References and Notes

* This investigation was supported by a grant (H-982) from the Division of Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service. We wish to acknowledge the technical assistance of Sarah Jane Baker and Helen Fung.

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31 March 1954.

Primary Isolation and Propagation of Influenza Virus in Cultures of Human Embryonic Renal Tissue*

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A virus of the influenza-A type has been isolated directly from the nasal washings of a patient by single passage in cultures of human embryonic renal tissue and has been propagated by additional passages in cultures of this same tissue. The primary isolation of influenza virus by tissue culture from which hemagglutinins can be demonstrated and utilized for serological identification of the virus has not been previously reported, nor has growth of influenza virus been demonstrated in cultures of human tissue. Moreover, this use of human tissue culture provides an opportunity to study the characteristics of influenza virus without the necessity of passage through cells of another host species.

Kidneys from a 25-cm human embryo obtained following a spontaneous abortion were preserved as small fragments, 1 to 2 mm in diameter, in Hanks-Simms solution (*1*) for 13 days at 4°C prior to preparation of the cultures; 15 to 20 fragments were planted on the walls of 50-ml glass tubes that had been preheated to 45°C (*2*). To each tube was added 3 ml of nutrient medium: bovine amniotic fluid, 90 percent; bovine embryo extract, 5 percent; inactivated horse serum, 5 percent; phenol red 0.002 percent; penicillin, 200 units/ml, and streptomycin, 200 μg/ml (*3*). The cultures were incubated at 35°C in a horizontal stationary position, and nutrient fluids were replaced at weekly intervals. By the seventh day there was epithelial outgrowth from most of the fragments, and this had attained a diameter of 0.5 to 1.0 cm at 14 days. Each culture was inoculated at this time with 0.1 ml of nasal washing obtained from a patient in Jan. 1953. Nutrient fluids were replaced twice weekly following inoculation.

The presence of virus was determined by hemagglutination at room temperature both with 0.25 percent human type-O erythrocytes and pooled fowl erythrocytes and also by growth in subsequent tissue culture and in the amniotic sac of 10- to 11-day chick embryos. Hemagglutination-inhibition tests using human type-O erythrocytes were carried out with standard influenza virus-immune rooster antiserums and hemagglutinating antigen, strains PR8, FM1, FW-1-50 and Lee, obtained through the Biological Depot, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D.C. All antiserums were treated with receptor-destroying enzyme prepared by culture filtrates of the Inaba strain of *Vibrio cholerae*.

Table 1 shows the hemagglutination titers of tissue-culture fluids as determined by the addition of 0.25 percent human type-O erythrocytes. Although hemagglutinins could be detected earlier at the second tissue-culture passage, there was no increase in titer. The lack of hemagglutination by subsequent fluids was probably due to a paucity of additional susceptible cells for viral multiplication. Treatment of these fluids with receptor-destroying enzyme of *Vibrio cholerae* did not increase the hemagglutinating titers, although fresh nutrient mediums had sufficient nonspecific inhibitor to prevent hemagglutination of human type-O red blood cells by 16 hemagglutinating units of infectious PR8 virus after overnight incubation at 37°C. Infectivity titrations in the amniotic sac of the chick embryo demonstrated an increase from $10^{2.6}$ EID_{50} in the nasal washing to $10^{5.0}$ EID_{50} in the pooled third-day fluids from the second tissue-culture passage. It is likely that these titers were an index of the number of viral particles capable of adaptation to the chick embryo rather than a measure of the total infectious virus.

In no instance were hemagglutinins for fowl erythrocytes demonstrated in tissue-culture fluids. However, the initial nasal washing and each tissue-culture fluid shown in Table 1 upon single passage through the amniotic sac of the chick embryo yielded hemagglutinins for fowl erythrocytes. Inoculation of these same tissue-culture fluids in the allantoic sac of the chick embryo did not result in viral growth as measured by hemagglutination. The amount of virus inhibited by nonspecific inhibitor of hemagglutination

Table 1. Hemagglutination titers of fluids from individual tissue cultures, expressed as the reciprocal of final virus dilution. The four cultures *a, b, c, d* were inoculated with 0.1 ml of original nasal washing; at second passage four more cultures, *w, x, y, z*, were inoculated with 0.1 ml of the third-day fluid from culture *c*.

Day	First tissue culture passage				Second tissue-culture passage			
	a	*b*	*c*	*d*	*w*	*x*	*y*	*z*
0	0	0	0	0	0	0	0	0
3	0	0	64	0	128	32	32	64
7	0	32	8	32	0	0	0	0
10	0	0	0	0	0	0	0	0

Table 2. Comparison of human tissue culture and chick embryo adapted GL1134 influenza-A virus with other influenza-A and -B viruses by hemagglutination-inhibition.

Antiserum	Hemagglutination-inhibition titer, expressed as the reciprocal of the initial serum dilution					
	PR8	FM1	FW 1-50	Lee	GL1134 TC	GL1134 E5
PR8	3200				< 50	< 50
FM1		800			< 50	< 50
FW-1-50			800		200	200
Lee				800	< 50	< 50
GL1134 Acute	400	50	50	50	50	50
GL1134 Convalescent	400	200	200	50	400	100

in fresh bovine amniotic fluid increased eightfold after one passage through the amniotic sac of the chick embryo. Therefore the virus grown in human embryonic renal tissue was in the O phase (*4*).

Virus propagated in tissue culture was compared with the same virus that had been through five chick-embryo passages from the original nasal washing. The results of hemagglutination-inhibition with known influenza-A and -B antiserums are shown in Table 2, and it can be seen that this strain (GL1134A-53) is related to FW-1-50 influenza-A virus. Although convalescent serum inhibited hemagglutination by virus grown in tissue culture to a greater degree than that grown in the chick embryo, no assumptions should be made on the basis of this single serum, which was obtained 2 mo after the onset of illness.

Further studies on the growth of influenza viruses in cultures of human tissues, the characteristics of the virus, and the effects of multiplication on human cells are in progress.

Addendum. Since submission of this paper, additional strains of influenza-A and -B viruses have been isolated and propagated directly from nasal washings in both human embryonic lung and kidney tissue cultures.

References and Notes

* From Research Project NM 005 051.24, the Bureau of Medicine and Surgery, Navy Department, Washington, D.C. This work was done in connection with other studies in collaboration with the Commission on Influenza of the Armed Forces Epidemiological Board. The opinions and assertions expressed herein are those of the authors and cannot be construed as indicating endorsement or approval by the Navy Department or the Naval Service at large.

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31 March 1954.

Communications

Lipogenesis by Cells of the Cornea

The capacity for neutral fat formation by nonadipose tissue is of possible relevance to the problems of fatty degeneration, atherosclerosis, and certain lipid histiocytoses. Simms *et al.* (*1–4*) have studied fat formation in chicken fibrocytes and concluded that serum contains lipoid soluble substances (called lipfanogens) that can be converted into fat.

The cornea is especially suitable for the study of such fat formation, since it is a relatively simple structure, is avascular, and contains no stainable fat. In a series of observations (*5*) on the cornea of the living rabbit and the incubated cornea, we have found that oleic acid and sodium oleate induce sudanophilic fat formation in all the cells of the cornea (epithelium, stroma, and endothelium) so long as serum is present. The experiments *in vivo* were made by injecting approximately 0.1 ml of oleic acid suspension or 10 percent sodium oleate solution into the corneal stroma. The experiments *in vitro* were made, for the most part, by incubating corneal buttons at 37°C in 2 ml of rabbit serum to which 2 drops of 10 percent sodium oleate were added. This technique was varied for specific purposes as will be evident in the conclusions.

Sudanophilic globules began to appear in the cytoplasm of the cells in approximately 6 hr and continued to increase in amount for at least 2 wk. This process appeared to be a true lipogenesis and was exclusively intracellular. Immersion of the corneal explants in 1 percent sodium fluoride or 1 percent potassium cyanide, or exposure of the buttons to 60°C for 1 hr prior to the incubation, completely blocked the lipogenesis. No effect was found, however, with similar heating of the serum prior to incubation. Variation of *p*H showed fat formation only in the range of 5.6–8.2 with maximal fat formation in 6.0–7.8. Serum was essential and could not be replaced by glucose-saline or glycerol-saline mediums.

The oleic acid or oleate salt appeared to be a specific and essential substrate: Of the other aliphatic acids tried unsuccessfully were elaidic acid, undecylenic acid, palmitic acid, arachidic acid, pelargonic acid, n-caproic acid, butyric acid, and acetic acid. Stearic acid resulted in a minimal fat formation (possibly attributable to contamination by oleic acid).

The lipogenesis did not result from contact with necrotizing agents other than oleic acid and sodium oleate. It did occur, however, abundantly with hydrolyzed rabbit fat and hydrolyzed olive oil.

Similar results were obtained with rabbit, beef, cat, and human corneas and with rabbit, horse, or human serum.