populations or abnormal deaths has been reported in areas where the fungus has been known to exist in the soil. Further experiments on the ultimate fate of naturally infected mice are now in progress in an attempt to elucidate this problem.

Summary. 1. Susceptibility of white Swiss mice to infection with *H. capsulatum* by inhalation of dust aerosol was determined by direct exposure of animals to known positive soil samples. 2. Disseminated histoplasmosis occurred in a large percentage of mice. 3. Naturally contaminated soil samples held 5 and 10 months at room temperature remained highly infectious. 4. Artificially seeded soil cultures were 100% infective to the white mouse at exposures of 1, 2, 4, and 7 days. 5. These data add support to the hypothesis that man and animals become infected with *H. capsulatum* by the respiratory route. 6. The safety hood and associated safety procedures proved completely effective in preventing infection of the control mice although they were placed in close proximity to animals exposed to known positive soil samples.

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Human Amnion Cell Cultures; Susceptibility to Viruses and Use in Primary Virus Isolations.* (22891)

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Multiplication of the 3 types of poliomyelitis viruses in tissue cultures of human amnion cells has been reported by Zitcer, *et al.* (1). Because of abundance and availability of placental membranes, the studies reported here were initiated to determine the susceptibility of amnion cell cultures to other viruses and to investigate the possibility of large scale use of these cells for primary isolation of viral agents.

Materials and methods. Preparation of amnion cultures. Membranes were collected over a 14-hour period and placed in jar containing 200 ml sterile balanced salt solution to which penicillin and streptomycin had been added at a concentration of 100 units and 100 μg/ml respectively.† The jar containing the membranes was kept at room temperature until brought to the laboratory. After separation from the chorion, the amnion was washed in several changes of sterile balanced salt solution, cut into approximately 10 cm pieces and freed of blood clots by digestion in 0.25% trypsin solution for 30 to 45 minutes. The supernatant solution containing blood cells was then discarded and fresh trypsin solution added to the minced tissues. The cells were thereafter liberated by gentle agitation with a magnetic stirrer for 6 to 14 hours without decanting. This simplified the trypsinization process and cells obtained by this method did not appear to be damaged by the prolonged digestion. Cells were then centrifuged at 1,000 rpm for 10 minutes and washed twice in sterile balanced salt solution before being suspended at a 1/150 dilution in Eagle's synthetic medium supplemented with 10% horse serum. The yield of packed cells from one membrane varied from 0.5 to 1.5 ml. A small percentage of membranes yielded

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cells which failed to grow out in culture. Tube cultures were grown in stationary racks after being implanted with 0.5 ml of cell suspension. After 48 hours when cells had adhered to the glass, the cultures were fed with fresh growth medium. A confluent sheet of cells was usually obtained in 4 to 7 days, and cultures were thereafter maintained in Eagle's medium with 4% horse serum. All media contained 100 units penicillin, 100 μg streptomycin, and 50 units mycostatin/ml. *Viral susceptibility.* All the viruses used were high titer prototype strains employed by various investigators at the National Institutes of Health. Cultures were inoculated with maximal non-toxic concentrations of virus and followed for cytopathic changes. Fluids from virus-inoculated tubes which showed degenerative changes were harvested after maximum destruction had occurred and carried through 5 successive passages. Fluids from tubes showing no effects after observation for 18 to 21 days were also passed "blindly" through 5 successive passages after which fluids from the last passage were inoculated into other susceptible hosts in order to determine the presence or absence of virus. *Virus Isolation.* Throat swabs and washings collected in bacteriological broth from cases of febrile respiratory illnesses occurring in a general population group in the Washington, D.C. area during the winter of 1955-56 were tested in amnion cultures by inoculation of 0.2 ml of untreated material/tube and the tubes observed for 14 days for cytopathic changes. Adenoviruses were identified by the method described by Rowe, *et al.*(2) and poliovirus by the neutralization test.

Results. As previously described by Zitcer, *et al.*, cells derived from the amnion were epithelial-like and of two sizes, approximately 90% of the cultures being made up of the smaller cell type. Both cell types were indistinguishable when grown in culture (Fig. 1). Table I shows the results of tests on susceptibility of amnion cultures to the viruses tested.

Cultures inoculated with the 8 types of adenoviruses characteristically showed marked nuclear granularity, and infection was usually first noted in cells on the outer periphery of the cell sheet or on the lower end of the tubes (Fig. 2). Rounding of infected cells then followed, and the entire culture was usually degenerated by 4th to 8th day. This slow process of degeneration and the absence of clumped, rounded cells is in contrast with infection of this group of agents in HeLa cell cultures. In addition, acid production which is usually observed in HeLa cell cultures infected with adenoviruses(3) was not noted in adenovirus-infected amnion cultures grown and maintained in Eagle's medium.

TABLE I. Viruses Tested in Human Amnion Cultures.

Virus	Source	Results*
Adenoviruses Types 1-8	HeLa cells	+
Coxsackie A1-8	Mouse	—
" A9	Monkey kidney cells	+
" B1, 3, 5	*Idem*	+
" B2†	"	±
" B4‡	"	—
Herpes simplex	HeLa cells	+
Influenza A, B	Monkey kidney cells	—
Dengue, Type 1	Mouse	—
Mumps	Chick embryo	—
Newcastle disease	*Idem*	—

* + = cytopathogenic through 5 passages. — = noncytopathogenic and negative for virus after 5 passages.
† One of 4 strains cytopathogenic.
‡ None of 4 " "

Herpes simplex virus readily produced cytopathic changes in amnion cultures and with undiluted inoculum, foci of infected cells could be observed within 24 hours. Most of the cells were involved after 48 hours and formed clumps of rounded cells. (Fig. 3).

Of the group A Coxsackie viruses tested in amnion cultures, only type A9 produced cytopathic changes. Types 1, 3, and 5 of the Coxsackie B group readily caused cell damage, while only one of 4 B2 strains tested was capable of multiplication in amnion cells. None of 4 B4 Coxsackie strains showed any evidence of cytopathic changes or multiplication. Cell destruction caused by the Coxsackie viruses was usually rapid and definite within 24 to 48 hours after infection (Fig. 4).

Infectivity titers of representative strains of viruses after 5 passages in amnion cultures calculated by the Reed and Muench method were as follows: Type 3 adenovirus, $10^{3.8}$;

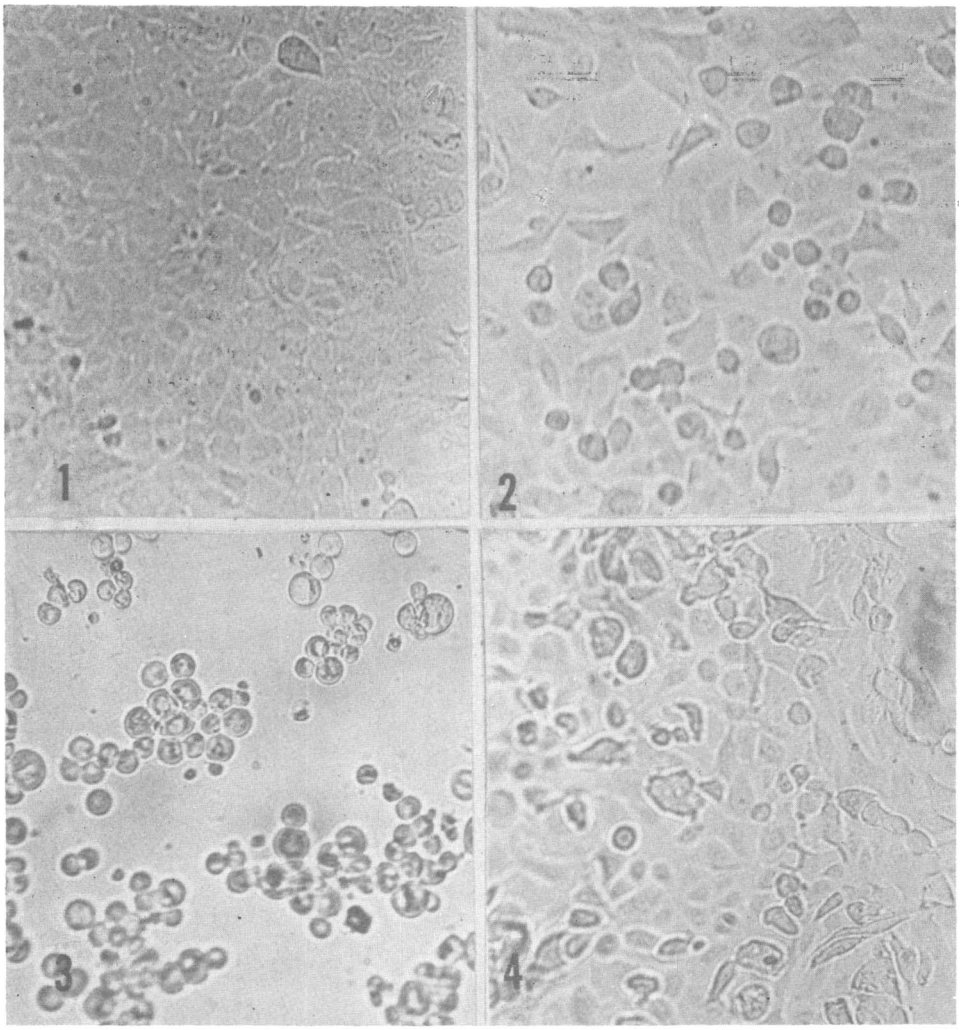

FIG. 1. 7-day-old culture of normal human amnion cells. Unstained. × 150.
FIG. 2. Early cytopathic changes in human amnion cell culture due to adenovirus type 3. Unstained. × 150.
FIG. 3. Cytopathic changes in human amnion cell culture 48 hr after inoculation with herpes simplex virus. Unstained. × 150.
FIG. 4. Early cytopathic changes in human amnion cell culture due to Coxsackie B1 virus. Unstained. × 150.

Coxsackie Bl, $10^{4.2}$; and herpes simplex virus, $10^{2.5}$. Titrations were made in amnion cultures and all tubes were observed for 14 days for cytopathic changes.

The high degree of susceptibility of amnion cells to the adenovirus group was further established in an experiment comparing the recovery of adenovirus types 1, 2, 3, and 5 from known positive throat specimens in amnion and HeLa cell cultures. The results of this experiment are shown in Table II. Of 16 specimens previously found positive for virus in HeLa cell cultures, 4 each of types 1, 2, 3, and 5 adenoviruses, virus was re-isolated in amnion cultures from all but one specimen, as compared with 12 isolations in HeLa cell cultures (obtained from the Microbiological Associates, Bethesda, Md.). Sixty unknown throat specimens taken from persons with febrile respiratory illnesses were also tested in both types of cultures, and adenoviruses were recovered from 5 in both amnion and

TABLE II. Comparative Isolations of Adenoviruses in Amnion and HeLa Cell Cultures.

Type of specimen	No.	No. isolations in Amnion	No. isolations in HeLa
Known positive	16	15	12
Unknown	60	5	5

HeLa cultures, all isolations being made from the same specimens.

Results of these experiments indicated that for isolation of adenoviruses, amnion cultures were as reliable as HeLa cultures. Moreover, the ease in maintaining amnion cultures made it seem that these cells would be of value for large scale use in routine screening for these and other viral agents. A total of 1,407 throat specimens, approximately half of which were taken from children, has been examined in amnion cultures and the results are shown in Table III. Forty isolations of adenovirus types 1, 2, 3, and 5 were made in amnion cultures. Except for one isolation of adenovirus type 5 from an adult, all strains isolated were from children whose ages ranged from 1 through 12. One type 1 poliovirus and 6 strains of herpes simplex virus were also recovered from the specimens tested.

For diagnostic purposes and for routine screening of materials for viral agents, amnion cultures may be a useful source of tissue culture cells. The range of viral susceptibility of amnion cells is very similar to that of the HeLa cell, and the results reported here are in accord with those of Weinstein, *et al.*(4).

TABLE III. Primary Virus Isolations in Human Amnion Cells.

No. specimens	Virus isolated	No.
1407	Adenovirus type 1	5
	Idem 2	13
	" 3	20
	" 5	2
	Poliovirus type 1	1
	Herpes simplex	6
	Total	47

Besides their susceptibility to a number of viruses, cultures of amnion cells can be readily prepared and easily maintained. In our hands, inoculated cultures can be maintained in a healthy state for periods as long as 3 to 4 weeks, with relatively infrequent medium changes. Spontaneous degeneration somewhat resembling viral effects was noted on 2 occasions, but no agent was recovered from culture fluids in amnion, HeLa, or monkey kidney cultures.

Preliminary experiments with cultures of human chorion have indicated that this tissue may also provide cultures suitable for viral studies. A mixed population of epithelial and fibroblastic cells usually is obtained from the chorion. The following viruses have been found to cause cytopathic changes in chorion cultures; adenoviruses (7 types), polioviruses types 1, 2, 3, herpes simplex virus, and the human salivary gland virus(5).

Summary. 1. Tissue cultures of human amnion cells were found to be susceptible to all adenoviruses tested, certain Coxsackie viruses, and herpes simplex virus. 2. Amnion cell cultures were employed in large scale routine testing for viral agents with recovery of 40 strains of adenoviruses types 1, 2, 3, and 5, one type 1 poliovirus, and 6 strains of herpes simplex virus.

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