Human Nasal Cells in Continuous Culture.* II. Virus Susceptibilities. (22639)

WILLIAM S. JORDAN, JR.†

Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University, and the University Hospitals, Cleveland, Ohio.

The cultivation of 2 strains of epithelial-like cells derived from normal nasal mucosa has been described(1). These cells, the DMB and DHov strains, can be distinguished morphologically, but share in common the capacity for rapid growth on glass and the presence of cytologic features characteristic of malignant cells(1). The present communication reports the susceptibility of these cells to a selected group of viruses.

Methods. The medium used for propagation of the cells consisted of 40% pooled human serum and 60% Hanks' balanced salt solution, the same feeding fluid used for the HeLa cells(2) with which the DMB and DHov strains were compared. Tubes for virus titrations were used 24 hours after inoculation of approximately 60,000 cells in 0.6 ml of media. Bottles for virus growth were used 4 to 5 days after the introduction of 10 ml of fluid containing 50,000 cells per ml. At the time of virus inoculation, these bottles contained approximately 2.5×10^6 cells. The maintenance fluid(3) contained 7.5% chicken serum, 17.5% tryptose phosphate broth and 75% Scherer's maintenance solution(4).

Viruses. The viruses employed were: 1) Adenoviruses types 1 through 7; type 4 was obtained from Dr. M. R. Hilleman, type 7 from Dr. T. O. Berge, and all other types from Dr. R. J. Huebner. 2) Poliomyelitis types 1, 2, and 3. 3) Coxsackie, Group B, type 3, originally isolated in HeLa cells(5). These agents had all been passed many times in HeLa cells. 4) Herpes simplex. 5) ECHO types 7 and 8, and 6) "chimp rhinitis", obtained from Dr. A. B. Sabin. 7) "CA" virus, Greer strain, obtained from Dr. R. M. Chanock(6). The ECHO and "CA" agents, originally isolated in monkey kidney cells, were passed two or three times in HeLa cells to provide the inocula used. 8) Measles (Enders' Edmonston strain) obtained from Dr. F. C. Robbins after passage in amnion cells, and passed once in HeLa cells. 9) Influenza A viruses (FW 1-53 and A/Clev/2/56) and influenza B viruses (Lee and GL/1/54). 10) Mumps virus (Habel strain). 11) Newcastle Disease Virus (4F and Hickham strains). These were all egg passage strains, and allantoic fluids were used as inocula for 18 × 150 mm roller tubes.

TABLE I. Comparative Virus Titrations* in HeLa Cells and 2 Lines of Nasal "Epithelial" Cells.

Virus		Cell line HeLa	DMB	DHov
Adenovirus	Type 1	5.25	4.25	4.75
	2	4.75	4.0	4.0
	3	4.0	3.75	3.25
	4	3.75	3.5	3.0
	5	6.5	4.75	5.0
	6	6.25	4.5	4.25
	7	3.75	3.75	3.75
Poliomyelitis	Type 1	7.0	7.0	7.0
	2	6.3	6.0	5.7
	3	5.7	6.0	6.0
Coxsackie	Group B, 3	4.0	4.5	5.0
ECHO	Type 7	5.0	4.5	5.5
	8	4.5	4.5	4.5
Herpes simplex		3.25	3.75	2.25
"CA" Greer		4.25	4.0	3.75
Chimp "rhinitis"		3.75	3.75	3.75
Measles		3.0	2.5	2.75

* $TCID_{50}$/ml (log).

Titrations. Dilutions (1 : 3.2 or $10^{-0.5}$) of inocula were made in Hanks' balanced salt solution, and 0.1 ml of each dilution was added simultaneously to 0.9 ml of maintenance fluid in 2 tubes of HeLa, DMB, and

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† Markle Scholar in Medical Science.

TABLE II. Growth of Viruses in 2 Strains of Nasal "Epithelial" Cells.

Virus	Cell line—TCD_{50}/ml (log) DMB Inoculum*	DMB Harvest	DHov Inoculum*	DHov Harvest
Adenovirus Type 2	1.5	3.75	1.5	3.5
4	1.7	3.5	1.7	3.0
7	1.0	2.75	1.0	2.75
Poliomyelitis Type 1	4.0	6.5	4.0	7.25
Coxsackie Group B, 3	3.3	5.25	3.3	5.0
ECHO Type 7	3.0	4.75	2.25	3.75
Herpes simplex	1.25	3.25	1.25	4.0
Chimp "rhinitis"	1.0	1.5	1.0	3.5

* Final concentration estimated from dilution in bottle.

DHov cells to give the comparisons shown in Table I. The tubes were inspected daily for the occurrence of degeneration, and alkalinized with $NaHCO_3$ if necessary. For the ECHO, "CA", and "chimp rhinitis" viruses end points were determined after 9 days; for the other viruses, after 6 days. With the exception of measles virus, end points were measured by the involvement of 50% or more of the cells. In the higher dilutions, measles virus produced plaques, but general involvement of the sheet of cells did not occur. Titer of measles virus is based on the highest dilution producing plaques.

Growth of virus. Multiplication of certain of the agents which produced definite cytopathogenic effects was assayed by harvesting the bottles after the appearance of maximum cytopathogenic effects. The cells were disrupted in the maintenance fluid by freezing and thawing 6 times. The supernatant was then titered in HeLa cells, and often in DMB and DHov cells as well. Each inoculum had previously been titrated in HeLa cells, and the amount of virus initially present in the bottles was calculated from the dilution of the inoculum (Table II).

Results. Fibroblasts predominated in the initial outgrowths from the explants that were the origin of both strains of nasal cells(1). With the first subcultures of these outgrowths, small tubes were made and the cells which grew, still seemingly all fibroblasts, were infected with type 4 adenovirus. Virus titering $10^{3.5}$ per ml was added in 0.1 ml amounts to 0.9 ml of maintenance fluid in DHov tubes. The maintenance fluid was completely replaced on the first and second days. Round cells slowly developed, and this time acidity was corrected by $NaHCO_3$. The titer of the material harvested on the seventh day was $10^{2.5}$ TCD_{50} per ml, indicating that at least all of the virus added to the tubes had been recovered. This harvest was then added in 0.1 ml amounts to tubes containing DMB fibroblasts. This amount of virus produces degeneration of HeLa cells (and of DMB and DHov epithelial-like cells) in 24 hours. The development of cytopathogenic changes in the fibroblasts was slow (Fig. 1), reaching its maximum in 6 days. The maintenance fluid was replaced once during this time. The titer of the harvest was $10^{3.5}$ TCD_{50} per ml, indicating a 10 to 100 fold increase in amount of virus. Thus, it was possible to demonstrate growth of an adenovirus in fibroblasts before the epithelial-like cells derived from the same explants of nasal mucosa had been established. Although its evolution was much slower, the cellular rounding and clumping that occurred (Fig. 1, D) was very similar to that noted with HeLa cells and the DMB and DHov epithelial-like strains. The fibroblasts could not be maintained in continuous culture, and further virus studies with these cells were not possible. All subsequent results were obtained with the epithelial-like cells.

Table I lists those agents which induced specific degeneration of DMB and DHov cells, and compares the titers obtained with these 2 strains with those measured with HeLa cells. These comparisons indicate that the sensitivities of these cells of nasal origin are virtually the same as those of HeLa cells. The DHov cells are least satisfactory because many round cells appear in the several maintenance fluids tried. There is no evidence as yet that this change is due to the presence of a latent virus in the DHov strain. Representative types of the various agents were used to determine whether cellular degeneration was accompanied by viral multiplication (Table II). As suggested by the comparative titrations, the yield of virus was similar to that obtained with HeLa cells. The change induced in DMB

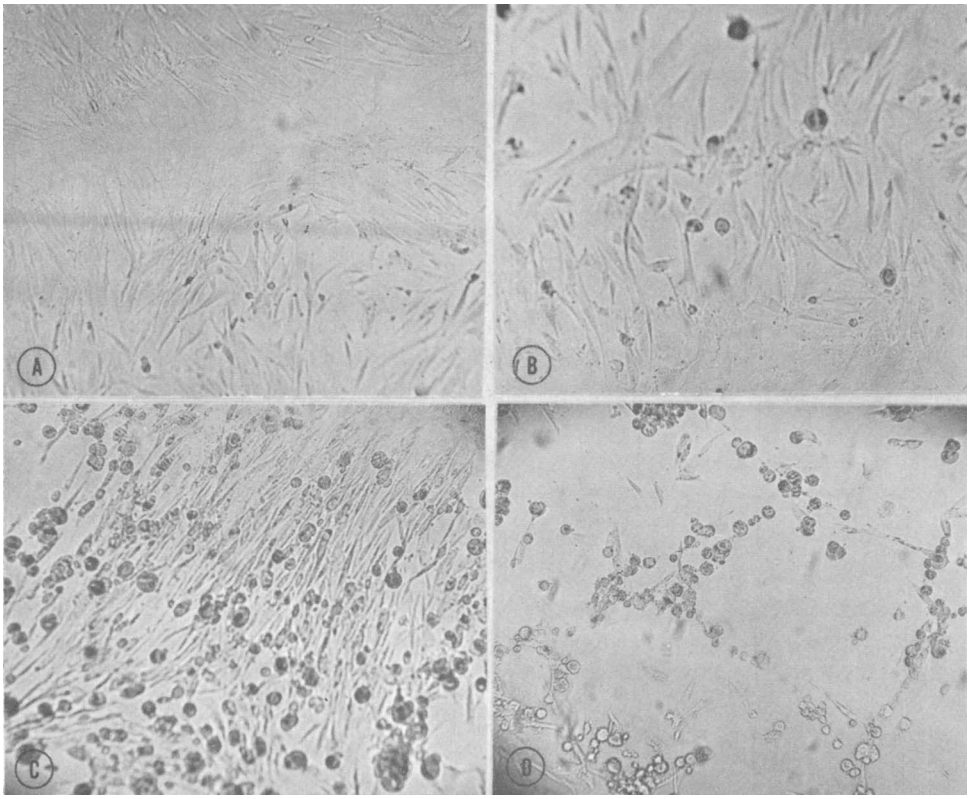

FIG. 1. Growth of type 4 adenovirus in early cultures of DMB cells when fibroblast was predominant cell type. A. Uninfected culture. B. 48 hr after addition of about 300 TCD_{50} of virus. C. 96 hr after infection. D. 144 hr after infection.

and Dhov cells by adenoviruses, polioviruses, Coxsackie, and herpes viruses were similar to those seen with HeLa cells(7). This was also true of the other agents, but the changes have not been so generally described. The ECHO viruses tested produced small round cells, most of which eventually fell off the glass. A similar effect was induced by the "chimp rhinitis" agent. The evolution of the change was slow, maximum titers being obtained in 8 to 9 days. The rounded cells tended to be "ragged" and were not so spherical or so dense as poliovirus infected cells.

"CA" virus also produced its maximum effect in 9 days, and the unique changes that developed beginning on the fourth day might easily have been mistaken for poor cell maintenance. There was a progression of changes which resembled the accumulation of debris plus the production of round cells. Certain areas of degeneration resembled the "spongy" effect noted on the kidney cells by Chanock (6). In other areas fragmented sheets of cells were plastered with debris which seemed to cling like iron filings on a magnet.

Measles virus produced syncytial giant cells, but the cytoplasmic vacuoles were less obvious than described for monkey kidney cells(8). In addition, collections of material resembling steel wool, reminiscent of the effect of "CA" virus, appeared. The $10^{-0.5}$ and $10^{-1.0}$ dilutions of inoculum with a titer of $10^{3.5}$ per ml led to degeneration of nearly all cells in 4 and 5 days, respectively. With the higher dilutions, as noted above, local areas developed, but generalized involvement of the sheet of cells did not occur.

Influenza and mumps viruses did not induce cytopathogenic effects. Newcastle disease virus caused death of cells on initial passage; this was interpreted as toxic degeneration since it did not appear on subsequent

passages.

Nasal secretions or washings collected from 6 adult donors with illnesses resembling the common cold have been passed 4 to 5 times in both DMB and DHov cells. A type 4 adenovirus was isolated from an individual who had just started to work with this agent in the laboratory, and who had an increase in titer of type 4 neutralizing antibody. Cytopathogenic agents have not been isolated from the other specimens.

Summary. (1) Two strains of epithelial-like cells derived from nasal mucosa have been found to be susceptible to a number of viruses. The fibroblasts which predominated in the early passages of these cell lines were susceptible, although degeneration occurred at a slower rate, to type 4 adenovirus. (2) Cytopathogenic effects were produced by 7 adenoviruses, 3 polioviruses, 2 ECHO viruses, a Group B Coxsackie virus, herpes simplex, measles, "CA", and "chimp rhinitis" viruses. Titers obtained in the nasal epithelial-like cells are similar to those measured with HeLa cells. Multiplication of certain of these agents was confirmed by titration of passage material. (3) Influenza and mumps viruses and nasal secretions from individuals with common colds were not cytopathogenic.

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Lysozyme: Its Characteristics in Human Parotid and Submaxillo-Lingual Saliva. (22640)

KIRK C. HOERMAN, HAROLD R. ENGLANDER, AND IRVING L. SHKLAIR.
(Introduced by L. W. Roth.)
The Dental Research Facility, U.S.N.T.C., Great Lakes, Ill.

Lysozyme activity in human saliva was reported by Fleming along with his discovery of the enzyme in 1922(1). It has been shown that human parotid saliva exhibits greater lysozyme activity than the corresponding whole saliva(2). This has led to the conclusion that the parotid glands are the major contributors to the total salivary lysozyme titer, if not the sole contributors. We were able to corroborate this finding employing turbidimetric assay methods(3). However, the lower lysozyme activity in whole saliva was due, in part, to increases in optical density resulting from differing concentrations of salivary sediment in the reaction systems. In this study, to avoid this source of error, salivas were collected by methods other than expectoration. Lysozyme activity was determined in sediment-free individual salivary gland secretions. As a result, lysozyme titers in the mixed secretions of the submaxillary and sublingual glands were found to be considerably higher than those in the corresponding parotid salivas. Data are presented regarding some of the characteristics of parotid and submaxillo-lingual* salivary lysozyme. Special emphasis is placed upon the interaction of parotid and SM-L saliva and the effect of this interaction upon the resulting total salivary lysozyme titer.

* Henceforth submaxillo-lingual will be abbreviated as SM-L.