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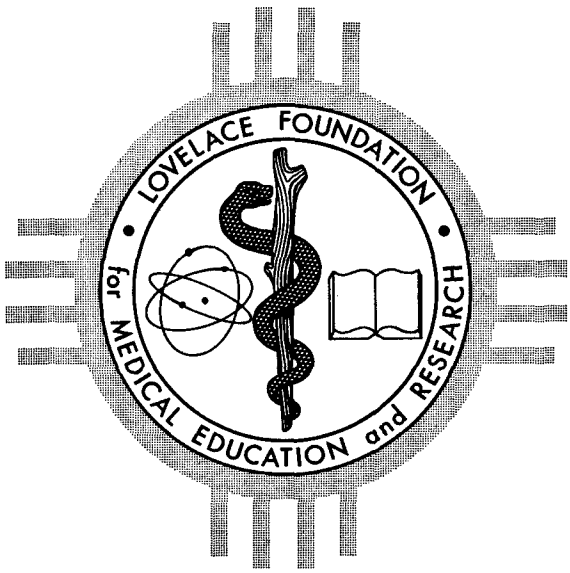


Albuquerque, New Mexico

# SUSCEPTIBILITY OF TISSUE CULTURES OF CANINE ORIGIN TO VIRUSES

by

FRANK F. PINDAK

AND

WILLIAM E. CLAPPER

August 1965



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# SUSCEPTIBILITY OF TISSUE CULTURES OF CANINE ORIGIN TO VIRUSES

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Frank F. Pindak and William E. Clapper

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## ABSTRACT

The suitability of tissue cultures of canine origin for isolation of viruses from dogs was studied. Primary cultures of kidney, lung, heart and whole dog embryo, low-passage tracheal cells and a continuous line of epithelial-like cells derived from dog kidney were challenged with 27 viruses. These included the infectious canine hepatitis virus, two coxsackie A and six coxsackie B viruses, three types of poliovirus, ten ECHO viruses, one adenovirus, the western and eastern strains of equine encephalitis virus, and A and B strains of influenza virus. Although each type of cells supported the growth of one or more viruses as evidenced by the cytopathic effect none of them appeared to be more suitable for virus isolation studies than HeLa and monkey kidney cells.

## ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. C.S. White for helpful suggestions in the preparation of the manuscript. They also thank the staff of the Department of Veterinary Medicine for supplying the tissues from which the various cell cultures were prepared.

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# SUSCEPTIBILITY OF TISSUE CULTURES OF CANINE ORIGIN TO VIRUSES

by

Frank F. Pindak and William E. Clapper

## INTRODUCTION

The frequent isolations of ECHO type 6 virus from the feces of healthy beagles, has been reported by the authors[1]. Although cultures of primary dog kidney were routinely inoculated, no viruses were recovered in these cells. References were found in the available literature stating that infectious canine hepatitis virus[2,3] and Visna virus[4] can produce cytopathic effects in primary cultures of dog kidney cells. The susceptibility of these cells and of cells derived from other dog tissues to human viruses was not fully investigated. Therefore, it was of interest to determine which human and other viruses may produce a cytopathic effect in tissue cultures of canine origin. Such information can be a useful guide in selecting the most suitable culture systems for isolation of viruses from dogs. It can also provide some basic information of possible changes in viral susceptibility of tissue cultures derived from irradiated animals.

## MATERIALS AND METHODS

### 1. Types of Tissue Cultures Used

Cultures of primary dog kidney (PDK), primary dog lung (PDL), primary dog heart (PDH) and trachea (Trac) from young beagles were employed in the study. The first three were used as primary cells, grown on glass directly from the trypsinized tissues. The tracheal cells, due to the scarcity of the original amount of cells, were grown in large bottles and harvested four to six times before they were seeded into test tubes

in which they were challenged with the viruses. In addition, primary cell cultures of whole dog embryo measuring 58 mm (Demb) and a continuous line of cells derived from normal adult dog kidney (DK) were also included in our study. The DK were first observed as colonies of epithelial-like cells in a bottle of primary dog kidney cells and obtained in pure culture by the procedure used for isolation of similar cells from dog liver and described earlier[5]. They were challenged with viruses in their 25th and subsequent passages.

2. Preparation of Primary Cell Cultures

The tissues were collected immediately after the donor animals were sacrificed. Following the removal of adjacent connective and adipose tissues they were minced with sterile scalpels, rinsed with Earle's solution and digested by trypsin at room temperature on a magnetic stirrer until free cells were obtained. The cells were washed with Earle's solution, resuspended in growth medium, planted into test tubes or bottles and incubated in a stationary position at 37°C until the sheets of cells were fully grown.

3. Media Used for Cell Cultures

All types of cells were cultivated in medium 199 containing 10% heat inactivated calf serum. Penicillin, streptomycin, neomycin and amphotericin B were added to prevent microbial growth. The same medium, but without calf serum and fungizone, was used for maintenance of the cultures in the infectivity studies.

4. Viruses Used

Each tube of cells was inoculated with 0.1 ml of undiluted viruses. These were coxsackie A-9 and A-16; coxsackie B-1, B-2, B-3, B-4, B-5, and B-6; polioviruses types 1, 2, and 3; ECHO viruses types 2, 4, 5, 6, 7, 9, 11, 14, 16, and 18; adenovirus type 4, influenza viruses A and B, western equine encephalitis virus (WEE), eastern equine encephalitis virus (EEE) and infectious canine hepatitis virus (ICH). The source of the original virus cultures has been given in a previous publication[5].

## RESULTS

### 1. Growth and Characteristics of the Cell Cultures

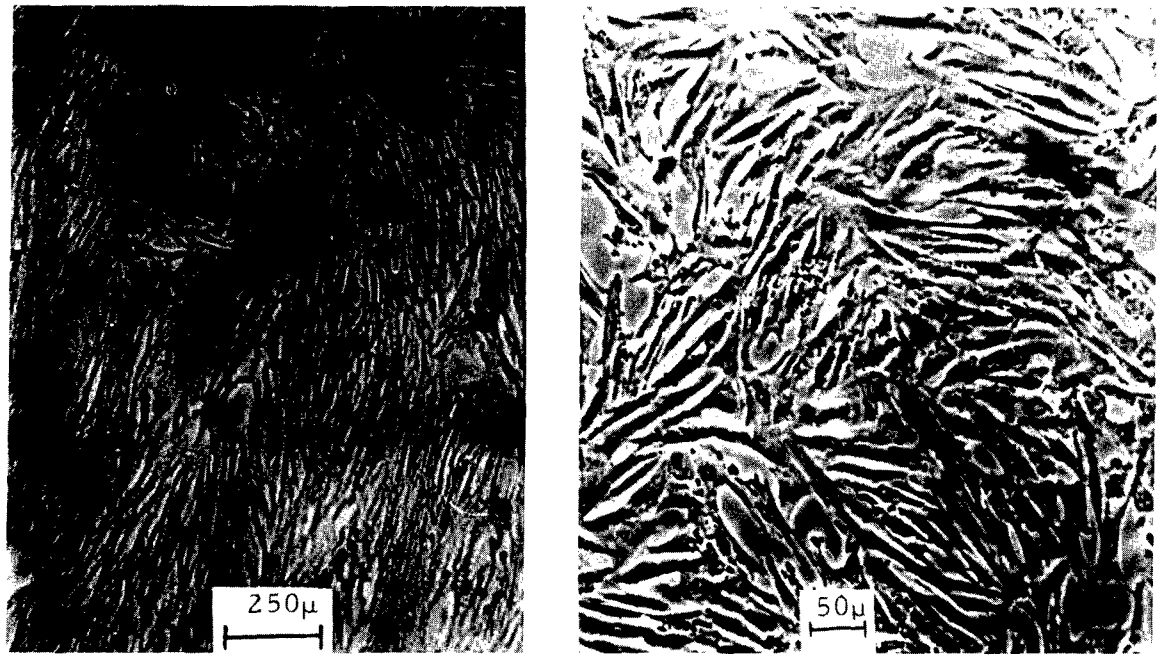
The PDK, PDL and PDH cells grew into a full sheet regularly within one week after seeding into test tubes or bottles. The cultures of PDL and PDH consisted only of fibroblast-like cells and had the tendency to arrange themselves unidirectionally (Figs. 1 and 2).

The suspension of cells obtained by tryptic digestion of trachea was seeded into two to three bottles. When the cells grew a full sheet, each bottle was harvested and the cells were replanted into two new bottles. Four to six such transfers resulted in an ample amount of cells to do the viral susceptibility studies. In all stages of growth, these cells resembled typical fibroblasts (Fig. 3).

The Demb cells were obtained in the following manner: The dog embryos were removed by cesarean section, the head and extremities were discarded, and the body was minced and digested with trypsin. The primary cultures were a mixture of epithelial-like and fibroblast-like cells (Fig. 4). These two types were later separated, but only their mixtures in the primary cultures were used in the experiment.

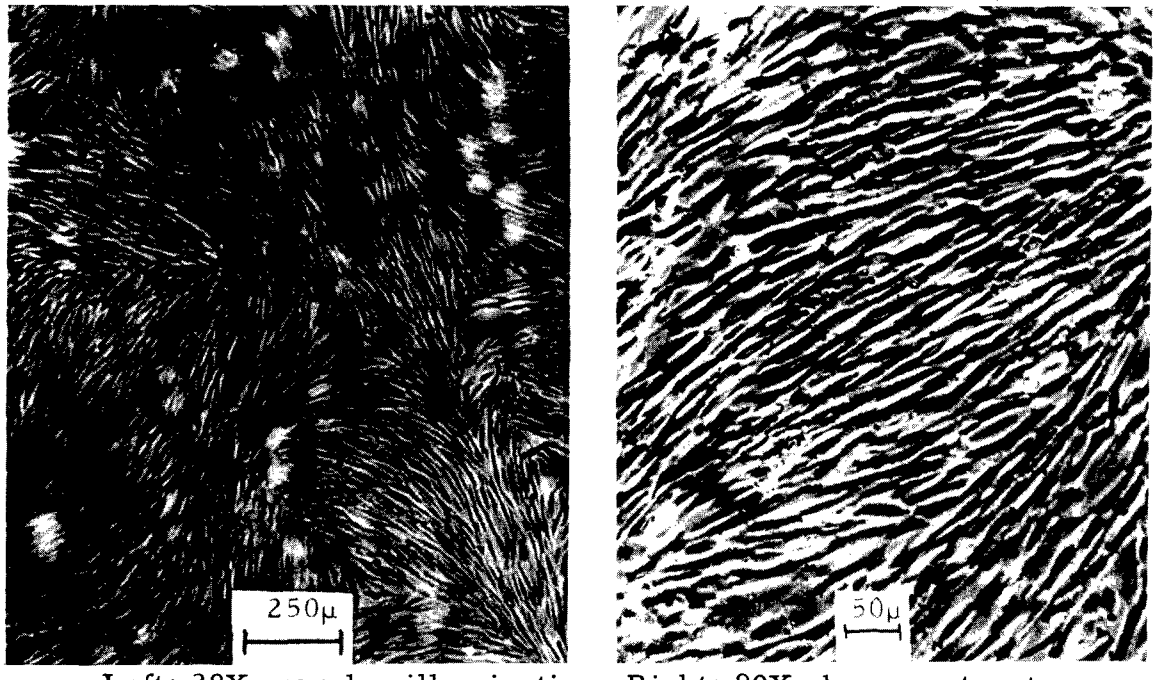
The PDK cells (Fig. 5) were also mostly fibroblast-like, but no definite pattern could be seen in their arrangement. When the outgrowth covered most of the glass surface, varying numbers of colonies of epithelioid cells could be observed scattered throughout the population of fibroblasts. After such cultures were harvested with trypsin and replanted into new bottles two to three times, the growth rate of those resembling fibroblasts was diminished and the usual degenerative changes encountered in most aging cell cultures were seen. At this time, there was a noticeable replacement of these cells with the epithelial cells which, as a rule, could be replanted six or seven times before they, too, showed the signs of degeneration. However, on two occasions, these maintained their normal growth rate and could be transplanted into new bottles at three to

Fig. 1. PRIMARY CULTURE OF DOG LUNG (PDL)



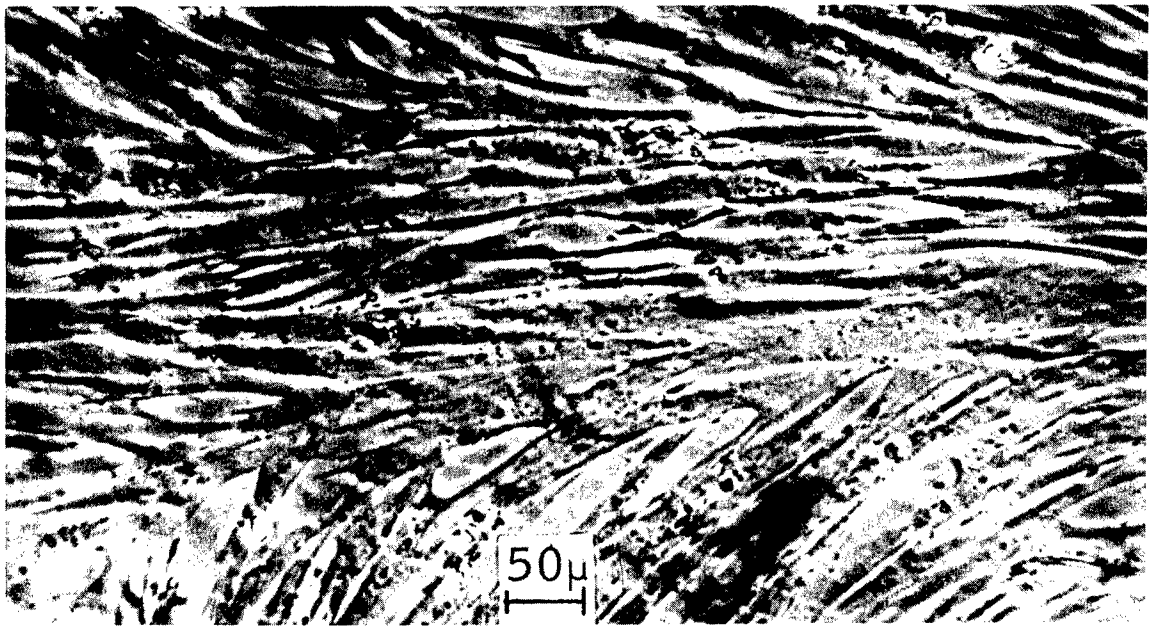

Left: 28 X regular illumination Right: 90 X phase contrast

Fig. 2. PRIMARY CULTURE OF DOG HEART (PDH)



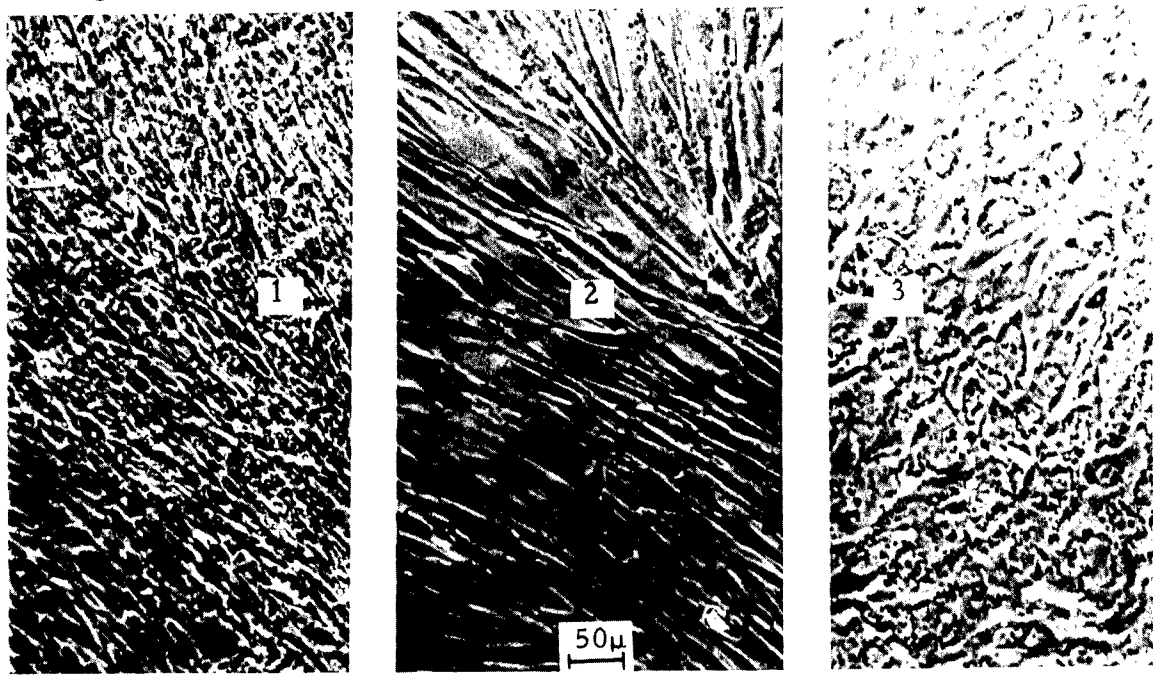

Left: 28X regular illumination Right: 90X phase contrast

Fig. 3. SECOND PASSAGE OF CELLS FROM DOG TRACHEA (Trac)



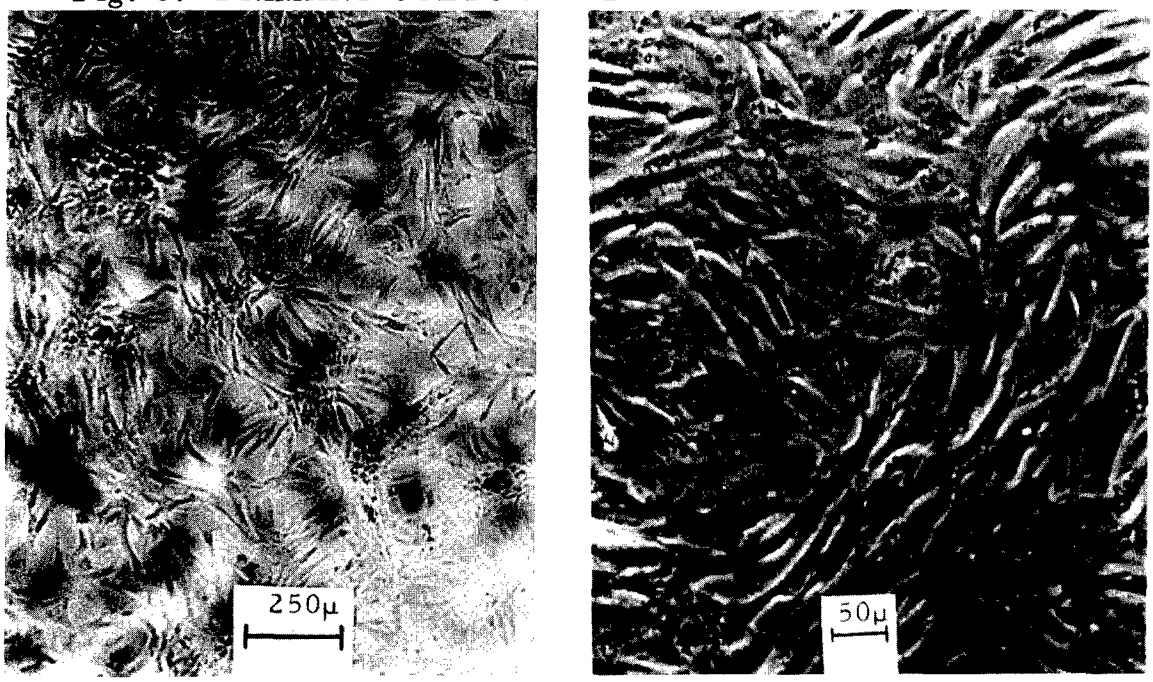

Phase contrast 90X

Fig. 4. CELL CULTURES FROM DOG EMBRYO (Demb)



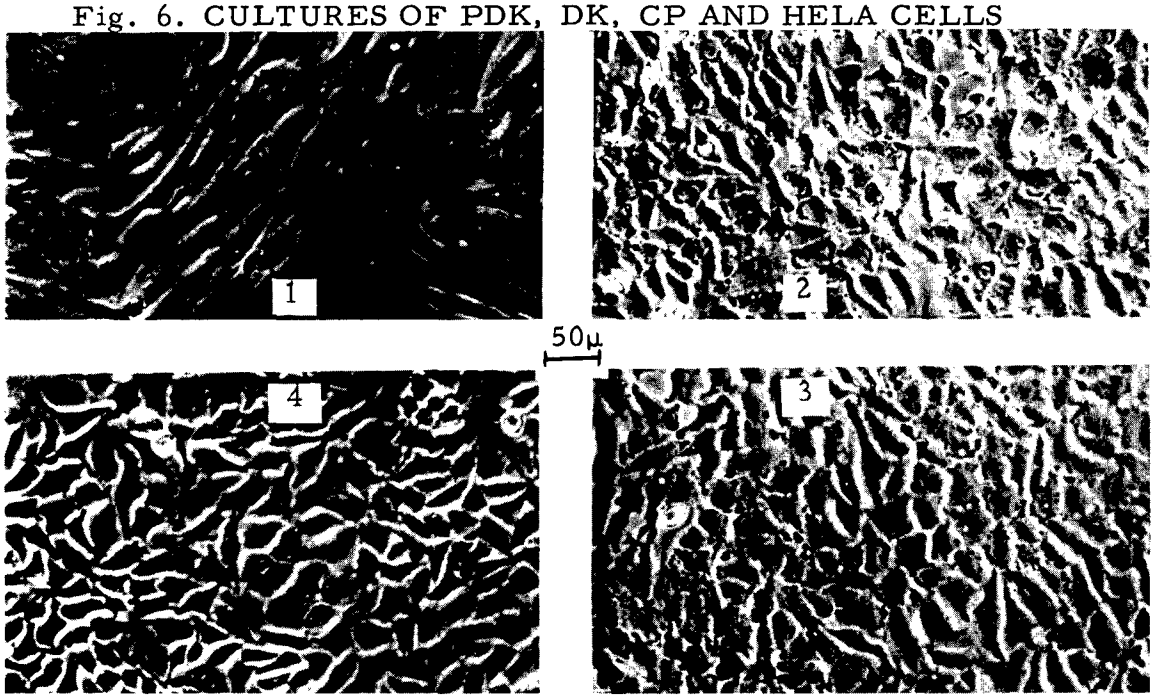

1. Primary culture of mixed cells. 2. Fibroblasts. 3. Epithelial cells

Fig. 5. PRIMARY CULTURE OF DOG KIDNEY CELLS




Left: 28 X, regular illumination Right: 90 X , phase contrast

Fig. 6. CULTURES OF PDK, DK, CP AND HELA CELLS




1. PDK 2. DK 3. CP 4. HeLa 90 X , phase contrast

five-day intervals. The continuous DK line stemmed from such cells.

The DK cells (Fig. 6) were a continuous line derived from PDK cells. Morphologically, they differed markedly from PDK and to some degree from HeLa cells, but had a close resemblance with the continuous line of dog liver (CP) cells described earlier[5]. They about doubled their population at a steady rate of once in three to five days.

2. The Effect of Viruses on the Cell Cultures

Maintenance medium was added to fully grown sheets of cells in test tubes and the cultures were inoculated with 0.1 ml of undiluted stock viruses. Coxsackie A-9 and A-16, and the ECHO viruses were previously maintained in primary monkey kidney cultures. Coxsackie B, adenovirus type 4 and polioviruses were from HeLa cultures, and the ICH virus was grown in PDK. WEE and EEE viruses were from cultures of CP cells. The sources of the influenza viruses was amniotic fluid of chick embryos. All inocula were known to contain live virus.

After inoculation with virus, the cultures were kept in a stationary position at 37°C and observed daily for cytopathic effect. The maintenance medium was changed when the pH of the cultures dropped markedly below 7.0. At the end of seven to ten days the cultures were frozen and passaged again in the same type of cells. A total of three passages was made of each virus in each type of cells. If there was no evident cell destruction in the last passage, the virus cultures were reinoculated into the cells in which they were normally maintained in order to establish the presence or absence of the virus. No virus was recovered from cell cultures which showed no cytopathic effect in the third passage. The results are given in Tables 1-3.

Coxsackie A-16 virus caused complete destruction of cells in cultures of PDK, PDL, PDH, and DK, but not in Trac and Demb. Neither coxsackie A-9 nor any of the six coxsackie B and ten ECHO viruses had any demonstrable effect on the cell cultures tested. Similarly, the cultures of PDK, PDL, PDH and Trac retained their normal appearance after inoculations

TABLE 1

CYTOPATHIC EFFECT OF COXSACKIE AND POLIO VIRUSES ON DOG CELL CULTURES

All Tubes Inoculated with 0.1 ml of Undiluted Virus

| Virus | PDK | PDL | PDH | Trac | Demb | DK |
|---|---|---|---|---|---|---|
| Cox. A-9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cox. A-16 | 4+ | 4+ | 4+ | 0 | 0 | 4+ |
| Cox. B-1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cox. B-2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cox. B-3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cox. B-4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cox. B-5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cox. B-6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polio 1 | 0 | 0 | 0 | 0 | $\pm$ | 2+ |
| Polio 2 | 0 | 0 | 0 | 0 | $\pm$ | 0 |
| Polio 3 | 0 | 0 | 0 | 0 | $\pm$ | 2+ |

PDK = primary dog kidney

PDL = primary dog lung

PDH = primary dog heart

Trac = Dog trachea cells in 3rd-8th passage

Demb = Dog embryo cells in 2nd passage

DK = Continuous line of cells derived from dog kidney

TABLE 2

CYTOPATHIC EFFECT OF ECHO VIRUSES ON DOG CELL CULTURES

All Tubes Inoculated with 0.1 ml of Undiluted Virus

| Virus | PDK | PDL | PDH | Trac | Demb | DK |
|---|---|---|---|---|---|---|
| ECHO 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECHO 18 | 0 | 0 | 0 | 0 | 0 | 0 |

PDK = primary dog kidney

PDL = primary dog lung

PDH = primary dog heart

Trac = Dog trachea cells in 3rd-8th passage

Demb = Dog embryo cells in 2nd passage

DK = Continuous line of cells derived from dog kidney

TABLE 3

CYTOPATHOGENIC EFFECT OF SELECTED VIRUSES ON DOG CELL CULTURES

All Tubes Inoculated with 0.1 ml of Undiluted Virus

| Virus | PDK | PDL | PDH | Trac | Demb | DK |
|---|---|---|---|---|---|---|
| Adeno. 4 | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| WEE | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| EEE | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| ICH | 4+ | 3+ | 4+ | 4+ | 3+ | + |
| Influenza A | 0 | 4+ | 4+ | 0 | 4+ | ND |
| Influenza B | 0 | 0 | 0 | 4+ | 4+ | ND |

PDK = primary dog kidney

PDL = primary dog lung

PDH = primary dog heart

Trac = Dog trachea cells in 3rd-8th passage

Demb = Dog embryo cells in 2nd passage

DK = Continuous line of cells derived from dog kidney

ND = not done

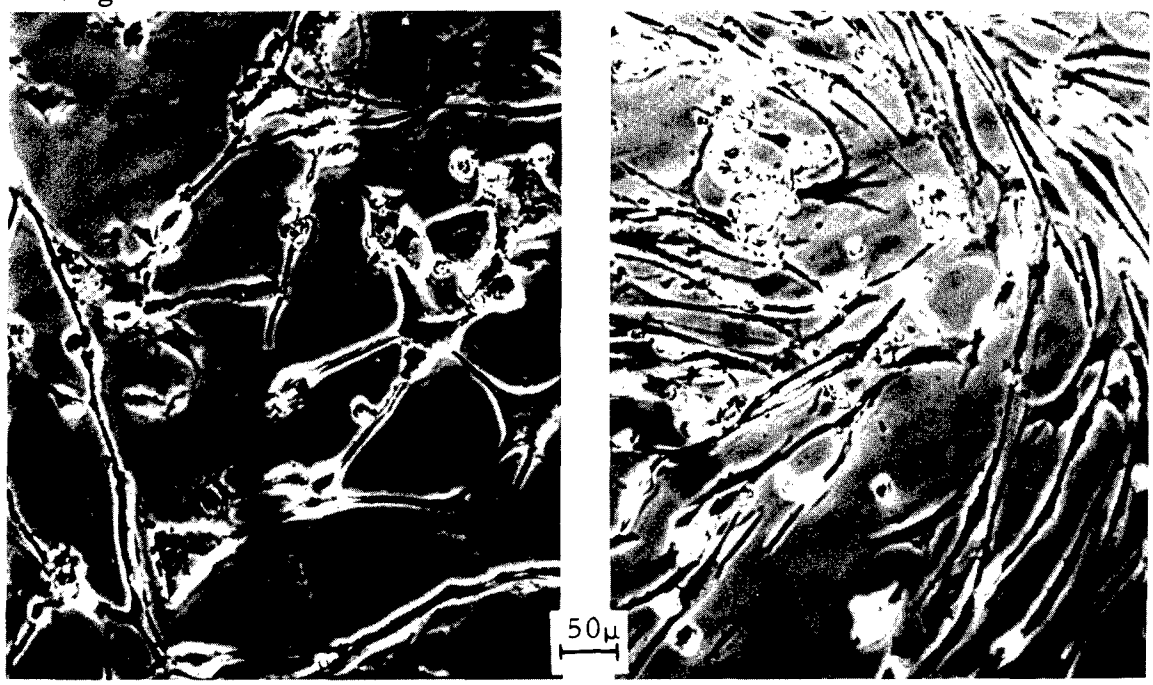
with polioviruses. In the cultures of Demb cells, however, all three polioviruses caused some "balooning" and partial cytopathic effect. Types 1 and 3 produced incomplete destruction in DK cells. Adenovirus type 4, WEE, EEE and ICH viruses destroyed the cells of all cultures tested. Cytopathic effect was also observed in cultures of PDL, PDH and Demb inoculated with influenza A and in Trac and Demb inoculated with influenza B viruses. The types of cell destruction produced by the different viruses can be seen in Figs. 7-10.

## DISCUSSION

During preliminary studies concerned with the effects of radiation on the viral flora of beagles, it became evident that detailed knowledge of susceptibilities of tissue cultures of canine origin to viruses was of prime importance. It was realized that dogs may harbor other than the commonly recognized rabies, distemper and infectious canine hepatitis viruses. Proper selection of tissue culture systems was necessary to insure that the widest possible range of viruses which might occur in either normal or experimental dogs could be recognized and isolated from various specimens. However, relatively few references were found in the available literature concerning the use of cells derived from dog tissues and those were mostly limited to the use of dog kidney cells. Inasmuch as these and other dog cells can be easily obtained, they were considered for routine use for virus isolations. However, their usefulness for this purpose had first to be determined by studying their susceptibility to known viruses. This work was limited to observation of the production of the cytopathic effect, since it is the most useful indicator of the presence of a virus.

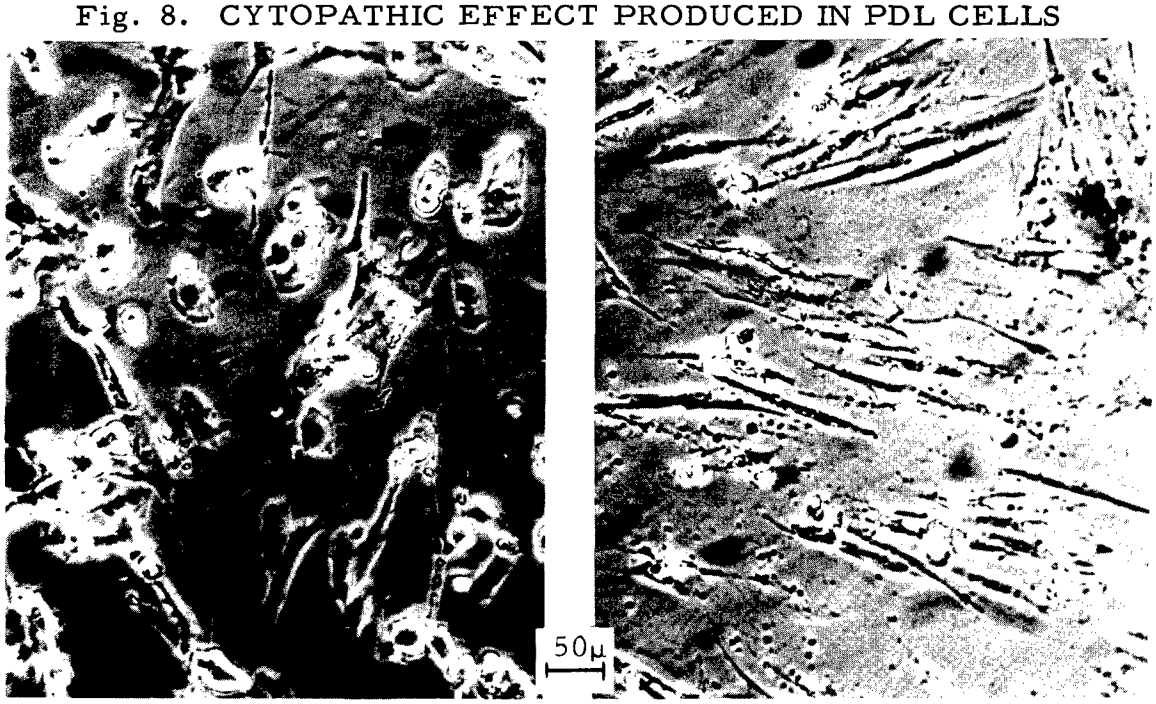
Cabasso et al.[2] have demonstrated that the ICH virus causes destruction of PDK cells. Lenahan and Wenner[6] challenged these cells with ECHO viruses types 1, 4, 9, and 10, coxsackie viruses A-7 and A-11, B-3, B-4 and B-5, adenoviruses types 2 and 6 and with WEE, EEE and SLE viruses. Of these, only ECHO virus type 10 and WEE virus produced the cytopathic effect. In a similar study, Hsiung[7] observed cell destruction with

Fig. 7. CYTOPATHIC EFFECT PRODUCED BY THE ICH VIRUS



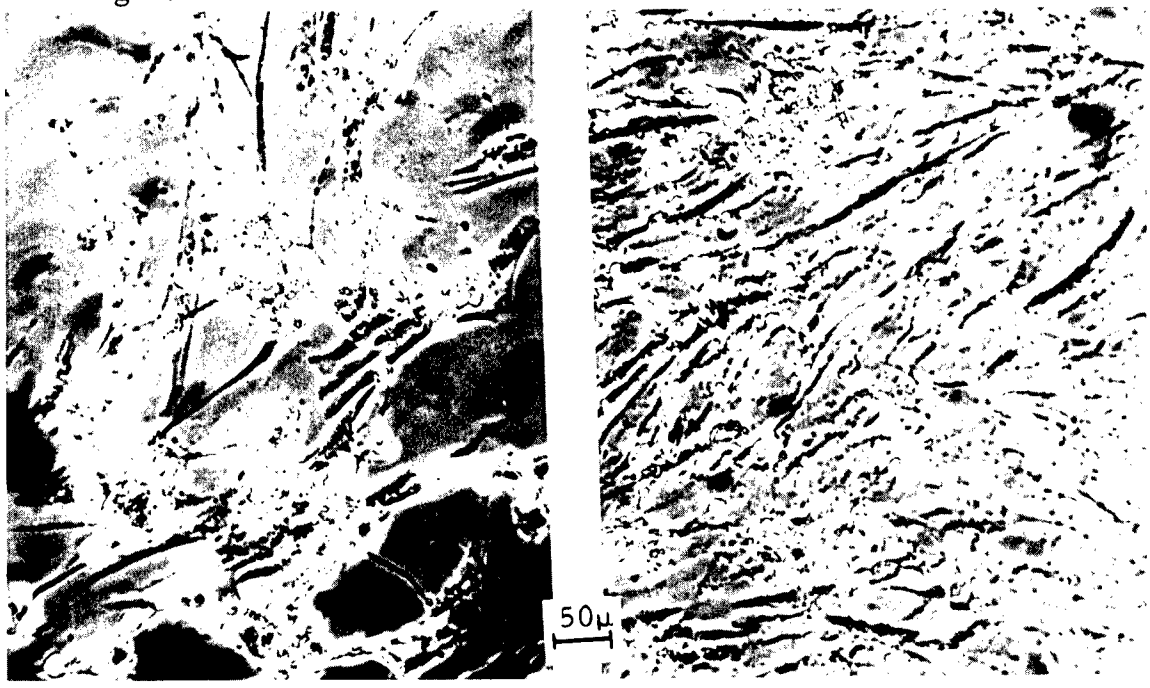

Left. in PDK cells Right. in PDH cells

Fig. 8. CYTOPATHIC EFFECT PRODUCED IN PDL CELLS




Left. Adenovirus type 4 Right. WEE virus

Fig. 9. CYTOPATHIC EFFECT PRODUCED IN PDH CELLS




Left. Coxsackie A-16 virus Right. EEE virus

Fig. 10. CYTOPATHIC EFFECT OF INFLUENZA A VIRUS IN PDH CELLS

ECHO type 10 and with an ECMO (enteric cytopathic monkey orphan) virus, but not with polioviruses types 1, 2, and 3, coxsackie A-9, B-1, B-2, B-3, B-4, and B-5 or with any of the thirteen remaining ECHO viruses tested. The inability of polioviruses to destroy dog kidney cells was also reported by Chen[8].

The data reported above concerning the PDK cells are in general agreement with the quoted literature. Of the 27 viruses inoculated into them only ICH, coxsackie A-16, adenovirus type 4, WEE and EEE viruses caused cell destruction. The stock EEE virus maintained in this laboratory appeared to be more active than that of Lenahan and Wenner[6]. This, however, may be explained by strain differences. In the remaining cells, only nine of the 27 viruses used produced any cytopathic effect.

It is generally believed that only those cells which are of human or simian origin are capable of reproducing polioviruses[6]. In this respect, the partial destruction of the DK cells (developed from a culture of primary dog cells) by polioviruses types 1 and 3, is of interest. The techniques of their handling were such that it is very unlikely that at any time the PDK culture could have been accidentally contaminated with HeLa cells. Their morphology appears to differ appreciably from HeLa cells (Fig. 6). Furthermore, whereas the HeLa cells are known to support the growth of coxsackie B viruses with resulting complete destruction of the cells[4, 20], none of them had any visible effect on the DK cells nor was there any growth of the virus since none were isolated in HeLa cells from the third passage in the DK cells. Recently, Moore et al.[9] reported that, in cultures of mouse skelatal muscle, they were able to propagate poliovirus type 2. It appears, therefore, that the possibility of growing polioviruses in non-primate cells needs further investigation.

The results here reported indicate that any of the tissue cells studied will allow the recognition and isolation of the ICH virus. Whether or not any of them can be of use in isolating other typical "dog" viruses remains

to be seen. PDK, PDL and Trac cells were used extensively in this laboratory for virus isolations from nose, throat and fecal specimens of dogs, with completely negative results (unpublished). None of these cells appear to offer an advantage over HeLa and monkey kidney cells for isolation of human viruses, as evidenced by data here presented, by results obtained previously[1] and by current studies on $Sr^{90}$ irradiated dogs, to be published in the near future.

The failure of ECHO and other human enteroviruses to grow in any canine tissue cultures was seemingly in conflict with an earlier investigation, during which ECHO type 6 virus was isolated on numerous occasions from normal beagles. One might speculate that, if a certain host can be infected with a given virus, his tissues could be expected to support its growth in vitro. However, evidence contrary to this supposition is available in the literature. Green, Lieberman and Mogabgab[10], working with human influenza viruses, reported that attempts to propagate influenza A or B in cultures of conjunctival fibroblasts, KB, HeLa or embryonic skin, all of human origin, were uniformly unsuccessful regardless of amount of virus inoculated or previous passage history. Growth of influenza C also could not be demonstrated in any of these cells. In a similar report, McConnell et al.[11] isolated a neurotropic agent from tissues of adult rats in L929 and mouse kidney cells, but obtained no growth of the virus in rat kidney cell cultures. It is evident, therefore, that cultures of cells of a host do not necessarily have the capability of growing viruses which infect his species.

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