The Pathogenesis of Respiratory Syncytial Virus Infection in Cotton Rats

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The cotton rat is susceptible to respiratory synctial virus infection in both the upper and lower portions of the respiratory tract. Virus replicates to high titer in the nose and lungs and to relatively low titer in the trachea. Immunofluorescence studies demonstrated viral antigen in the nasal epithelium and the bronchial and bronchiolar epithelium but not in the trachea or the alveolar cells of the lungs. Histopathologic changes included a desquamative, exudative rhinitis of moderate severity and a mild proliferative bronchiolitis. Serum neutralizing antibody developed in all animals by the ninth day after infection, reaching extremely high titer in several instances. Unlike the previously described response of experimentally infected infant ferrets, cotton rats are uniformly susceptible to pulmonary infection throughout life, thereby offering a model for long-term pulmonary studies heretofore not available. (Am J Pathol 93:771–792, 1978)

THE IMPORTANCE of respiratory synctial (RS) virus as the prime respiratory pathogen of infancy [1] has not been matched by an equivalent understanding of the pathogenesis of, and immunity to, infection by this agent. Perhaps the chief reason for the lag in our understanding has been the lack of a small experimental animal in which to study RS virus disease. The recent description of experimental RS virus infection of infant ferrets [2] provided such a model, which bears several similarities to human RS virus infection. However, the most interesting aspect of RS virus pathogenesis in ferrets, ie, the age dependence of pulmonary infection, is also its chief disadvantage as a model of human disease, since the rapid loss of pulmonary susceptibility makes impossible the long-term study of the effect of RS virus on the ferret's lungs.

Several years ago Dreǐzin and co-workers [3] described RS virus infection in the cotton rat (*Sigmodon hispidus*) and suggested its use as an experimental model. This report confirms and extends their findings. The patterns of nasal and pulmonary infection are described as are the influence of age on viral replication and the immunologic response to infection.

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Materials and Methods

Animals

Cotton rats (*Sigmodon hispidus*) were obtained from a nucleus colony of the Veterinary Resources Branch, Division of Research Services, National Institutes of Health. All animals were bred under germ-free conditions. They were housed in large polycarbonate rat cages, with a bedding of hardwood chips, and fed a standard rat chow. Apples were used to provide water. Litters ranged in size from 2 to 10, with an average of 6 animals.

Virus

The Long strain of RS virus, obtained from the American Type Culture Collection, was used in all experiments. Virus suspensions were prepared by infecting monolayer cultures of HEp-2 cells at a multiplicity of infection of approximately 0.02; adding a nutrient medium consisting of Eagle's minimal essential medium (EMEM), 2.5% heat-inactivated chicken serum (HICS), and gentamicin sulfate (50 μg/ml); and incubating cultures at 37 C in an atmosphere of 5% CO_2. When cytopathic effect was maximal (3 to 4 days), virus was harvested by one freeze-thaw cycle followed by centrifugation at 450*g* to remove particulate material. The virus suspension was divided into aliquots and stored at −70 C.

Mode of Infection

Cotton rats were infected at different ages: at 3, 14, or 28 days or as adults. Animals were infected intranasally under light ether anesthesia with 10^4 plaque-forming units (pfu) of virus. The volume of inoculum was adjusted with Hanks' balanced salt solution (BSS) according to the size of the animal to ensure that the inoculum reached the lungs at the time of infection, being either 0.025 ml (3 day), 0.05 ml (14 day), 0.10 ml (28 day), or 0.20 ml (adult). Dialyzed India ink was used to verify that each volume was sufficient to reach the lungs.

Cell Culture

HEp-2 cells were propagated in monolayer culture in EMEM supplemented with 10% heat-inactivated fetal bovine serum and gentamicin sulfate (50 μg/ml). Frozen stocks of cells were maintained at −70 C in this same medium, with the addition of 10% dimethyl sulfoxide. Cells used for virus assay were continually monitored for sensitivity to RS virus by plaque assay.

Methylcellulose Overlay

A 2% methylcellulose suspension was prepared and autoclaved according to the method of Schulze.[4] After cooling to 43 C, the suspension was combined with an equal volume of double-strength modified Eagle's medium (Gibco) and was supplemented with 3% HICS, 50 μg/ml gentamicin sulfate, and 2.0 mM L-glutamine. Aliquots of the overlay were stored at −20 C until used.

Virus Assay

At intervals following infection, animals were anesthetized with pentobarbital and exsanguinated by cardiac puncture. Within each age group, 3 animals were used to obtain the data for each time interval. Each of these animals was from a separate experiment. Serum was stored at −20 C until analysis for neutralizing antibody. The chest organs were removed and the heart, esophagus, and thymus were separated and discarded. The trachea was also removed, and the lungs and trachea were stored separately. The nasal tissue studied included the nasal passage and the nasal turbinates. Tissue was quick-frozen and stored at −70 C until assayed.

Thawed tissue suspensions were diluted 1:10 with BSS. Lungs and trachea were homogenized in a Tenbroeck tissue grinder (Bellco Glass Co.); nasal tissue was combined with sterilized sand and ground in a porcelain mortar and pestle. Following brief centrifugation at 450g to sediment particulate matter, decimal dilutions of supernatant were added in 0.05-ml volumes to HEp-2 monolayers in 16-mm diameter wells (24-well plates, Costar Plastics). After 1 hour of incubation at 37 C in a CO_2 incubator, with intermittent agitation, the plates were washed with EMEM (without serum) and overlayed with the methylcellulose medium. Plates were then incubated at 37 C in a 5% CO_2 atmosphere. After 5 days the cultures were fixed with 10% formalin, washed, and stained with 0.1% aqueous crystal violet. Plaques were counted with the aid of a dissecting microscope, and virus titer was expressed as plaque-forming units (pfu) per gram of fresh tissue.

Serum Antibody Assay

Serum neutralizing antibody to RS virus was assayed using a 60% plaque-reduction neutralization test.[5]

Histology

Nasal, pulmonary, and tracheal tissues were obtained on 10 consecutive days following infection and from control animals every other day for 10 days. All tissues were fixed in 10% formalin for 24 hours and embedded in paraffin. Lungs were inflated by intratracheal injection of formalin at the time of fixation to preserve the pulmonary architecture in an expanded state. Histologic sections were stained with hematoxylin and eosin. Control tissues were obtained from animals inoculated intranasally with an appropriate volume of virus-free tissue culture supernatant.

Immunofluorescence Reagents

Ferret antiserum to RS virus was obtained from adult animals 3 to 4 weeks following intranasal infection. Rabbit antibody to ferret IgG was prepared by techniques previously described.[2] This antibody was fractionated and labeled with fluorescein isothiocyanate (FITC).[6] After absorption with liver powder, the labeled antiserum was used for indirect immunofluorescent staining.

Immunofluorescence Microscopy

Tissues were removed from the animal, quick-frozen on dry ice, and stored at −70 C until sectioned. Cryostat sections were cut at 10 μ. To preserve lung tissue in an expanded state, intratracheal injection of OCT compound (Ames Company, Eckhart, Ind), diluted 1:2 with phosphate-buffered saline (PBS), was performed immediately prior to freezing.[7] The indirect immunofluorescence technique was utilized to localize viral antigen in tissue. Ferret antiserum to RS virus, diluted 1:10 with PBS, was applied to the acetone-fixed tissue sections and incubated for 30 minutes at room temperature. Following serial washings with PBS, the tissue was then overlaid with the FITC-labeled rabbit antibody directed against ferret IgG. After 30 minutes of incubation at room temperature, the tissue was again washed in PBS, rinsed in distilled water, dried, and mounted in elvanol.[8]

Sections were examined with a Standard Universal Zeiss microscope illuminated by an HBO 200 mercury lamp, with KP490 and BG12 excitation filters and an LP528 barrier filter. Photographs were taken using Kodak high-speed (ASA 125) Ektachrome film (Tungsten).

Results

Response to Infection

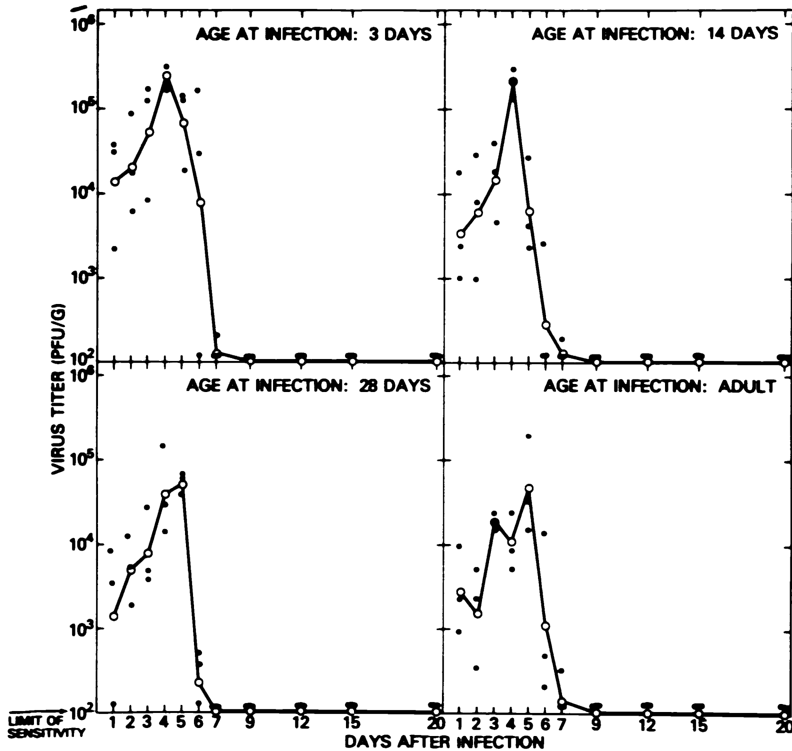
Infected animals were observed daily by the principle investigator. Nasal discharge was not observed. Throughout the course of the experi-

ment, infected animals did not differ from controls in terms of alertness, weight gain, or general physical activity. None of the animals died.

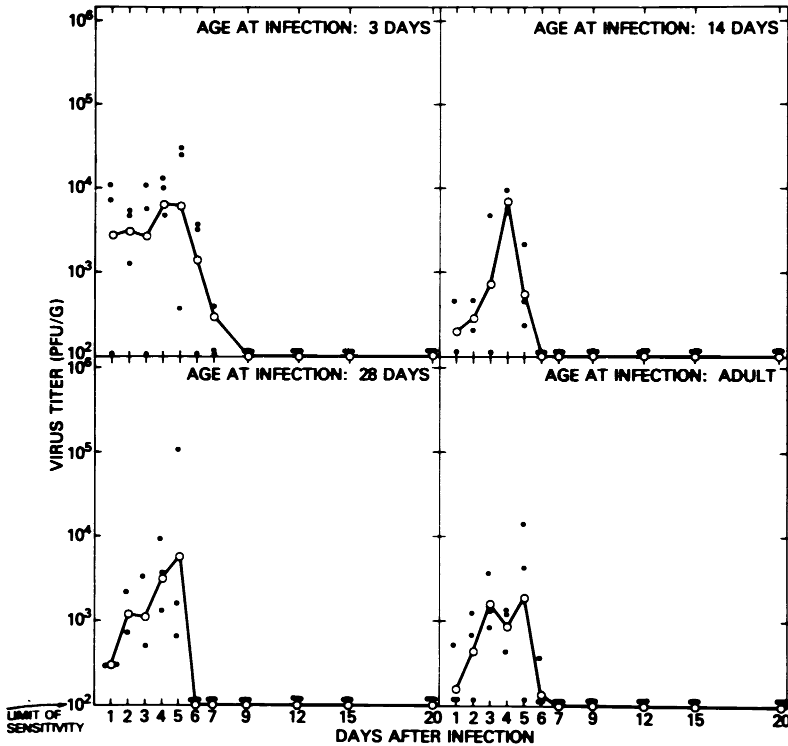
Pattern of Viral Replication

Replication of RS virus in infected cotton rats is shown in Text-figures 1, 2, and 3, which depict growth of virus in lungs, trachea, and nasal tissues, respectively.

The course of pulmonary infection appeared virtually identical in all of the age groups studied (Text-figure 1). Infectious virus was present by the day after infection and increased rapidly to a maximum of approximately 10^5 pfu/g by the fourth day after infection. The titer then fell sharply, reaching or approaching an undetectable level by the seventh day and



TEXT-FIGURE 1—Viral replication in lungs of cotton rats infected with RS virus. Tissues were homogenized in balanced salt solution, and cell-free supernatants were applied to HEp-2 cell monolayers. Cultures were incubated at 37 C in a 5% CO_2 atmosphere for 5 days, fixed in formalin, and stained with 0.1% aqueous crystal violet. Virus titers are expressed in terms of plaque-forming units per gram of fresh tissue. *Closed circles*, titers of individual animals; *open circles*, geometric mean of titers from 3 animals.

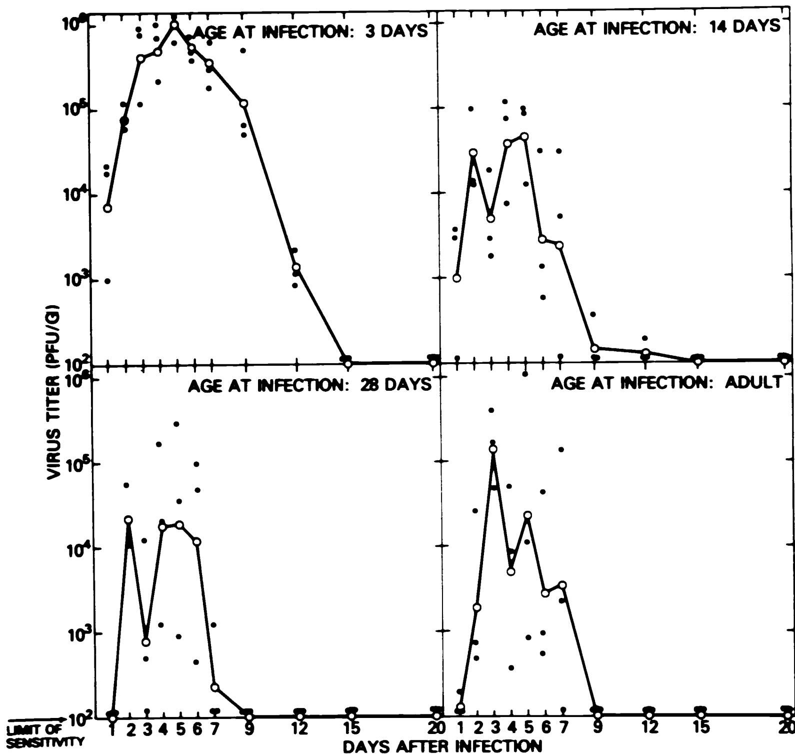


TEXT-FIGURE 2—Viral replication in trachea of cotton rats infected with RS virus. Tissues were treated as in Text-figure 1.

remaining undetectable for the remainder of the experiment (20 days after infection).

Tracheal infection, although much less extensive than pulmonary infection, also showed little variation among the age groups studied (Text-figure 2). Peak titers in all cases were approximately the same, approximately $10^{3.7}$ pfu/g, and were reached on Days 4 and 5 after infection. By the seventh day, nearly all infectious virus had been cleared, and none was detectable from the 9th to 20th day.

Infection was most prolonged in the nose in all age groups, with infectious virus detectable as long as 12 days after inoculation (Text-figure 3). The most extensive nasal infection was seen in animals infected at 3 days of age; these rats had highest peak titer (10^6 pfu/g) and longest duration of infection (12 days). The older age groups varied little from each other, each showing a slightly shorter and less extensive infection than the 3-day-old animals. A curious dip in virus titer was consistently

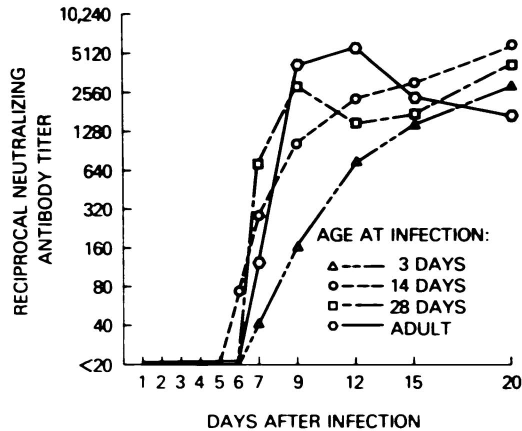


TEXT-FIGURE 3—Viral replication in nose of cotton rats infected with RS virus. Tissues were treated as in Text-figure 1.

seen on the third and fourth days after inoculation in the three older age groups. We have no explanation for this phenomenon.

Antibody Response

The serum neutralizing antibody response following infection was consistent and quite pronounced (Text-figure 4). In all age groups neutralizing antibody could not be detected for the first 5 days after infection. On the sixth day, 20% of the animals showed a measurable antibody response, while on the seventh day 90% had developed antibody, and 100% were positive on the ninth day. From the 6th to the 12th day the antibody response in the 3-day-old animals, which grew virus to the highest titer, lagged behind the other age groups. By Day 15, however, all four age groups had attained the same titer, which remained relatively constant for the remainder of the experiment (20 days after infection). Many animals showed an extremely high specific antibody response: 1 animal developed a 60% plaque-reduction titer of 1:20,560.



TEXT-FIGURE 4—Neutralizing serum antibody response of cotton rats infected with RS virus. Antibody titers were determined by a 60% plaque-reduction neutralization test. The value at each time interval represents the geometric mean titer of 3 animals.

Localization of Viral Antigen

Sections of nasal turbinate, trachea, and lung were examined, using indirect immunofluorescence staining, to localize viral antigen. Lung sections showed the same pattern in all animals, regardless of age at infection. Viral antigen was found in many of the bronchi and bronchioles and was restricted to the luminal epithelial cells in these areas (Figure 1). Exfoliated epithelial cells containing viral antigen were commonly seen (Figure 2), although no instance of complete bronchiolar obstruction was observed. Viral antigen was seen rarely in alveoli, and then only in single, isolated cells.

Viral antigen was not detected in sections of trachea. Sections of nasal turbinates showed intense staining of epithelial cells, with 100% of such cells in many areas containing viral antigen (Figures 3 and 4). Only the epithelial cells contained viral antigen.

Histology

Lungs

Histopathologic evidence of RS virus infection in the lungs was seen in the epithelium of the bronchi and bronchioles as early as 2 days and as late as 9 days after infection (Figure 5). Some bronchi and bronchioles were unaffected (Figure 5A), whereas others exhibited extensive changes. Even when the epithelium was extensively involved, normal ciliated epithelial cells were interspersed among degenerating and proliferating epithelial cells.

Epithelial cells altered by RS virus infection appeared to undergo one of two changes. The most prominent change was a ballooning of the cyto-

plasm, probably a form of hydropic degeneration. The other type of change was seen in small clusters of rounded cells with an eosinophilic condensed cytoplasm. The affected cells appeared severely degenerated or dead and were usually located at the surface adjacent to regenerating epithelial cells. The former cells were probably injured irreversibly by RS virus. There was mild exfoliation of cells (Figure 5C) but no evidence of giant cell formation or squamous metaplasia.

There were three general patterns of pathologic changes which appeared to depend on the stage of infection. The first changes seen on Days 2 and 3 following inoculation with virus included a progressive loss of cilia which paralleled an increase in ballooned or vacuolated epithelial cells. The normal basal layering of nuclei was lost and pseudostratification of the columnar epithelium became apparent. Degenerating eosinophilic epithelial cells, sometimes interspersed with neutrophils, became prominent as they were pushed peripherally toward the lumen by regenerating epithelial cells. The most extensive changes were noted between Days 4 and 6 following infection (Figures 5B through 5D). Regenerating epithelial cells intermixed with ballooning epithelial cells and clusters of degenerating and dying eosinophilic cells at the surface of the lumen gave the epithelium the appearance of stratification. In some terminal bronchioles the changes were so marked that the alveolar duct and connecting alveoli appeared collapsed. Although neutrophils were frequently seen in these areas, the pathologic process was consistent with that of reabsorption atelectasis. After Day 6, the cellularity of the epithelium decreased. The epithelium became pseudostratified, cilia began to reappear in large numbers, most of the degenerating eosinophilic cells were lysed or exfoliated, the ballooned epithelial cells decreased in number, and the alveoli of the lungs appeared normal. By Day 10 the lungs were essentially normal (Figure 5E).

Nose

The nasal mucosa consists of four types of epithelium: squamous, cuboid to columnar (Figure 6A), columnar (Figure 7A), and pseudostratified columnar with reserve cell hyperplasia (Figure 8A). Of the four types, only the squamous epithelium did not appear to be affected by RS virus infection. The cuboid and columnar epithelia showed mild to moderate histopathologic changes followed by rapid recovery (Figures 6B, 6C, and 7B through 7D), whereas the pseudostratified epithelium showed severe changes with very slow recovery (Figures 8B through 8E). The histopathologic changes were largely confined to the mucosa, with edema being the only abnormality in the submucosa. Although exudate con-

sisting of neutrophils and occasional desquamated epithelial cells was commonly seen, ulceration of the mucosa was infrequent. The anterior and middle portions of the nasal cavity appeared to be more extensively involved than the posterior portion, which showed only focal changes.

Histologic evidence of infection was noted in cuboid and columnar epithelia as early as 2 days after infection. There was beginning disruption of the normal tissue architecture, including vacuolar changes in some cells, eosinophilic condensation of the cytoplasm in others, and neutrophils interspersed throughout the epithelium. After 4 to 5 days, degenerating and rounded-up epithelial cells were seen at the luminal surface of the mucosa (Figures 6B and 7C). These damaged and presumably infected epithelial cells appeared to be forced into the nasal cavity by large numbers of regenerating epithelial cells. The regenerating cells often showed squamous metaplasia within the cuboid epithelium (Figure 6C) and a basal cell hyperplasia within the columnar epithelium (Figure 7C). By 8 days after infection the columnar epithelium essentially returned to normal (Figure 7D), whereas the metaplastic squamous epithelium still appeared to be in an earlier stage of repair (Figure 6C).

The earliest histologic evidence of infection in the pseudostratified columnar epithelium was segmental clefting which was apparent 3 to 4 days after inoculation (Figure 8B). The clefting effect was apparently due to lysis of cells throughout the full thickness of the epithelium, with segments of normal-appearing epithelium separating the clefts. However, after several days, much of the remaining epithelium appeared damaged (Figure 8C) and eventually collapsed to approximately one third of its normal thickness (Figure 8D). These changes persisted through Day 10 without histologic evidence of epithelial regeneration (Figure 8E).

Discussion

Respiratory syncytial virus infection of cotton rats may be characterized as an acute respiratory infection, involving both upper and lower portions of the respiratory tract. Virus replication, as monitored by immunofluorescence microscopy, is restricted to the epithelium. There is an excellent correlation between histologic findings, immunofluorescent observations, and viral replication in each of the three portions of the respiratory tract examined. Virus grows to high titer in nasal turbinates, infects the majority of the cells, and produces a moderately severe desquamative, exudative rhinitis. The trachea is relatively resistant to infection: virus grows to a low titer and does not induce any histologic changes. The lungs, like the nose, support viral replication to high titer. Infection, as monitored by immunofluorescence microscopy, is restricted to bron-

chial and bronchiolar epithelium and is associated with mild bronchitis and bronchiolitis.

An earlier report by Dreĭzin and co-workers [8] described the course of RS virus infection in 1.5- to 2-week-old cotton rats. Comparing our results in 14-day-old animals with theirs, we find several similarities and some significant differences. Both groups found viral replication in lungs, trachea, and nose. Peak viral titers and maximal histopathologic changes were seen at approximately the same time after infection, pulmonary infection was shown to involve the bronchi and bronchioles but not the alveoli, and virus was cleared from the respiratory tract at approximately the same interval after infection.

We differ, however, in some observations of the trachea and lungs. Dreĭzin detected more extensive tracheal involvement, with high viral titers, abundant viral antigen, and significant histopathologic change. In contrast, we found no histologic or immunofluorescent evidence of tracheal infection and viral titers 100- to 1000-fold lower, despite finding lung titers equivalent to theirs. Dreĭzin detected more severe pulmonary pathology, including syncytia in the bronchi and bronchioles, which we did not see. These variations may be attributable to differences in the animals used in the two laboratories. Although no information is given regarding the origin of their cotton rat colony, we presume it to be derived from animals trapped within the Soviet Union. Since our colony was derived from feral animals from the eastern United States, it is possible that the observed variations reflect phenotypic differences in the two colonies.

In addition to confirming Dreĭzin's basic observations, we have extended the scope of the cotton rat model. We have studied the virologic, histopathologic, and immunofluorescent components of nasal RS virus infection and have monitored the serologic response of the animals. The pathogenesis of RS virus infection was studied in three other age groups, ranging from early infants to adults, to determine if the cotton rat shows an age-dependent response to infection similar to that seen in humans [1] and ferrets.[2]

Experimental RS virus infection in cotton rats bears several similarities to natural RS virus infection in humans. First, rhinitis is a prominent pathologic response to infection in the cotton rat. Similarly, rhinitis is a common clinical response observed during human infection. The restriction of viral replication to epithelial cells also agrees with the very limited data available from immunofluorescence studies of specimens from infants with RS virus disease.[9] The time course of infection in humans and cotton rats is also similar.[10,11] Finally, the pulmonary component of RS

virus infection in cotton rats resembles that of the severely affected young infant with RS virus disease. It should be noted, however, that bronchiolitis in the latter instance is quite extensive, while in the cotton rat bronchiolar changes are considerably less severe.

Comparison of the cotton rat model with the infant ferret model[2] indicates some similarities and some important differences. Both animals undergo a mild, acute respiratory infection, with involvement of both the upper and lower portions of the respiratory tract. Pattern of viral replication in the nose is similar in both animals, as are histologic findings (although pathologic changes are more pronounced in the cotton rat).

Although the infant ferret and the cotton rat both support viral growth in the lungs, the pattern of infection differs markedly. Viral antigen in the ferret lung is almost exclusively restricted to the alveolar cells, with only rare, single-cell involvement of bronchiolar epithelium. The opposite is true in the cotton rat, in which viral antigen is seen only in bronchial and bronchiolar epithelium, with only rare, isolated involvement of alveolar cells. We have no explanation for this difference; however, it is of interest that each of these two species develops one form of pulmonary disease resembling that seen in RS-virus–infected infants, ie, bronchiolitis (cotton rat) and interstitial pneumonia (infant ferret).[1] It is possible that these two animals may allow the study of both forms of human lung disease.

Another striking difference between the ferret and cotton rat is the extent of the serum antibody response. Ferrets infected as early as the day of birth or as late as 28 days develop little or no neutralizing antibody.[2] In contrast, cotton rats infected shortly after birth develop neutralizing antibody, in some instances to extremely high titer, by the ninth day after infection. Antibody response is not influenced by age at the time of infection. When ferrets and cotton rats are infected at 3 days of age, both support RS virus replication in the lungs and nose to the same level and for the same interval. However, the antibody response of the rat is 100-fold or more greater than that of the ferret.

The most significant difference between the two experimental models of infection involves the effect of age on susceptibility of the lungs to virus and the level of viral replication in this site. Whereas 28-day-old ferrets are completely refractory to pulmonary RS virus infection,[2] cotton rats are uniformly susceptible to infection of the lungs throughout life. The mechanism of this age-dependence effect in ferret lung is unknown. Continuous susceptibility of the cotton rats lungs offers several possibilities to explore aspects of RS virus pathogenesis previously not amenable to experimental study.

First, the continuous susceptibility of the lungs to RS virus infection

makes it possible to study the effect of vaccination on resistance to infection as well as potentiation of disease. Use of this animal model might elucidate the mechanism by which formalin-inactivated, alum-precipitated RS virus vaccine potentiated rather than protected against disease in young children.[12-14]

Second, this model should yield answers about the duration of immunity following infection. Studies in humans have made it clear that immunity to RS infection is transient and incomplete,[1] but the reasons are not well defined.

Other characteristics of the cotton rat also serve to enhance its desirability as an experimental model. In contrast to ferrets, which require careful attention to housing and breeding conditions,[2] cotton rats do not need special caging, do very well on standard rat chow without supplements, require no special lighting cycles, and breed spontaneously throughout the year. This last characteristic offers the possibility of a detailed study of maternal-infant transfer of resistance and the effects of these immunologic factors on RS virus infection.

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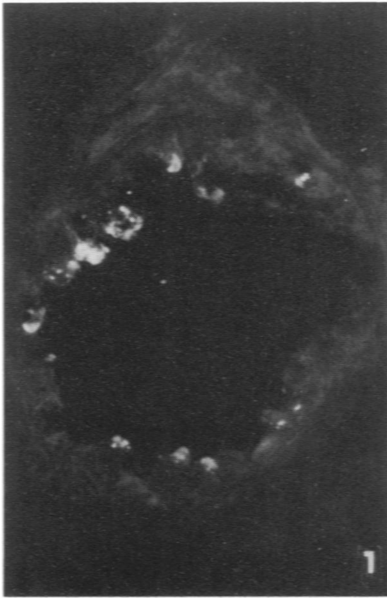
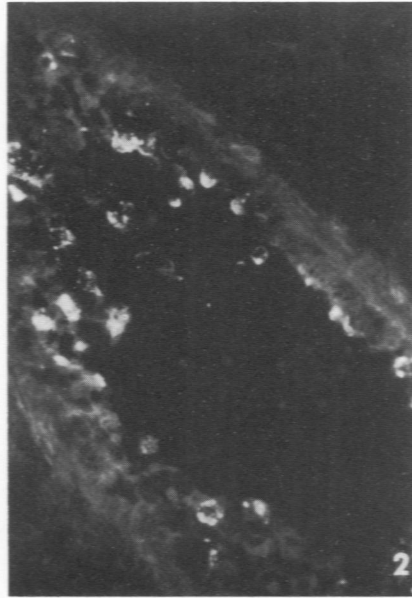
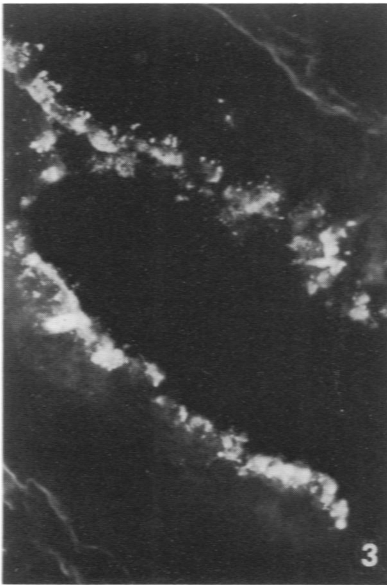
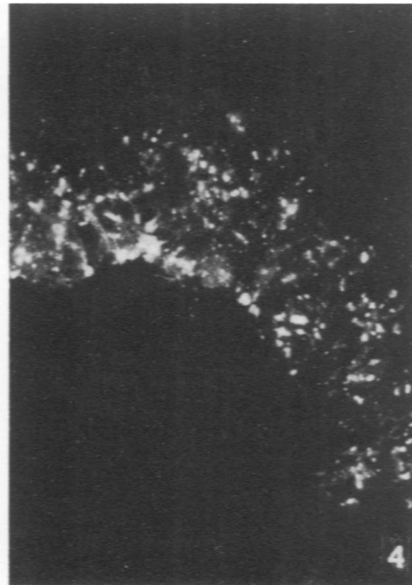
[*Illustrations follow*]









Figures 1 and 2—Immunofluorescence photomicrographs of RS-virus-infected cotton rat lungs (4 days after infection). Cryostat sections were cut at 10 μ and were stained using indirect immunofluorescence technique. Figure 1 shows several bronchiolar epithelial cells containing viral antigen. In Figure 2 more cells are infected, and several containing viral antigen have been exfoliated into the lumen of the bronchiole. (× 380) **Figures 3 and 4**—Immunofluorescence photomicrographs of RS-virus-infected cotton rat nose (4 days after infection). Cryostat sections were cut at 10 μ and were stained using indirect immunofluorescence technique. Figure 3 shows columnar epithelium. Virtually all of the surface epithelial cells contain viral antigen. Figure 4 depicts an area of pseudostratified columnar epithelium. Viral antigen is seen in most cells throughout the epithelium, both surface and subsurface. However, as in Figure 3, viral antigen is seen only in epithelial cells, with other cell types showing no evidence of infection. (×380)

Figure 5—Histopathologic changes occurring in cotton rat lung during RS virus infection. A—Portion of normal bronchus. Epithelial cells are uniform in appearance, with layering of nuclei in basal portion of cells. B—Portion of bronchus 5 days after infection. Epithelial cells show proliferative changes, hydropic degeneration, and direct cytopathic effect of RS virus infection (*arrows*). The surrounding alveoli appear normal. C—Bronchiolar epithelial cells, 5 days after infection, show segmental proliferation and desquamation of infected cells into the lumen. D—Portion of bronchus, 6 days after infection, showing proliferation and/or degeneration of epithelial cells, stratification of cell nuclei, and normal cells interspersed throughout the epithelium. E—Portion of bronchus 9 days after inoculation. Epithelium has returned toward normal with layering of nuclei, but some vacuolization is apparent between nuclei and basement membrane. (H&E, ×380)

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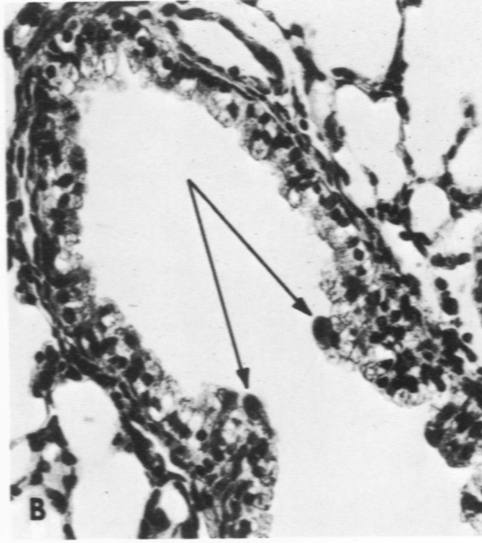
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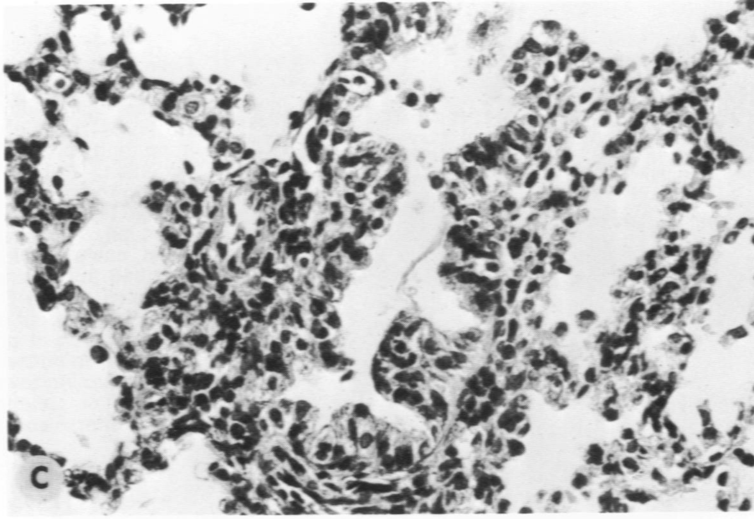
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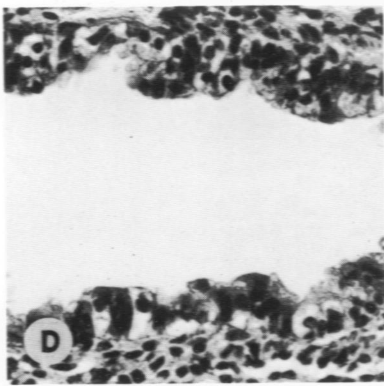
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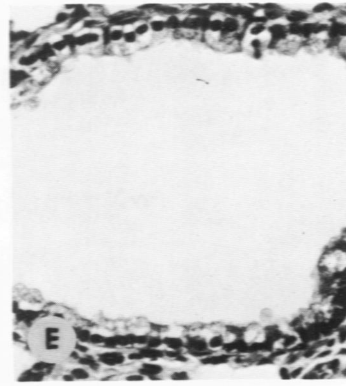
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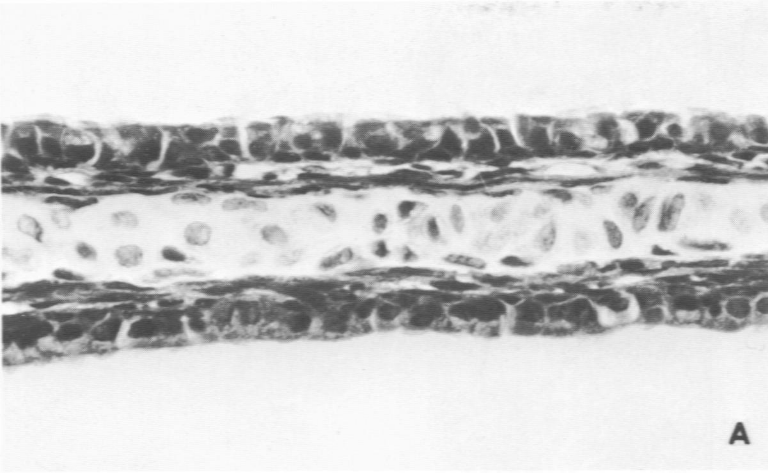
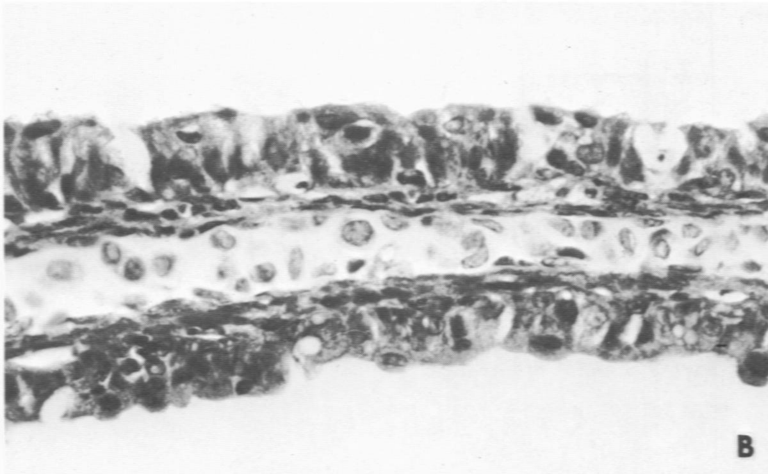




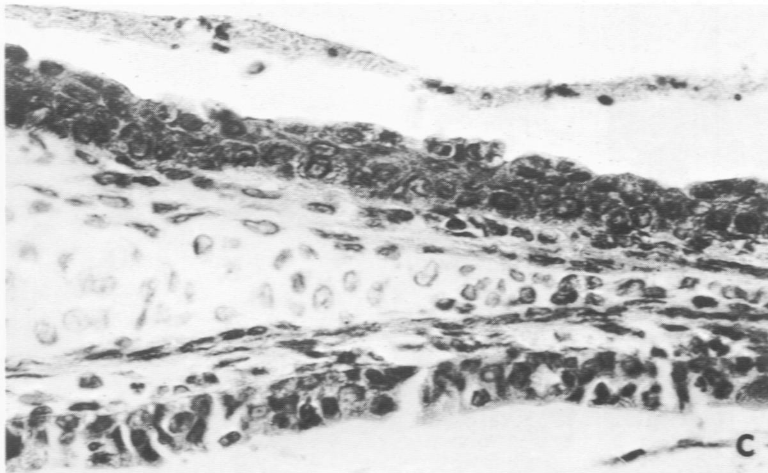


Figure 6—Histopathologic changes occurring in cuboidal-columnar respiratory epithelium of nose during RS virus infection. **A**—Normal appearing epithelium 1 day after inoculation. **B**—Basal cells appear to force rounded-up, presumably infected, epithelial cells to eventually desquamate into the nasal cavity, giving epithelium a stratified appearance (4 days after infection). **C**—Squamous metaplasia is apparent in epithelial cells in *top portion* of photomicrograph, while cells in *bottom portion* appear to be returning toward their normal cuboidal or columnar appearance (8 days after infection). (H&E, ×680)

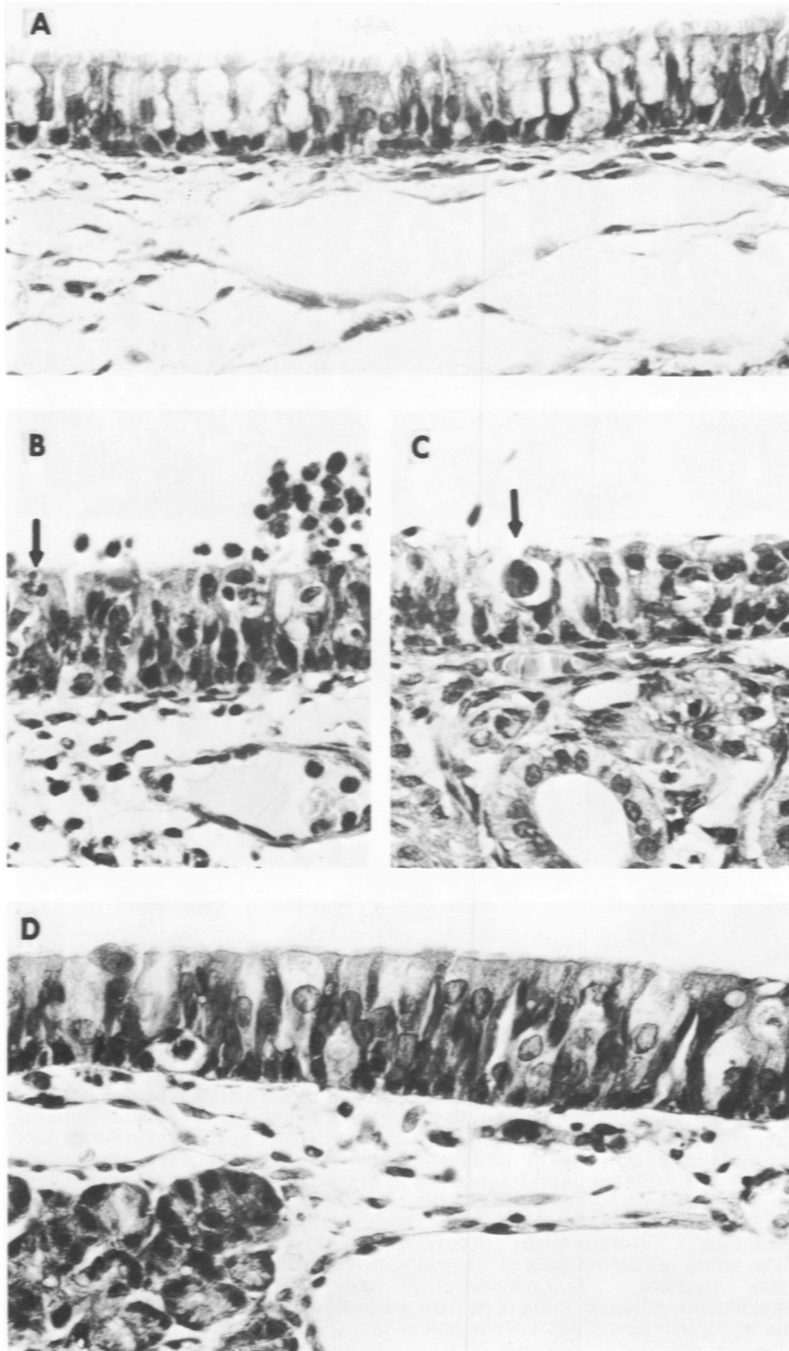
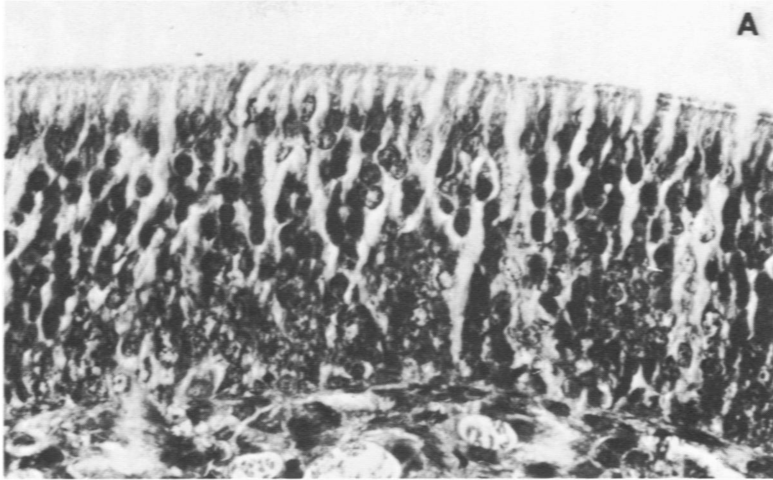


Figure 7—Histopathologic changes occurring in columnar respiratory epithelium of nose during RS virus infection. **A**—Normal columnar epithelium has prominent cilia. **B**—Neutrophils are observed either migrating (*arrow*) through epithelial cells showing cytopathic effect of RS virus infection or as part of an acute inflammatory exudate (5 days after infection). **C**—Basal cell hyperplasia with desquamation of involved epithelial cells is the most common host reparative response to injury by RS virus (5 days after infection). Large multinucleated cells (*arrow*), which might be syncytial giant cells, were only rarely seen. **D**—Elongated, regenerating epithelial cells were seen as early as 8 days after inoculation. (H&E, ×680)



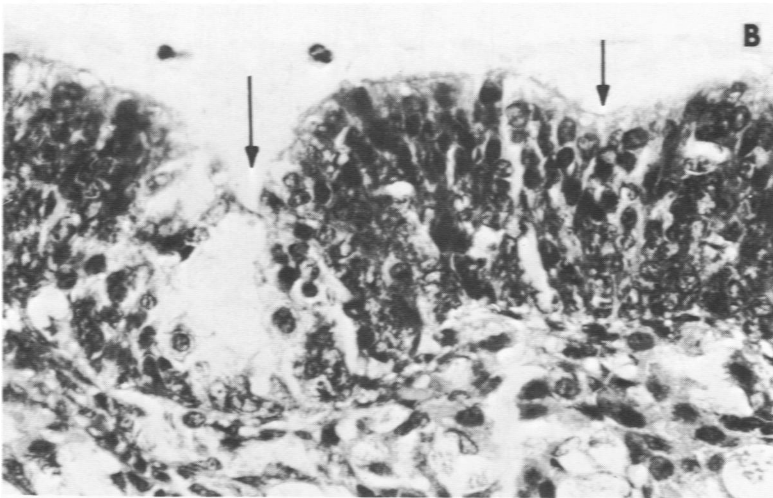
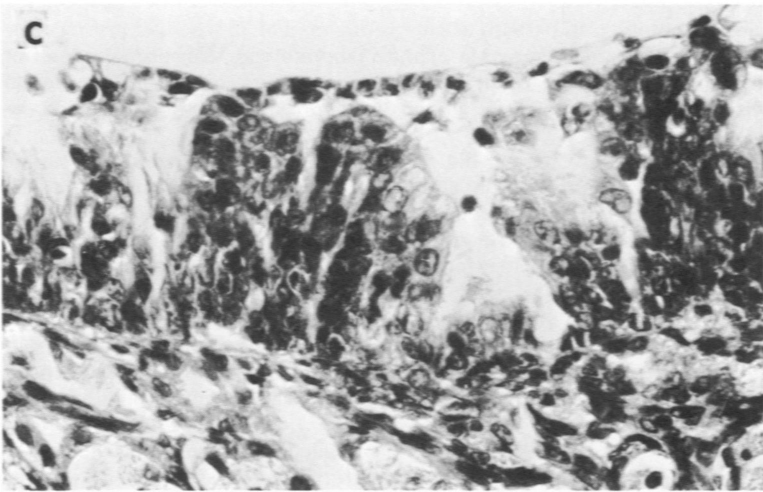


Figure 8—Histopathologic changes occurring in pseudostratified columnar respiratory epithelium of nose during RS virus infection. **A**—Normal-appearing epithelium. **B**—Segmental clefting (*arrows*) of epithelium is secondary to lysis and collapse of underlying epithelial cells (4 days after infection). **C**—Clefting and fragmentation of epithelium (5 days after infection) more extensively involved by RS virus infection than in Figure B. Desquamated epithelial cells intermixed with acute inflammatory cells are conspicuous. **D**—Complete collapse of pseudostratified epithelial cell layer with some remaining cells still showing evidence of cytopathic effect (8 days after infection). **E**—Collapse of cell layer still persists at 10 days after inoculation. Although there is no obvious cytopathic effect of RS virus, there is no apparent regeneration of the epithelium. (H&E, ×680)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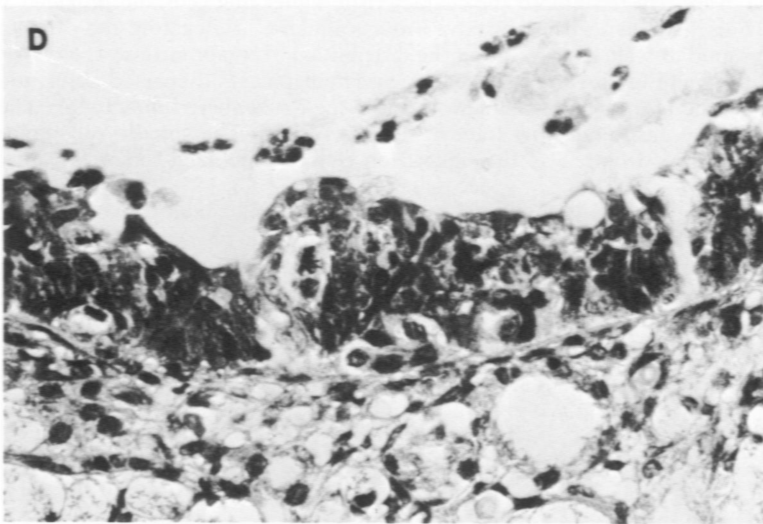
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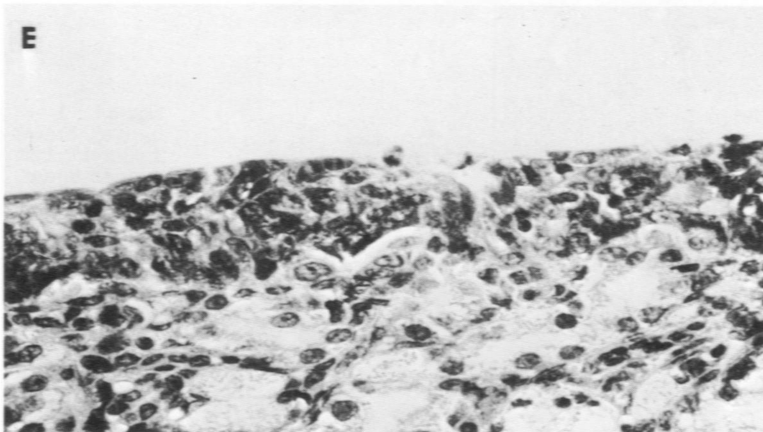
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