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Effect of Squamous Metaplasia on Infection of Hamster Trachea Organ Cultures with *Mycoplasma pneumoniae*

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An organ culture system for hamster trachea was developed for maintenance of the ciliated respiratory epithelium during periods of extended cultivation (i.e., >20 days). Evaluation of five serum types showed that horse serum and fetal calf serum were best for the maintenance of epithelial ciliary activity and morphology. Rings that were opened on one side ("split rings") had the best maintenance of the ciliated epithelium as judged by the retention of ciliary activity and normal histological appearance after 3 to 4 weeks in culture. The in vitro induction of squamous metaplasia was achieved by cultivating explants in Waymouth MAB 87/3 (vitamin A-free) medium, without serum. This system allowed a direct comparision of the effects of *Mycoplasma pneumoniae* infection in two epithelial types, ciliated pseudostratified columnar and keratinizing squamous. Attachment of ^{14}C-labeled mycoplasmas was more than twofold greater in the normal epithelium. Pretreatment of explants with neuraminidase decreased attachment for both squamous and pseudostratified epithelial surfaces to a similar basal level. Recovery of viable organisms from infected tissue of both epithelial types indicated that the organism titer remained essentially constant during the infection period, but was significantly higher for the pseudostratified ciliated epithelium. These results suggest that specific receptor sites for *M. pneumoniae* are markedly reduced by the induction of squamous metaplasia and, hence, appear to be specific for the normal respiratory surface containing goblet cells and pseudostratified, ciliated epithelial cells.

Vitamin A, a lipid-soluble carotenoid, affects a variety of biological systems by interacting with cell membranes (1). It also exerts a controlling influence on the process of differentiation in normal epithelium. In the mammalian respiratory tract, physiological doses of vitamin A promote the maintenance of a columnar epithelium with numerous mucous-producing goblet cells and ciliated cells (16). Low levels of vitamin A cause a decrease or elimination of goblet cells, in addition to replacement of columnar cells with squamous cells capable of producing keratin—a process termed squamous metaplasia (13, 14).

Mycoplasmas are extracellular microbial parasites with a propensity for attachment to epithelial surfaces (21). Tvedton et al. (22) examined the effect of vitamin A deprivation in rats by noting the responses to subsequent challenge with viable *Mycoplasma pulmonis*. The incidence of infection and the degree of gross and histopathologically detectable damage were increased in animals maintained on a vitamin A-deficient diet. Clinical signs and lesions were similar for conventional or germfree rats fed the vitamin A-deficient diet and were more pronounced than in animals fed conventional diets.

Aside from this report on animal mycoplasmas and another study done with walled eubacteria (6), there is little information available regarding the effect of squamous metaplasia on host-parasite interactions. The current study was designed to provide a system to study the induction of squamous metaplasia in hamster trachea organ cultures. In addition, attention was devoted to the consequences of vitamin A deficiency-induced metaplasia on the attachment of *M. pneumoniae* to the tracheal epithelium.

MATERIALS AND METHODS

Mycoplasmas. The source and methods for cultivation of *M. pneumoniae* PI 1428 in G-199 medium were described previously (9). For labeling studies, cells were cultivated in G-199 medium supplemented by 1.5 μCi of [^{14}C]oleic acid (specific activity, 50.0 mCi/mmol; New England Nuclear Corp., Boston, Mass.) per ml. Log-phase cells were harvested by centrifugation at 16,000 $\times g$ for 45 min and were then washed three times in phosphate-buffered

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saline (PBS; pH 7.4). The final pellet was suspended in organ culture medium. A 100-μl portion of each wash and the final cell suspension were counted to determine the amount of label incorporated. Incorporation efficiency was approximately 1.4% (291,000 cpm/2.12 × 10^7 colony-forming units [CFU]).

Organ cultures. Tracheal explants were prepared from hamsters as described previously (8). They were maintained in minimal essential medium (MEM; autoclavable, with spinner salts; GIBCO, Grand Island, N.Y.) complete with glutamine and sodium bicarbonate and supplemented with sterile horse serum (10%) and penicillin G (200 U/ml). Relative activity measurements, i.e., percentage of the epithelial layer that remained intact (0 to 100%) multiplied by the vigor of ciliary beating (0 to 3+), were made every 2 days during observations with phase optics (×225). Medium and culture vessels were replaced every 4 days.

Serum vitamin A assay. The sera assayed were commercially available lots of: human serum (lot 719A312; International Scientific Industries, Inc., Cary, Ill.); chicken serum (lot 4060045; Flow Laboratories, Rockville, Md.); calf serum (lot 713R313; GIBCO); horse serum (lot R251709; GIBCO); and fetal calf serum (lot R053211; International Scientific Industries, Inc.). Assays were conducted by the trifluoracetic acid method of Neeld and Pearson (15). Standards were prepared from crystalline vitamin A acetate (molecular weight, 328.5; Nutrional Biochemicals Corp., Cleveland, Ohio) after the crystals were suspended in cotton seed oil.

Induction of squamous metaplasia. Tracheal half-ring explants were maintained in Waymouth MAB 87/3 medium (GIBCO) and were incubated as described above. After an incubation period of 20 to 30 days, histological (8) and scanning electron microscopy examinations (M. G. Gabridge, C. Agee, and A. Cameron, J. Infect. Dis., in press) were conducted.

Attachment of organisms to epithelial surfaces. Before infection with labeled organisms, half of the tracheal explants were pretreated with neuraminidase (*Clostridium perfringens*, purified type V; Sigma Chemical Co., St. Louis, Mo.) at a concentration of 5 U/ml in PBS. Control explants were treated with PBS only. Explants were incubated with or without enzyme for 60 min at 36°C in 5% CO_2 in water-saturated air. After treatment, explants were rinsed in PBS and placed into screw-cap test tubes (16 by 125 mm) (six per tube) with 0.8 ml of labeled organisms (ca. 10^7 CFU/ml). Tubes were placed in a roller drum (1 rpm) and incubated at 37°C in air for 1 h. After incubation, the rings were rinsed three times in separate PBS baths and placed on tared Parafilm squares. The rings were then dried in vacuo over a silica gel bed for 24 h. Dried rings were weighed and placed in scintillation vials. The epithelium was dissolved with Bio-Solv (Beckman BBS-3) for 24 h at 37° C. A 5-ml volume of scintillation cocktail (2) was then added, and samples were counted in an ISOCAP/300 liquid scintillation system (Nuclear-Chicago, Des Plaines, Ill.).

Recovery of attached *M. pneumoniae*. Explants were infected with an appropriate dilution of *M. pneumoniae* (ca. 1.2 × 10^7 CFU). On days 0, 2, 4, 6, and 8, five explants from each group were rinsed in PBS and homogenized with a Ten Broeck tissue grinder (held in an ice bath to retard any degradative enzyme activity) (19). Ten-fold serial dilutions were made immediately in PPLO broth, and 0.1-ml samples were spread in duplicate on Hayflick agar plates to determine the number of CFU recovered from the explants.

RESULTS

Serum supplement for long-term cultivation. To determine the effect of serum source on the maintenance of hamster tracheal epithelium, explants were cultivated in organ culture medium that contained a 10% concentration of either human, chicken, calf, horse, or fetal calf serum. Each of the sera was capable of sustaining some ciliary activity over a 12-day period (Table 1). Human serum caused the greatest drop in relative ciliary activity (from 270 to 11), whereas chicken and calf sera gave somewhat better maintenance (from ca. 271 to 34). Horse and fetal calf sera were significantly better in terms of overall maintenance of ciliary action, since the decrease over the observation period averaged only about 68%.

It was noted that the rank ordering of sera in terms of ciliary maintenance was the inverse of that obtained when the vitamin A content was measured (Table 1). Serum with the highest vitamin A content (human) was poorest for maintaining trachea organ cultures. Chicken and calf sera had intermediate vitamin A levels and were only fair for maintaining explants. Horse and fetal calf sera had the lowest vitamin A content and gave the best results for maintaining ciliary activity. It was noted, however, that other serum factors besides vitamin A might be participating in promoting explant survival.

TABLE 1. *Effect of serum source and vitamin A concentration on relative ciliary activity of hamster trachea intact-ring explants maintained in MEM medium*

Serum	Vitamin A concn[a]	Relative activity[b]	
		Day 1	Day 12
Human	1.60 ± 0.04	270	11
Chicken	0.58 ± 0.02	267	32
Calf	0.54 ± 0.03	272	36
Horse	0.14 ± 0.01	270	85
Fetal calf	0.13 ± 0.01	269	90

[a] Micrograms per milliliter of serum; mean data (± standard deviation) from three separate assays run in duplicate.

[b] Mean data from nine rings per serum from three separate trials.

Configuration of explants. In an attempt to maximize the length of time that the ciliated epithelium could be maintained in culture, tracheal rings were cut on the ventral side and split into a "W" shape to increase their exposure to nutrients in the thin layer of medium. Relative activity of split- and intact-ring explants was monitored for 12 days. A total of 18 rings from two separate trials was used to generate the data for each group shown in Fig. 1. Split-ring explants had a consistently and markedly higher relative ciliary activity than did the whole-ring explants. After 6 days of cultivation, the split rings had a relative activity of ca. 250, whereas the intact rings had a relative activity of 225. By day 12, these values were 250 and 75, respectively, for a relative decrease of 70% (apparently due to the minimal exposure to fresh nutrients in the intact rings). Thus, for short-term experiments (<6 days), either intact or split rings could be used to provide an actively beating ciliary epithelium, whereas split rings were obviously superior when cultures were to be maintained for extended periods.

Variations in the amount of serum in organ culture medium had a significant effect on maintenance of the mucociliary epithelium of split-ring explants. Concentrations of 5, 10, and 20% fetal calf and horse sera were added to the standard organ culture medium, and relative activity was monitored over a 20-day period. Decreases in the relative ciliary activity were observed in nearly all samples (Fig. 2; mean values from 18 explants per serum concentration). A supplement of 10% horse serum provided the best maintenance of the ciliary activity over long periods of time, with a relative activity of ca. 270 on day 20. A similar concentration of fetal calf serum reduced activity to 225. For both sera, the 10% concentration was optimal, since a 5% supplement reduced ciliary activity to less than 100, and a 20% supplement lowered the relative activity to less than 200.

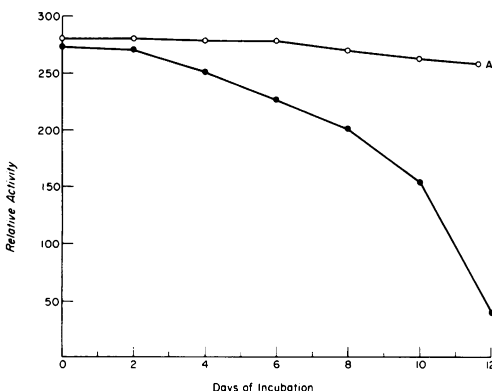

FIG. 1. *Effect of explant configuration on ciliary activity of hamster trachea organ cultures. (A) Split-ring explants; (B) intact-ring explants. Each point represents mean data from 18 cultures.*

When intact- or split-ring explants were infected with 5×10^6 CFU of virulent *M. pneumoniae* per ml, the rates of change and final relative activity values were quite different in each group. The data (Fig. 3; mean values from 36 explants in two separate experiments) indicate that the ciliary activity of infected, intact rings was significantly lower than the infected, split rings. The split rings, both infected and control, maintained a higher relative activity than did comparable groups of intact rings. Eight days after infection, the relative activity of split rings was 150 ($\pm$19), whereas that of intact rings was 50 ($\pm$8). Infection with *M. pneumoniae* caused a drop in relative ciliary activity of 40% in the split rings and 78% in the intact rings.

In related studies, it was found that control explants maintained in MEM (with 10% horse serum) had even greater ciliary activity for extended periods when trace amounts of retinol were added. Accordingly, the remainder of the studies on normal ciliated epithelium utilized tracheal split rings maintained in MEM medium supplemented with 10% horse serum and 0.2 μg of *trans*-retinol (Sigma Chemical Co., St. Louis, Mo.) per ml with a final concentration of 0.1% ethanol ("supplemented MEM").

Induction of squamous metaplasia. Squamous metaplasia was induced in hamster tracheal explants (split ring) by cultivation in Waymouth MAB 87/3 medium for 20 to 30 days. Cultivation of explants in this vitamin A-free medium caused a progressive, significant decrease in detectable ciliary activity as the epithelial type changed from pseudostratified and ciliated to squamous and nonciliated. The data in Fig. 4 (mean vaues from two experiments, for a total of 55 explants for each medium) show a comparison of relative ciliary activity values for control (supplemented MEM medium) and metaplastic (MAB 87/3 medium) explants. Some decrease in activity for the MAB 87/3 explants was noted within the first few days, and the reduction progressed in a regular fashion until the relative ciliary activity was less than 5% of the control level at 30 days. Control cultures (i.e., those maintained in supplemented MEM medium) had some decrease in ciliary activity, but even after cultivation for 20 to 30 days the majority of the surface was covered with actively beating cilia.

Histopathologically, it could be seen that the epithelium was intact and apparently viable in

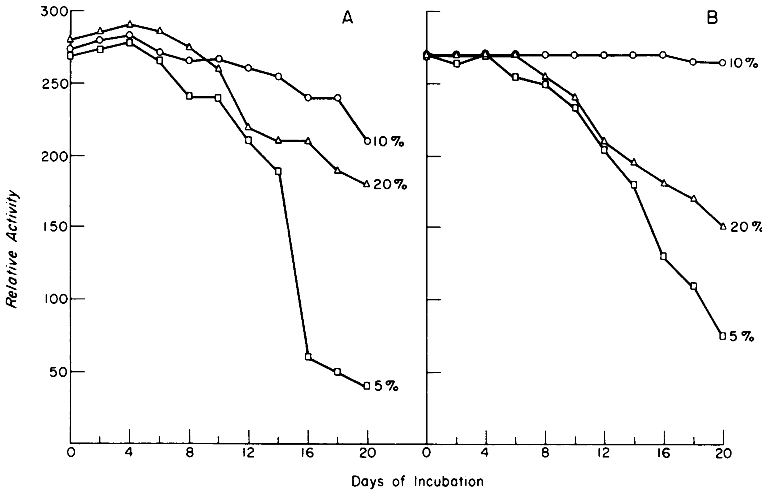

FIG. 2. *Role of serum source and concentration (MEM medium) in maintaining ciliary activity in split-ring tracheal explants. Each curve represents mean data from nine cultures; (A) fetal calf serum; (B) horse serum.*

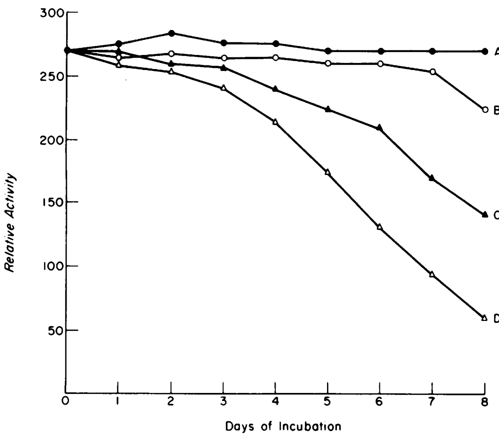

FIG. 3. *Effects of infection with M. pneumoniae on ciliary activity in intact and split tracheal ring explants. (A and C) Split rings; (B and D) Intact rings; (A and B) uninfected controls; (C and D) mycoplasma infected. Mean data from nine explants per treatment.*

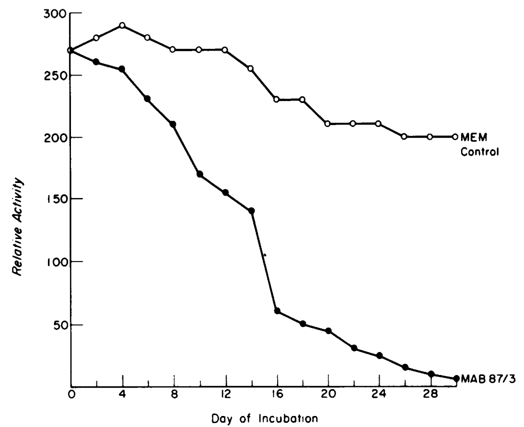

FIG. 4. *Induction of squamous metaplasia in tracheal split-ring explants by cultivation in Waymouth MAB 87/3 medium, and effects on ciliary activity. Each curve represents mean data from 55 explants in two separate experiments.*

both types of epithelium, and that the explants maintained in the 87/3 medium had undergone squamous metaplasia (Fig. 5). Tracheal tissue obtained directly from adult hamsters had a cartilage base and a thin lamina propria covered with a pseudostratified, ciliated epithelium containing goblet cells. Tracheal explants maintained for 30 days in supplemented MEM medium also had the cartilage base, but the lamina propria was thicker, and there was obvious hyperplasia in the epithelium. The cells, however, were epithelial-like, and occasional cilia and mucous could be seen over the epithelium. In contrast, the cultures kept for 30 days in 87/3 media underwent a distinctive squamous metaplastic change. The elongated and polygonal epithelial cells were replaced by multiple layers of squamous epithelium. Occasional patches of keratin could be observed, as could flat, desquamating epithelial cells.

The induction of squamous metaplasia was just as obvious in a scanning electron microscopic study of similar tissue samples (Fig. 6). The surface of control (supplemented MEM) explants was covered with patches of cilia and

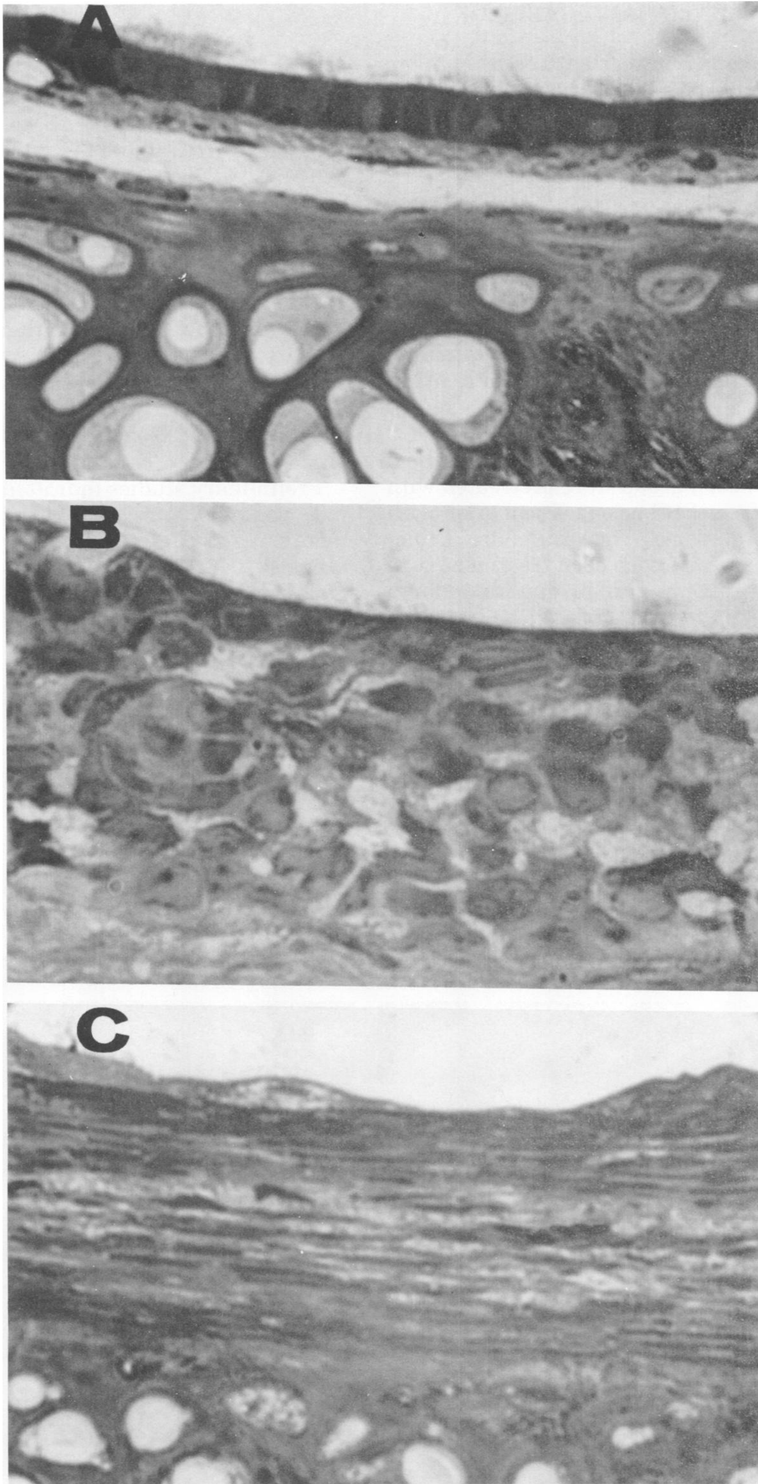

FIG. 5. *Histopathological appearance of: (A) normal tracheal epithelium freshly prepared from an adult hamster; (B) tracheal explant maintained for 30 days in MEM medium with 10% fetal calf serum and 0.2 μg of retinol per ml; (C) tracheal explant maintained for 30 days in Waymouth MAB 87/3 medium (note layers of squamous epithelial cells indicating metaplasia). Toluidine blue stain. ×200.*

granular, nonciliated areas with occasional microvilli. In contrast, the tissue maintained in MAB 87/3 medium was covered with large, smooth, flat cells. Cilia, microvilli, and granular-appearing cells were relatively rare. When present, cilia appeared to arise from small, isolated cells with a granular surface and not from the predominant, smooth, platelike cells.

Attachment of organisms. To determine whether *M. pneumoniae* cells would attach to squamous epithelium with a different efficiency relative to attachment on ciliated epithelium, split-ring tracheal explants from both MEM and Waymouth MAB 87/3 groups were exposed to [^{14}C]oleic acid-labeled organisms. Table 2 contains the results of studies in which tracheal explants from each group were pretreated with neuraminidase or PBS prior to incubation with the labeled organisms. The results are a composite of three trials, with each treatment tube of six explants being run in duplicate, for a total of 144 explants. The presence of a squamous epithelial surface caused a significant reduction in the attachment of organisms. For example, the mean counts per minute per milligram in normal, pseudostratified columnar epithelium was ca. 2,200, compared with 975 cpm/mg on the metaplastic epithelium. Thus, the induction of squamous metaplasia was associated with a 57% decrease in the attachment of labeled organisms. When either group of explants was pretreated with neuraminidase, attachment of mycoplasmas was lowered to a basal level of ca. 600 cpm/mg, presumably representing nonspecific attachment or adsorption. Neuraminidase pretreatment reduced attachment by 71% in normal pseudostratified epithelium (explants in supplemented MEM) and by 35% in squamous, metaplastic epithelium (Waymouth MAB 87/3 culture).

Growth of organisms. Because mycoplasmas attached to squamous epithelium to a much lesser degree than to ciliated epithelium, recovery experiments were performed to determine the extent of multiplication. Explants cultivated for ca. 30 days in either supplemented MEM (control) or MAB 87/3 (metaplastic) media were exposed to viable *M. pneumoniae* cells (1×10^7 to 2×10^7 CFU/ml), and CFU counts were calculated from homogenates of explants on days 0, 2, 4, 6, and 8.

Preliminary studies indicated that mycoplasmas would not survive in either MEM or Waymouth MAB 87/3 medium, since CFU titers dropped 100-fold over an 8-day period (Table 3). However, organisms could survive on tracheal explants. Immediately after infection, ca. 5 ×

TABLE 2. *Attachment of radioactive ([^{14}C]oleic acid) M. pneumoniae cells to hamster tracheal explants*[a]

Pretreatment	Epithelium	
	Ciliated	Squamous
PBS (control)	2,221 ± 185	975 ± 151
Neuraminidase (5 U/ml)	637 ± 28	635 ± 32

[a] Counts per minute per milligram of tissue; mean data (± standard deviation) from 36 explants per group from three separate experiments.

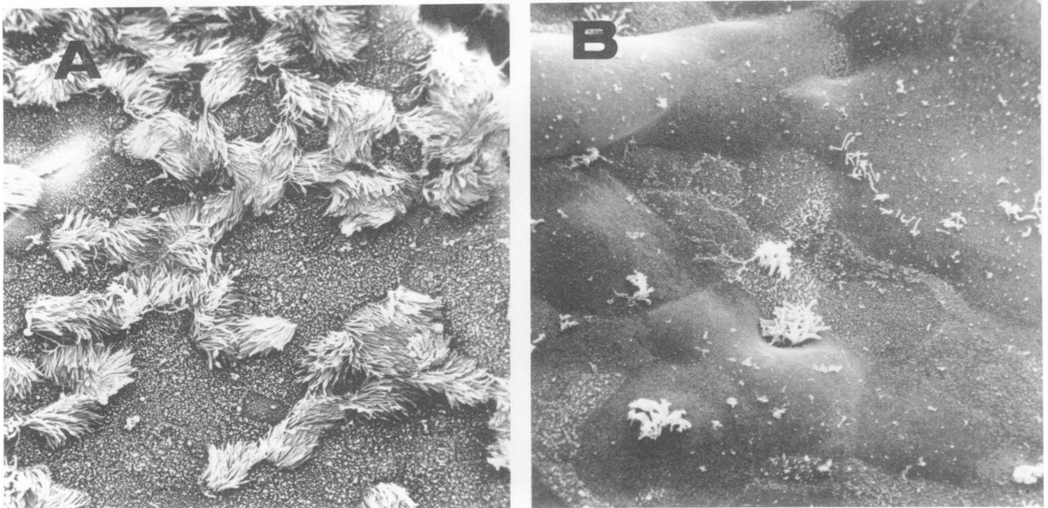

FIG. 6. *Scanning electron micrographs of hamster tracheal explants after 30 days in culture. (A) Control in MEM medium; (B) metaplastic from 87/3 medium. Note near absence of ciliation and the flattened, platelike epithelial cells covering the surface in squamous metaplastic tissue.* ×720 *(approx.).*

TABLE 3. *Viable cell titers of M. pneumoniae recovered from organ culture media or hamster tracheal explants* [a]

Source	*M. pneumoniae* titer [b]	
	Day 0	Day 8
MEM medium	9.8×10^4	1.6×10^2
Explants (10) in MEM medium	5.2×10^5	5.15×10^5
MAB 87/3 medium	9.8×10^4	1.2×10^2
Explants (10) in MAB 87/3 medium	2.6×10^5	2.5×10^5

[a] Mean data from two separate experiments.
[b] CFU per milliliter or per explant.

10^5 CFU could be recovered from normal epithelium, and 3×10^5 CFU could be recovered from squamous epithelium. These titers remained constant or decreased slightly (less than twofold) during the 8-day observation period (Table 3). Thus, mycoplasmas attached in greater numbers to the pseudostratified epithelium, and organisms survived (but apparently did not multiply) on both types of epithelium.

DISCUSSION

One of the major impediments to progress in studies on the attachment of *M. pneumoniae* to different epithelial types has been the absence of an organ culture system suitable for continuous observation and long-term maintenance of ciliated tracheal explants. Some attempts have been made at extended cultivation of mammalian respiratory epithelium (13, 14), but these studies did not employ ring explants (i.e., tracheal slices), which provide an opportunity to continually monitor and quantitate the ciliary activity.

The current study led to the development of an organ culture system in which tracheal rings could be maintained for more than 20 to 30 days. The optimal medium for ciliated epithelium was MEM supplemented with 10% horse serum and 0.2 μg of retinol per ml. The optimal medium for obtaining squamous metaplasia was Waymouth MAB 87/3 medium, without supplements. However, the most critical element for the maintenance of tracheal ring cultures was the configuration of the explant. Tissue left in the form of an intact ring lost much of its ciliary activity after a week in culture. Because the ring is normally maintained in a thin layer of medium, the top surface of the ring is at the air-liquid interface. This tends to block any circulation of fresh medium into the lumen. Consequently, access to nutrients is limited, and waste products can accumulate. We found that splitting the rings on one side and then forcing them open resulted in a much healthier explant. Ciliary beating would continue in a very vigorous fashion for more than 30 days. Split-ring explants were somewhat harder to damage with viable mycoplasmas, apparently because the ciliated epithelium had minimal environmentally induced necrosis, and it was able to resist attachment by the microorganisms. This implies that perhaps some of the damage noted in other studies (11, 17) with intact rings may be artifactual; i.e., cultural conditions may have potentiated some of the cytotoxic effects attributed to *M. pneumoniae*. It appears that split-ring tracheal explants may serve as a more accurate model system with which to study host-parasite interactions that occur at the mucociliary surface.

For studies dealing with the in vitro induction of squamous metaplasia, the split-ring explants are especially valuable. One can follow the progressive loss of ciliated epithelial cells through a visual monitoring of relative ciliary activity. When Waymouth MAB 87/3 medium was used, relative ciliary activity (i.e., the percentage of the surface that is ciliated, multiplied by the relative vigor of beating) was about one-half of the maximum after 12 days in culture. Ciliary activity continued to subside, until it was less than 3% of the theoretical maximum on day 30.

Explants from such low-activity cultures had obvious squamous metaplasia as seen in histological and electron microscopic examinations. Few signs of goblet or ciliated cells were evident, and the inner surface was covered in a laminar fashion with keratinizing, desquamating squamous cells. Reference cultures maintained in MEM medium for similar 30-day periods were ciliated, but certain changes were obvious. A marked hyperplasia was evident, and the ciliated cells were fewer in number. The latter observation was consistent with the decreased relative ciliary activity (e.g., 200) noted with phase optics.

When squamous and ciliated respiratory epithelial surfaces were compared with regard to attachment by *M. pneumoniae*, the ciliated epithelium could accommodate more than twice as many organisms. Attachment of isotopically labeled mycoplasmas was corrected to a weight basis to compensate for any differences that might have been generated while the rings were being sliced or cultured. Attachment to the ciliated epithelium in terms of counts per minute per milligram was almost fourfold that of the nonspecific, background attachment noted in neuraminidase-treated rings. This specific attachment is considerably greater than that reported in a similar study (7) and is prob-

ably related to an improved labeling technique. Our method involves exposure of explants to mycoplasmas while the target tissue and parasite are in a slowly rotating tube in order to provide repeated exposure and maximum contact.

The data on incorporation and attachment efficiency indicate that we obtained ca. 0.0137 cpm/CFU and that ca. 1.6×10^5 CFU attached to each explant. This is consistent with earlier reports of Collier and Baseman (7), who found 0.015 cpm/CFU and 2×10^4 to 2.6×10^4 CFU/explant, and also agrees with Powell et al. (17), who found 0.0025 to 0.05 cpm/CFU and 1×10^6 to 3×10^6 CFU/explant. In each of these studies, the relative attachment efficiency (i.e., CFU attached to explants/total CFU available) is less than 10%. This seemingly low efficiency may be due to damage or alterations induced in the pathogen while the mycoplasmas are cultivated, collected, and washed. It may also be due to the artificiality of the organ culture system, with its aqueous environment and altered mucociliary flow. Conversely, this low attachment efficiency may accurately reflect the natural situation, since each instance of physical contact between host and parasite need not necessarily result in perfect attachment, especially when receptor sites are involved. The final analysis must await further in vivo experiments to determine the attachment efficiency seen in disease induced in animal hosts.

It is significant that the attachment of labeled mycoplasmas to the normal squamous epithelium was only 53% greater than to the neuraminidase-treated squamous surface. This indicates that there were relatively few specific sites (18) at which *M. pneumoniae* could attach once squamous metaplasia has occurred. Although squamous metaplasia may have simply blocked or modified the receptor sites, or had an even larger than normal number of inefficient sites, it seems most plausible that receptor sites are simply not present in the squamous cells. If this conclusion can be verified, the induction of squamous metaplasia in tracheal explants, theoretically a reversible phenomenon (16), may provide a valuable system with which to study the synthesis of the neuraminic acid receptor site (12) specific for *M. pneumoniae*.

Organisms that did attach to either ciliated or squamous epithelial surfaces did not increase significantly in number. This may indicate that the organisms actually multiplied, but this process was accompanied by a concomitant release, or death, of progeny. Most probably, multiplication was relatively slow or completely absent. This latter conclusion would be compatible with other reports of similar static or slowly increasing cell populations in various types of tracheal tissue infected with *M. pneumoniae* (5), *M. mycoides* (4), *M. dispar* (10), *M. salivarium* (3), *M. gallinarum* (20), and *M. gallisepticum* (20).

It is also important to recognize potential artifacts inherent in the in vitro study of any host-parasite interaction. For example, it is conceivable that even though *M. pneumoniae* did not replicate in plain MEM or Waymouth medium (which contained no yeast extract, and only 10% or 0% serum, respectively), it could multiply in media (either in the fluid or on vessel surfaces) containing explants. This extracellular growth could then indirectly induce pathological effects. The likelihood of this occurrence is questionable, however, since others (7, 8, 11) have shown that cytopathology is only associated with the intimate contact of mycoplasma cells or membranes and epithelial cell surfaces. Nevertheless, the possibility of microbes multiplying in tissue-conditioned medium should be considered in any such in vitro study.

Collectively, these data suggest that the increase in susceptibility to respiratory infections noted in vitamin A-deprived animals (6, 13, 14) is not due to an increase in attachment and multiplication of organisms on the squamous epithelial cells. In fact, organisms appear less able to attach to squamous than to ciliated cells. Increased rates of infection in animals with squamous metaplasia most likely result from increases in attachment to the remaining ciliated cells due to the decrease in protective mucous and less efficient flow over a surface with fewer than normal ciliated cells. This would be consistent with a recent study of Gabridge et al. (in press) in which they noted that attachment of *M. pneumoniae* organisms was inversely correlated with degree of ciliation in various areas of the normal hamster trachea.

Data from the study just described indicate that tracheal explants can be used for long-term culture studies. Improvements in medium composition, explant configuration, and labeling techniques permitted mycoplasma attachment studies to be conducted after tracheal explants had been maintained for over 4 weeks in culture while the ciliary activity was monitored periodically. Since this facilitates the induction of squamous metaplasia through limiting access to vitamin A, this organ culture system should prove useful in studies of receptor site synthesis and the role of epithelial cell type in host-parasite interactions.

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