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Rhinovirus Growth in Nasal Polyp Organ Culture (39854)[1]

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Little information on the pathophysiology of rhinovirus infection of the respiratory tract is available. The mechanisms by which the symptoms associated with rhinoviral colds are produced and the events which initiate recovery from colds are not known. Since human rhinoviruses do not grow in subprimates, a practical animal model for studying rhinoviral disease is unavailable. Organ culture, an alternative system, has been tried using human fetal tissue (1-4) but this material is in short supply. The behavior of bovine rhinoviruses has been studied in bovine tracheal organ cultures by Reed and Boyde (5).

This report describes the development of an organ culture system for growing rhinoviruses in human nasal polyps which are readily available from elective polypectomies. The technique provides a means for studying rhinovirus pathogenesis in human tissue.

Materials and methods. Cultures of nasal polyp fragments. Nasal polyps from patients undergoing elective polypectomy were placed in Hanks' balanced salt solution (HBSS) containing penicillin (100 units/ml), streptomycin (100 μg/ml), and mycostatin (50 μg/ml) for transport to the laboratory. Within 24 hr of removal, polyps were rinsed with two changes of HBSS, and the mucosa was cut into small pieces approximately 2 mm on a side. Three to five fragments were placed in a screw-capped tube containing 1.0 ml of Leibowitz (L-15) medium [Grand Island Biological Company, Grand Island, New York] with 10% fetal calf serum, 100 units of penicillin, 100 μg of streptomycin, and 50 μg of mycostatin/ml. Tubes were incubated at 34° in a roller drum turning at 10 revolutions/hr. Medium was changed every 3 days until the fragments were infected or used in an experiment as a control.

Fragments which were floating in the medium were examined microscopically at 48 hr for evidence of ciliary motion on the surface. Tubes with at least one polyp fragment having ciliary activity were selected for use.

Virus. Laboratory strains of rhinovirus types 1A, 3, 6, 14, 38, 39, 43, and a recent isolate of rhinovirus type 10 were used. A strain of Coxsackie A21 (Coe) was also used in two experiments. Infectivity titrations were done by a standard method in WI-38 tubes.

Histologic examination. Polyp fragments were fixed in formalin and sectioned. Serial sections were stained with hematoxylin and eosin and examined for the amount of surface covered by epithelium and cellular destruction. Infected and uninfected specimens were coded and read blindly.

Fluorescent antibody staining. Polyp fragments were rapidly frozen in isopentane and stored at −70°; 2- to 4-μm sections were cut with a cryostat. Type-specific antisera to rhinovirus types [obtained from the Reference Reagents Branch of the National Institute of Allergy and Infectious Diseases] were utilized for indirect fluorescent antibody (FA) staining of polyp sections. Antisera were absorbed six times with acetone-extracted human liver powder and once with WI-38 cells to reduce background staining (6). Goat antirabbit IgG and rabbit anti-guinea pig IgG conjugated with fluorescein isothiocyanate were obtained commercially [Behring Diagnostics, Incorporated, Somerville, New Jersey].

Polyp sections were fixed in ethanol at 4° for 5 min and overlaid with type-specific rhinovirus antiserum. After rinsing with phosphate-buffered saline (PBS), sections were stained for 30 min with the appropri-

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ate fluorescein conjugate in PBS containing 1 mg/100 ml of fetal bovine serum. Four to six drops of 1% Evans blue were added to 200 ml of PBS in the final rinse as a counter stain.

The specificity of the FA technique for staining rhinoviruses was first established with WI-38 cell monolayers in Leighton tube cultures infected with rhinovirus. Monolayers showing early cytopathic effect (i.e., rounded cells) were found to have specific staining of infected cells. This specific staining was characterized by the presence of fluorescent granules limited to the cytoplasm in infected cells (7). The specificity of the staining pattern was confirmed by the absence of staining of uninfected cells by preimmune serum or of infected cells by antiserum against a different rhinovirus type.

Experimental design. Six to 24 tubes containing fragments from a single polyp were each inoculated with 0.1 ml of a dilution of virus which contained 10^2–10^4 $TCID_{50}$/ml. An equal number of uninfected tubes from the same polyp served as controls. After mixing, 0.1 ml of medium was removed from each tube, pooled with medium from similar tubes, and titered. The cultures were incubated on a roller drum at 34°. After 3 hr, fragments were washed four times with 1 ml of HBSS. The fourth wash was pooled and titered. The polyp tubes were refed and returned to the roller drum.

At 12, 24, 36, and 48 hr after inoculation of the virus, 0.1 ml of medium from each infected tube was removed, pooled, and titered. Fragments from two to three infected tubes and an equal number of control tubes were removed at these times and processed for histologic examination and fluorescent staining.

Results. Viral replication in polyp cultures. Cultures of fragments from 19 different nasal polyps were inoculated with one or more rhinoviruses (Table I). Two of the cultures were also inoculated with Coxsackie A21 virus. Seventeen of the 19 polyps were found to support replication of at least one rhinovirus type, as evidenced by an increase in the titer of the virus in the medium containing the fragments to 10^2 $TCID_{50}$/ml or greater. No virus was detected in medium removed from the control (uninoculated) tubes.

TABLE I. RHINOVIRUS REPLICATION IN NASAL POLYP ORGAN CULTURE AT 24 OR 48 HOURS AFTER INOCULATION

Polyp number	Rhinovirus type	Replication (peak titer $TCID_{50}$/ml)
279	6	10^2
283	24	10^2
285	24	$>10^2$
	10	$>10^2$
287	10	$>10^3$
289	10	$10^{3.5}$
20	10	$10^{3.25}$
	1A	$10^{3.25}$
23	10	$10^{2.5}$
	Coxsackie A21	$10^{3.0}$
25	10	$10^{2.5}$
	Coxsackie A21	$\geq 10^4$
	38	$\geq 10^4$
	43	$\geq 10^4$
29	14	NG[a]
30	14	NG
31	14	NG
	10	$10^{3.25}$
32	10	$10^{3.5}$
33	10	$10^{4.0}$
	3	NG
34	10	$10^{4.0}$
	3	NG
35	3	NG
	39	$\geq 10^2$
	10	NG
36	3	NG
	39	NG
	10	$\geq 10^2$
37	3	NG
	39	$\geq 10^2$
	10	$\geq 10^2$
38	3	NG
	39	$\geq 10^2$
	10	$\geq 10^2$
39	3	NG
	39	NG
	10	$\geq 10^2$

[a] No detectable growth.

Seven of the nine different rhinovirus types inoculated into polyp cultures grew in at least one polyp; type 14 did not grow in any of three polyps, and type 3 did not grow in any of seven polyps. The two polyps which did not support detectable rhinovirus growth received type 14 only, suggesting that the lack of growth of a rhinovirus in these two polyps was related to the virus rather than the polyps. Type 10 grew in 14 out of 15 trials, type 39 in 3 out of 5, type 24 and Coxsackie A21 in 2 out of 2. Types

1A, 6, 38, and 43 all grew in one polyp.

The kinetics of growth of rhinovirus type 10 in seven different polyp cultures are shown in Fig. 1A. Immediately following inoculation the medium contained $10^{1.5}$–10^4 $TCID_{50}$/ml. After the fragments were washed at 3 hr, the amount of virus present was reduced to undetectable levels. Rhinovirus was regularly recovered from the culture medium at 24 hr after inoculation (21 hr after the wash) and often by 12 hr after inoculation. Viral titers persisted at relatively constant levels (10^2–10^4 $TCID_{50}$/ml) for 36 hr. Experiments with rhinovirus types 38, 39, and 43 demonstrated similar growth curves.

Rhinovirus type 10 incubated under similar conditions in tubes with no polyp fragments showed a decline in infectivity titer to $<10^1$ $TCID_{50}$/ml at 72 hr (Fig. 1B). Similar findings were seen with rhinovirus type 24.

Histology. Histologic sections of uninfected polyp fragments stained with hematoxylin and eosin maintained in culture for 5 to 14 days showed an epithelial border which varied in amount from fragment to fragment. More than 50% of the surface of most fragments was covered with epithelium (Fig. 2). Ciliated columnar epithelial cells were present in many areas (Fig. 3), but nonciliated columnar and cuboidal cells predominated. The interior of the polyp appeared to contain fibrous tissue and some mesenchymal cells.

Stained sections of infected and uninfected polyps were indistinguishable when examined under the light microscope. No areas of epithelial erosion or cell damage, which might represent viral foci, were seen.

Fluorescent antibody staining. Multiple frozen sections of rhinovirus-infected and control polyp fragments were examined at 12, 24, 36, and 48 hr after infection for specific FA staining. Intense autofluorescence was present in the interior of all polyp fragments which prevented adequate examination of that portion of the specimen for specific fluorescence. The epithelial border could be examined; no areas with epithelial cells showing specific staining were seen in any of the infected fragments.

Discussion. Seven of the nine rhinovirus

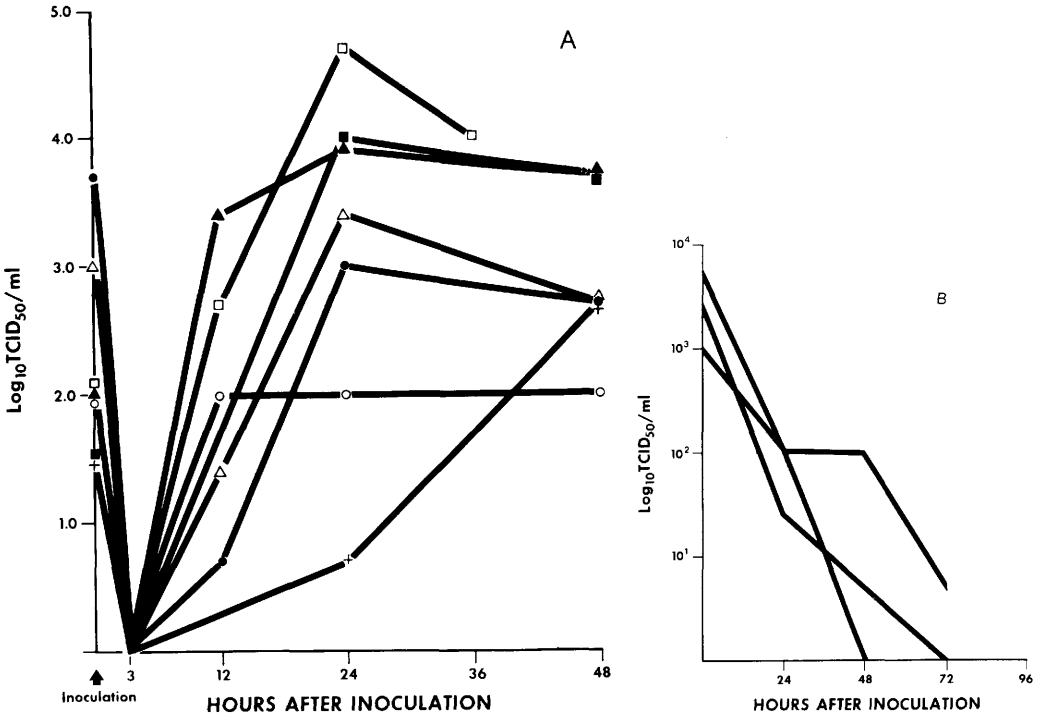

FIG. 1. A. Replication of rhinovirus type 10 in nasal polyp organ culture. Results with seven different polyps. B. Loss of infectivity of rhinovirus type 10 control tubes with no polyp fragments. Results of three experiments.

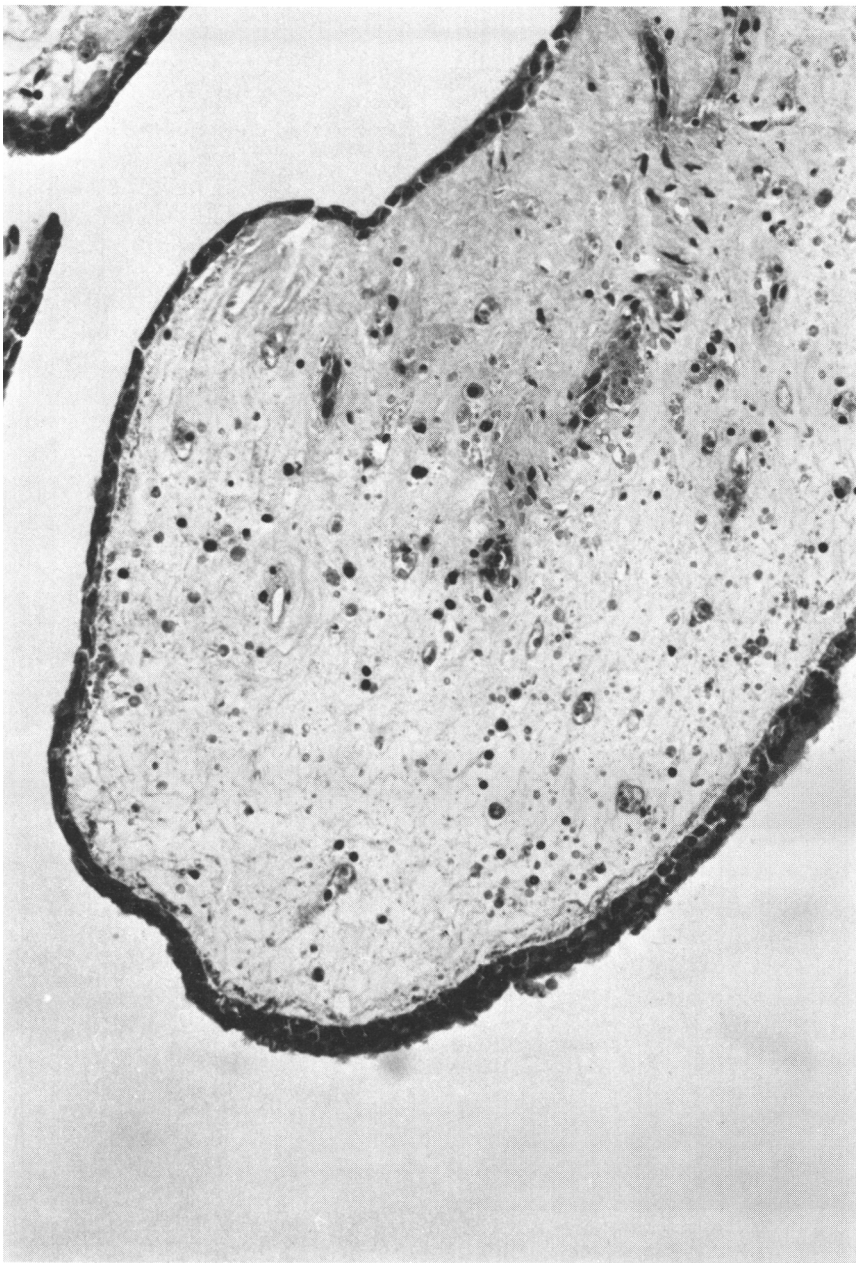

FIG. 2. Hematoxylin and eosin-stained section of uninfected nasal polyp fragment after 5 days in culture. The border shows areas of ciliated and nonciliated epithelium (× 100).

types tested grew in nasal polyp culture, but not all virus strains grew in each polyp. Whether the failure of a particular rhinovirus type to replicate in a particular polyp was due to lack of a suitable receptor site for the virus or another mechanism such as prior immunity of the donor of the polyp is unknown. In relation to the latter possibility, Hoorn noted that cells from an animal which was immune to a particular virus were susceptible to that virus in organ culture (8).

In this model, gross appearance of ciliary activity was not altered in infected fragments nor was any obvious histologic

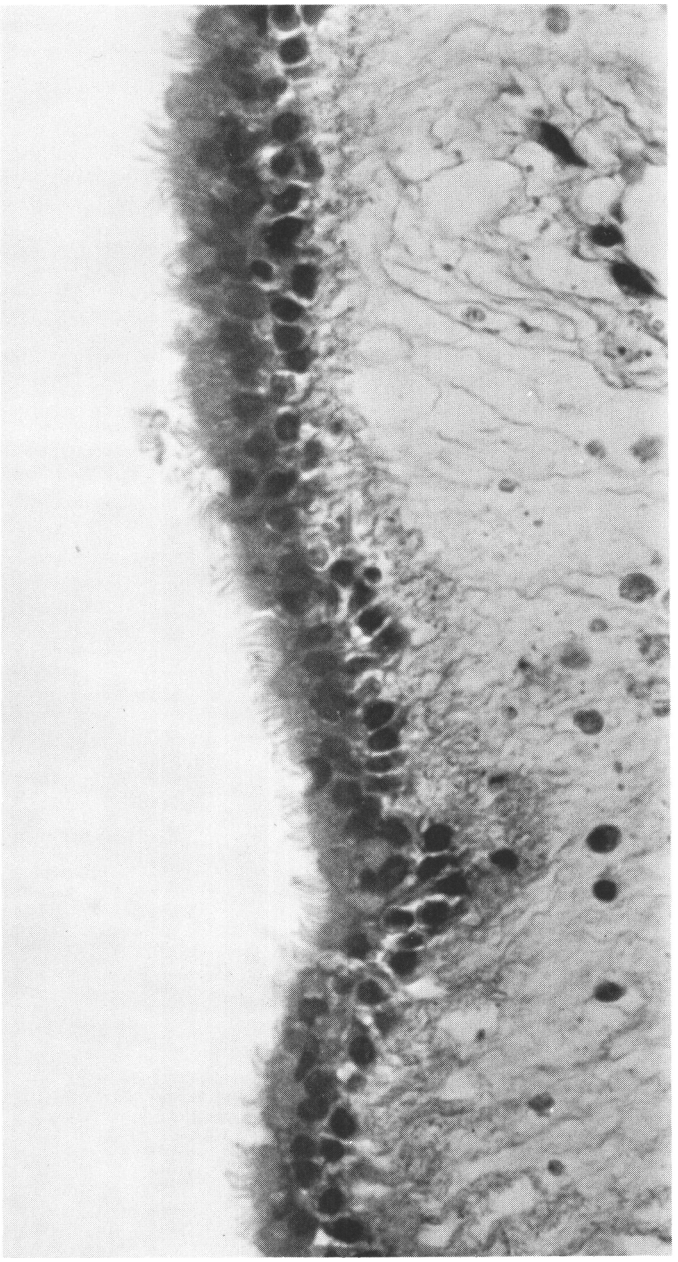

FIG. 3. Ciliated epithelial border of uninfected nasal polyp fragment in culture (× 400).

change seen in infected fragments compared to controls. These findings are in keeping with observations in individuals with experimental rhinovirus colds. Douglas and coworkers examined scrapings of nasal mucosa from volunteers with rhinovirus colds (9). Mucosal specimens from control, noninfected subjects were not markedly different histologically from those who were infected, although rhinovirus was isolated from biopsies from infected subjects. These observations suggest that direct cellular destruction may not be the major factor associated with symptomatic illness due to rhinovirus infection.

Rhinovirus antigen was not found in in-

fected polyp fragments with an indirect FA technique. Possible explanations for this include the presence of only a small number of infected cells which were missed when the serial sections were cut, viral replication in the interior of the polyp, or sloughing of infected epithelial cells with rapid replacement by new growth.

This technique of nasal polyp organ culture for rhinovirus growth shows promise as a tool for studying rhinovirus pathogenesis in human respiratory epithelium. Modification of the cultural conditions or increasing amount of virus used as the inoculum may enable demonstration of the site of viral growth.

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