a finding that is supported by other work in our laboratory, demonstrating preferred adherence of F^+ bacteria to human buccal epithelial cells in contrast to preferred adherence of F^- bacteria to HEp-2 cells (17).

More consistent data were obtained in the radioactive assay when F^+ bacteria were labeled with [^{3}H]leucine and F^- bacteria were labeled with [^{14}C]acetate than when the labels were reversed. Ideally the same chemical should have been used with both isotopes. However our choice of the isotopically labeled chemicals reflects both the cost and efficiency of incorporation of each precursor. The labeling pattern of [^{3}H]acetate would have been quite different from that of [^{14}C]acetate, making that pair inappropriate, and the expense of sufficient amounts of [^{14}C]leucine precluded its use as an alternate to [^{3}H]leucine. Thus, the different results obtained when the labels were switched may in some unknown way be due to the nature of the radioactive precursors.

Only two experiments were done with the reverse label. As noted above, in experiment 4 the adenoids of a 4-year-old patient were later shown to be colonized with *H. influenzae* type b that were nonfimbriated, as determined by electron microscopy of negatively stained preparations. In the experiment 2b the two pieces of tissue from the same dish gave quite different results, as described above. Obviously these two tissue pieces differed from each other in an unknown way, an event that was unique among all pairs of tissue pieces examined in the adherence assays.

The third assay, scanning electron microscopy, in addition to showing that adherence was to nonciliated cells, also showed highly preferential adherence of F^+ bacteria compared with F^- bacteria. In fact in this assay it was difficult to detect adherent F^- bacteria. Although this would appear to be in conflict with the results of Farley et al. (5), the differences are probably due to their use of bacteria suspended from overnight culture on solid medium, as compared to our use of freshly grown log-phase bacteria. In a single experiment in which we used bacteria prepared by their method, scanning electron micrographs showed approximately equal adherence of F^- and F^+ bacteria (data not shown). The reason for our finding so few adherent log-phase F^- bacteria by scanning electron microscopy is unknown. Possibly, because of their different mechanisms of adherence, log-phase F^- bacteria become less tightly bound to the tissue during tissue preparation. Alternatively, even though the nonmucosal sides of the tissue were trimmed before assay of adherent bacteria by the first two assays, perhaps F^- bacteria had gained entrance to the organ culture through these nonmucosal surfaces. The somewhat disparate data obtained by the two quantitative assays compared

with the electron microscopy assay emphasize the need to measure adherence by more than one assay, as also noted by others (11).

This study, which used organ cultures of human nasopharyngeal tissue to represent the host target, has demonstrated that F^+ bacteria usually adhere better than, but occasionally adhere only as well as, F^- bacteria to their natural site of colonization. Overall, the data suggest that fimbriae function to enhance the attachment of *H. influenzae* type b to host tissue.

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