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Organ Cultures of Human Fallopian Tubes for the Propagation of Viruses

By

J. CASAL[1], D. RUBENSTEIN, M. VOTAVA[2], and D. A. J. TYRRELL

Clinical Research Centre, Common Cold Unit, Salisbury, Wilts., England

With 1 Figure

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Summary

Attempts were made to grow certain viruses in organ cultures of Fallopian tube. Influenza A_2 and B, parainfluenza type 2, rhinovirus type 2, ECHO virus type 11 and adenovirus type 7 were observed to multiply, but poliovirus type 1, a human "coronavirus" and rhinovirus type 3 did not.

1. Introduction

It has been found useful to employ organ cultures in virology in order to propagate viruses in differentiated cells (1); such studies may help in understanding the pathogenesis and pathology of infection and also in the cultivation of viruses which do not grow readily in experimental animals or in tissue cultures.

We have found ciliated epithelium from the respiratory tract of human embryos valuable in the study of respiratory viruses, but it is often difficult to obtain. Since apparently normal Fallopian tubes are frequently obtained in gynaecological surgery we have studied the growth of viruses in them; we thought they might be useful in addition to, or instead of the respiratory epithelium.

2. Materials and Methods

2.1. Tissue

Fallopian tubes were obtained at operation and quickly placed in cold Hanks' saline or in culture medium. Within a few hours tissue which had not been clamped or bruised was dissected out and cut into pieces about 3 mm square. These were planted or scratched areas on the bottom of a 60 mm plastic Petri dish containing 4 ml of medium. Two pieces of tissue were used in each dish. The medium was usually that shown to be optimum in earlier studies by TYRRELL and BLAMIRE (2), namely, 0.2% bovine plasma albumin in Eagle's medium. The dish cultures were incubated

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in 5% CO_2 in air, the tube cultures in air, and both were held at 33° or at 37°C, depending upon the virus tested. Cultures were only used if distinct ciliary activity was seen one or two days after they were prepared. It was found that ciliary activity was retained for several weeks after the cultures were set up.

2.2. Viruses

Most of the viruses were standard laboratory strains, but some were in the form of nasal washings collected from experimentally infected volunteers; a few were tissue culture fluids from early passages of field strains.

2.3. Virus Titrations

Organ culture fluids were mixed with an equal volume of broth and stored at −70°C as soon as they were harvested. They were thawed later and titrated, usually by standard endpoint titrations using 10-fold dilutions in roller tube tissue cultures and two to four tubes per dilution. Influenza B was titrated in primary monkey kidney cells, adenovirus, parainfluenza and ECHO virus in L 132 cells.

Table 1. *Results of Growth Curve Experiments in Organ Cultures of Fallopian Tubes Using Laboratory Grown Viruses*

Virus multiplied		Virus did not multiply
Strain	Peak titre ID_{50}/ml	
Influenza A_2	$10^{3.2}$	Poliovirus type 1 Lsc 2 ab[1]
Influenza B	$10^{3.2}$	"Coronavirus" L. P. strain
Parainfluenza type 2	$10^{6.2}$	Rhinovirus type 3 (F.E.B.)
Adenovirus type 7	$>10^{4.2}$	
ECHO virus type 11	$10^{4.7}$	
Rhinovirus type 2 (H.G.P.)	$10^{3.6}$	

[1] Two separate experiments.

3. Results

Altogether thirteen growth curves of varying degrees of completeness were performed. A representative example of a successful attempt to grow a virus in organ culture of Fallopian tube is shown in Fig. 1. A volume of 0.2 ml of diluted virus was dropped onto the tissue. After about 2 hours the medium was changed 3 times to reduce the amount of unadsorbed virus. It can be seen that virus was shed into the medium and that more virus was recovered than was added. The cilia continued to beat as well in the infected cultures as in the uninoculated controls and virus continued to appear in the medium for sixteen days.

The results of further experiments of this type are shown in Table 1. This indicates that viruses of several different biological groups multiplied in the cultures. The form of the growth curves was roughly similar to that shown in Fig. 1, but most experiments were discontinued after 7 days. It can be seen that although myxoviruses such as influenza and parainfluenza virus multiply freely as in respiratory epithelium, only one of the two rhinoviruses did so. It was rather surprising that although one enterovirus, ECHO virus type 11, multiplied a vaccine strain of poliovirus did not, even though a substantial inoculum (10^3 TCD_{50}) was given. It was not surprising that the "coronavirus" did not multiply since these are well known to be very fastidious organisms (3).

In further studies we attempted to grow the virus present in clinical specimens by inoculating nasal secretions (about 0.2 ml per culture) by dropping them onto the pieces of tissue. Table 2 summarizes the results of these and the growth of rhinovirus type 2 is shown in Fig. 1. This indicates that virus isolation from clinical specimens is possible.

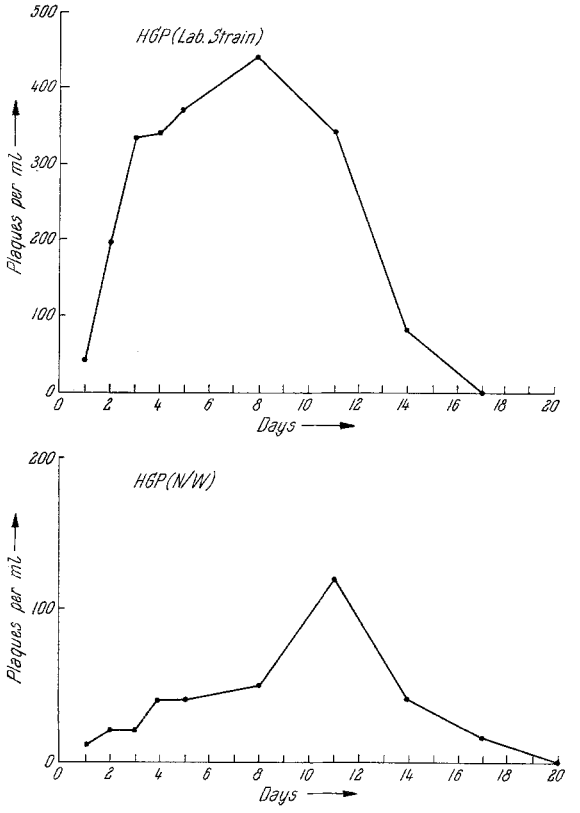

Fig. 1. Upper half: The multiplication of a laboratory strain of rhinovirus type 2 in organ cultures of human Fallopian tube. Virus was titrated by plaque assay in H-HeLa cells (6). Approximately 10^3 PFU were inoculated. Lower half: Similar experiment using nasal washings as inoculum. Approximately 10 PFU were inoculated

Table 2. *Results of Growth Curve Experiments Using Viruses Obtained from Clinical Specimens*

Strain	Peak titre ID_{50}/ml
Influenza A_2	$10^{5.2}$
Influenza B	$10^{4.2}$
Rhinovirus type 2 (H.G.P.)	10^2

4. Discussion

It is technically quite easy to set up cultures of Fallopian tubes, provided the tissue is not damaged at or after surgery. On the other hand, it seems to be less useful for growing a wide range of respiratory viruses than nasal or tracheal epithelium. It has been found possible to propagate mycoplasmas in similar cultures (4).

It was interesting that the ciliary activity was not noticeably reduced by any of the viruses. Perhaps this indicates that there are subtle differences between ciliated epithelium of the genital and respiratory tract; although they look rather similar morphologically. HOORN and TYRRELL noted similar but less marked differences between oesophageal and tracheal epithelium (5).

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Authors' address: Dr. D. A. J. TYRRELL, Common Cold Research Unit, Coombe Road, Salisbury, Wilts., England.