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DISSERTATION

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by


The Ohio State University

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Approved by

[Signature]
Adviser
Department of Veterinary Pathology
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VITA

April 22, 1939 • Born—Toledo, Ohio

1963 . . . . . . D.V.M., The Ohio State University, Columbus, Ohio

1965 . . . . . . M.Sc., The Ohio State University, Columbus, Ohio

1963-1967 . . . Postdoctoral Fellow, Department of Veterinary Pathology, The Ohio State University, Columbus, Ohio

PUBLICATIONS

"Host Range Studies of Two Porcine Polioencephalomyelitis Viruses."

FIELDS OF STUDY

Major Field: Veterinary Pathology

Studies in Comparative Neuropathology.

Professor Adalbert Koestner

Studies in Animal Oncology. Professor Adalbert Koestner

Studies in Animal Virology. Professors Louis Kasza and

Richard A. Griesemer

Studies in Medical Pathology. Professor Dante G. Scarpelli
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CHAPTER I

EFFECTS OF A PATHOGENIC PORCINE ADENOVIRUS ON PRIMARY AND ESTABLISHED PORCINE KIDNEY CELL CULTURES

Introduction

In 1966 Kasza (15) reported the isolation and characterization of an adenovirus from the brain of a pig with signs and lesions of encephalitis. The virus was serologically unrelated to several types of human and animal adenoviruses (15). The pathogenicity of the virus for germfree and pathogen-free pigs has recently been demonstrated (see Chapter III). Other porcine adenovirus isolates have recently been reported (4, 12, 13, 16, 17) but there is no detailed description of their sequential effects on cell cultures. In addition, many studies may have been hampered by the fact that primary porcine cell cultures are often contaminated with adenoviruses. A description of the effects of the porcine adenovirus isolated in this laboratory on porcine kidney cells can provide a basis for comparing cellular lesions caused by this porcine adenovirus with lesions caused by other animal and human adenoviruses and can assist in understanding the events occurring during porcine adenoviral replication. By comparing the effect of the virus on primary and established porcine kidney cells it can be shown that the established cell culture is useful for the study of a porcine adenovirus.
The objectives of this study were:

(1) To describe the sequential development of the lesions produced by our porcine adenovirus in primary porcine kidney cell cultures and in an established porcine kidney cell line.

(2) To develop an immunofluorescence technique for demonstrating the viral antigen.

(3) To provide cytochemical data for correlation with the results of a parallel study of the ultrastructural alterations caused by this virus.

Materials and Methods

Virus

The stock virus used in this study was in its ninth passage in primary porcine kidney cell cultures. The TCID$_{50}$/ml was $10^{3.2}$ as indicated by the cytopathic effect in primary porcine kidney cells on the fourteenth day after inoculation.

Porcine kidney cell cultures

Primary monolayer cell cultures were prepared from swine kidney cells by trypsinization following the technique described by Youngner (23). The cells were grown on coverslips in Leighton tubes containing 2.0 ml of medium. The components of the medium have been described previously (14).

A swine kidney cell line was established from a primary swine kidney cell culture. The primary culture was removed from the culture flasks using 0.25% trypsin and the cells were resuspended in fresh medium to their original concentration. The cells were
transferred in this manner for 10 passages before significant proliferation occurred. At this time the proliferative capacity improved.

Cells were diluted to 50,000 cells/ml using growth medium. This cell suspension was dispensed into Leighton tubes (2.0 ml each) and 100 ml prescription bottles (20.0 ml each). The medium was changed on the third day and the cultures were confluent on the fifth to sixth day. Medium was changed again in the cultures destined to be used to prepare the next passage and a new passage was prepared on the tenth day.

This swine kidney cell line is now in its eighty-first passage. The cells multiply readily under the conditions described and form uniform monolayers of epitheloid cells in 5-6 days. The cultures can be satisfactorily maintained without transfer or medium change for 12-14 days. With weekly medium change the cultures survive at least 6 months without transfer. Hypogammaglobulinemic calf serum can be substituted for the normal calf serum without noticeable adverse effect but when rabbit serum is used at the same concentrations the cells degenerate in 7-9 days.

Numerous samples of this cell line have been examined for contaminating microorganisms using May-Grünwald Giemsa stain, acridine orange fluorochrome, and immunofluorescence. Samples have also been inoculated onto mycoplasma medium according to the technique of Girardi et al. (9) and also into various cell cultures including primary porcine kidney cell cultures, canine melanoma
and canine thyroid adenocarcinoma cell lines. No adventitious organisms have been detected by these methods.

**Inoculation**

When the monolayers were confluent the cultures were inoculated by removing the old medium, adding 0.2 ml of adenovirus stock, incubating one hour at 37 C, and adding 2.0 ml of fresh medium. Coverslips were removed at the times indicated below.

**Immunofluorescence techniques**

Indirect immunofluorescence was performed using the technique of Boyer et al. (6). Antiviral globulin was prepared by standard techniques from the serum of rabbits immunized by three weekly intravenous injections of virus grown in primary swine kidney cells. Commercially prepared and conjugated goat antirabbit globulin was used. A modification of this technique involved the use of globulin prepared from the serum of pigs one month after intranasal or oral inoculation of the virus suspension. In this case commercially labeled rabbit antipig globulin was employed. A direct technique was also used in which the convalescent pig globulin was labeled with 0.03 mg fluorescein isothiocyanate per 1 mg protein. The conjugated protein was separated from the unbound dye by eluting it with phosphate-buffered saline (pH 7.2) from a Sephadex column (G-25 resin).

Cells were fixed in cold acetone and washed in phosphate-buffered saline (pH 7.2). Slides were incubated at 37 C for 30-40 minutes in moist chambers, mounted in buffered glycerin and
examined immediately. Rhodamine-conjugated bovine serum albumin was included in all labeled globulins as a counter-stain.

The following controls were used:

1. Uninfected tissue culture cells

2. Rabbit antikidney-cell-culture globulin instead of rabbit antivirus globulin

3. Rabbit antivirus globulin after incubation for one hour at 37°C with excess virus instead of uninhibited rabbit antivirus globulin

4. Rabbit antihuman-adenovirus (types 1-4) globulin instead of rabbit antivirus globulin

5. Labeled goat antirabbit globulin globulin alone

6. Porcine antikidney-cell-culture globulin instead of porcine antivirus globulin

7. Porcine antivirus globulin after incubation for one hour at 37°C with excess virus instead of uninhibited porcine adenovirus globulin.

The direct and indirect methods in which convalescent pig serum was used consistently gave better results than the indirect method using rabbit serum. The advantages consisted of less non-specific staining of cell cytoplasm and brighter, better defined fluorescing material. The two methods using pig globulin were equally satisfactory and one or the other was routinely used in this study.
Acridine orange stain

The technique described by Mayor was used (18). Best results were obtained when staining times in acridine orange were reduced to 6 or 8 minutes from the 10 minutes suggested by Mayor.

Phase microscopy

Coverslips were removed from the tubes, mounted in a drop of culture medium, ringed with fingernail polish and examined immediately.

Enzyme digestion

Enzyme digestions using deoxyribonuclease, ribonuclease and pepsin were performed according to the description of Mayor (18).

Collection of cells for staining

Coverslips were removed for staining at 7, 12, 18, 24, 36, 48, 72, 96, 102, 120, 126, 134, 144, 168, 192, and 216 hours after inoculation. All staining procedures were begun immediately except for the May-Grünwald Giemsa stain. Cells destined for this procedure were fixed in absolute methyl alcohol and stained within 3 days.

Cell enumeration

At least 1000 cells were counted on each of two coverslips from each collection (May-Grünwald Giemsa stain). Since the early cytopathic effect tended to occur along the edges of the coverslip the following counting procedure was adopted. An eyepiece insert was used which covered an area 0.1 x 0.1 mm. Counting was begun at
one edge of the coverslip. After all the nuclei included in the
square had been counted the slide was advanced 1 mm and another
count taken. When the opposite edge of the coverslip was reached
the slide was moved 1 cm and the count continued. This was repeated
until at least 1000 cells had been counted and the slide had been
completely traversed at least three times. Counts were never dis­
continued until an edge was reached. During the counting procedure
the cells with nuclear lesions were counted and categorized
according to their morphologic type.

Results

Effects of the virus on porcine kidney cells

Different cellular lesions first appeared at different times
following inoculation and cells showing these lesions predominated
during particular periods of time. The time at which a lesion first
appeared was the criterion used to define the lesion as an early,
middle, or late manifestation of virus effect. The length of time
a particular lesion predominated was used to determine the length
of the early, middle, and late phases. These procedures were
adopted since the cytopathic effect was asynchronous and, thus,
except in the earliest phase, cells with lesions characteristic of
any phase were found at any time. Using these criteria the effects
of this virus on porcine kidney cells were divided into three phases.

Early phase.--The earliest time at which lesions were
regularly detected was 18 hours after inoculation. Before this time
affected cells were found only occasionally and only in the primary
cultures.
The first lesion observed was a moderate nuclear enlargement (2-3 times normal size) with derangement of the nuclear chromatin (Fig. 1). The nucleus fluoresced intensely green with acridine orange, demonstrating a diffuse granular pattern of deoxyribonucleic acid (DNA) which nearly filled the nucleus. The nucleoli were intensely flame red and had increased in size proportionally with the nucleus. In many cells the nucleoli were no longer identifiable, but many small masses of intensely red ribonucleic acid (RNA) were seen scattered among the DNA and grouped beneath the nuclear membrane (Fig. 6). These cells did not contain antigen. When stained with May-Grünwald Giemsa these cells were identifiable by the increased size and basophilia of their nuclei.

This phase appeared the same in both the primary and established kidney cell cultures and it lasted only about 6-12 hours. Very few cells were affected, the number ranging from 0.17 to 0.38% in different slides examined. The cellular alterations described above were the ones seen most frequently during this time.

At 24 hours cells were observed in which the nucleus stained intensely for RNA. The nucleus was somewhat enlarged with a diffusely red, slightly granular or filamentous appearance. This red material was sensitive to the action of ribonuclease and was completely removed before nucleolar and cytoplasmic RNA were entirely removed. Cells such as these were seen in the later part of the early phase and throughout the middle phase.

Another cellular lesion was seen at 24 hours in which there was an intense red cytoplasmic fluorescence and a diffuse green
Fig. 1. Acridine orange. Primary porcine kidney cell culture—18 hours after inoculation. The cell has an enlarged nucleus with an intense green fluorescence and a granular pattern typical of the earliest phase of viral effect which occurs from 18 to 24 hours after inoculation.

Fig. 2. Immunofluorescence. Primary porcine kidney cell culture—24 hours after inoculation. Bright fluorescence of the nucleus and numerous fluorescent cytoplasmic granules. Many of these cells were found between 18 and 36 hours after inoculation.
nuclear fluorescence using acridine orange. When the immunofluorescence technique was applied these cells had numerous brightly fluorescing flecks of antigen in the cytoplasm as well as a brilliant nuclear fluorescence (Fig. 2).

**Middle phase.**—By 36 hours after inoculation cells with another type of lesion predominated. Here the intranuclear chromatin was arranged in a distinctly reticular pattern when stained with acridine orange (Fig. 3). In the early stages of this phase many cells had an enlarged nucleus with multiple, thin, orange to red threads of RNA criss-crossing the nucleus with unstained areas between them. This appeared to be the transitional phase between the nucleus with diffuse bright red fluorescence (RNA) and the next phase in which these threads fluoresced intensely green (DNA). The areas unstained with acridine orange fluoresced bright green with the immunofluorescence technique (Fig. 4). Cells with an exaggerated reticulated pattern accompanied the predominant lesion of this phase. Here the nucleus was markedly enlarged and had a vacuolated appearance when stained with acridine orange or May-Grünwald Giemsa. The threads of DNA were thin and much fewer in number and the vacuoles were large. The pattern with immunofluorescence was the same, the antigen appearing early in the vacuoles and later in the threads surrounding the vacuoles. These cells with the reticulated pattern had thickened nuclear membranes to which clumps of DNA were attached.

Cells with this reticulated pattern of intranuclear chromatin and antigen predominated in the primary kidney cell.
Fig. 3. Acridine orange. Established porcine kidney cell culture—36 hours after inoculation. Enlarged nucleus with threads of brightly green fluorescing material (DNA) and scattered small masses of red fluorescing material (RNA) producing a distinctly reticulated appearance. Bright clumps of material on the nuclear membrane are also DNA. Nucleoli (arrows) fluoresce red. Cells with this type of lesion were characteristic of the middle phase of the virus effect.

Fig. 4. Immunofluorescence. Established porcine kidney cell culture—36 hours after inoculation. Nucleus has numerous clumps and granules of brightly fluorescent material representing viral antigen. Cells with this distribution of antigen were typical of the middle phase of viral effect.
cultures until the 134th hour. During this time the number of affected cells increased from about 0.75% to 25% of the cells on the coverslip. In addition, many cells had been lost from the coverslip so that by 134 hours there were only about 0.325 million cells on a coverslip compared to 1.5 million at 36 hours or in control preparations at 134 hours (Fig. 5).

A similar picture was seen in the porcine kidney cell line except that the cells with the reticulated nuclear pattern predominated only until 120 hours. At this time the number of affected cells was 15.5% of the cells still remaining on the coverslip. There were about 0.75 million cells on the coverslip at 120 hours compared with 1.5 million at 36 hours and 2.3 million in the control at 120 hours (Fig. 5).

These times (134 hours for primary kidney cells and 120 hours for the kidney cell line) were taken as the end of the middle phase of the cytopathic effect. However, cells with lesions typical of the late phase had appeared many hours previously, so there was a long transition period during which cells with lesions typical of both middle and late phases were present but cells with the reticulated nuclear pattern predominated. Typical adenoviral cytopathic effect as seen by ordinary light microscopy of unstained cells also appeared during this transition period. A distinct effect was first noted at 120 hours in both types of cultures and small foci of rounded cells were observed at 102 hours.

Late phase.—The cellular lesion predominating in this phase consisted of a large, basophilic, centrally located nuclear mass
Fig. 5. Development of porcine adenovirus cytopathic effect in primary and established porcine kidney cell cultures. Open symbols show the number of cells with inclusion bodies as a per cent of the cells remaining on the coverslip. Solid symbols show number of cells on coverslips from infected cultures as a per cent of the number of cells on coverslips from control cultures. At least 1000 cells were counted on each of two coverslips for each determination. The curves were drawn by inspection.
PORCINE ADENOVIRUS CYTOPATHIC EFFECT

PER CENT CELLS REMAINING ON COVERSIP

ESTABLISHED PORCINE KIDNEY CELL CULTURE

PRIMARY PORCINE KIDNEY CELL CULTURE

PER CENT CELLS WITH INCLUSION BODIES

HOURS AFTER INOCULATION

Fig. 5
separated from a thickened nuclear membrane by a distinct, sharply
demarcated zone which was not stained by the May-Grünwald Giemsa
technique. Acridine orange staining revealed an intense yellow-
green fluorescence of the central nuclear mass and clumps and
patches of slightly less brilliant green in the zone beneath the
nuclear membrane and attached to it (Fig. 6). There was an indi-
cation of reticulation in the central mass and immunofluorescence
demonstrated an irregular distribution of viral antigen. The most
intense fluorescence of viral antigen was usually observed in the
zone between the nuclear membrane and the central mass. Here a
homogeneous bright fluorescence was seen which often markedly
exceeded in intensity the fluorescence of the central mass (Fig. 7).

Cells with this type of alteration appeared to develop from
the reticulated pattern described earlier. As early as 36 hours
after inoculation a few cells showing a small zone of demarcation
and a central nuclear mass with a distinct reticulated pattern were
found in both types of cultures. The number of cells with this type
of lesion increased continuously from this time on. In the primary
cultures the number increased from about 3% of affected cells at 36
hours to 55% at 134 hours. In the porcine kidney cell line the
percentage of cells showing this pattern was consistently higher.
Beginning at 36 hours 40% of the affected cells had a clearly
demarcated central mass and by 120 hours 65% of the affected cells
had such a lesion.

As time progressed these cells became more round and began
to detach from the glass. They were seen on the surface of the
Fig. 6. Acridine orange. Primary porcine kidney cell culture—13\(^\frac{1}{4}\) hours after inoculation. The cell in the center contains an intensely green fluorescent central mass separated from the nuclear membrane by a clear zone containing clumps of brilliant green fluorescent material. Cells with such lesions were typical of the late phase of virus effect. The cells to the right (arrows) have numerous scattered clumps of DNA and RNA typical of the early phase of viral effect and illustrating the asynchronous development of viral cytopathic effect.

Fig. 7. Immunofluorescence. Primary porcine kidney cell culture—13\(^\frac{1}{4}\) hours after inoculation. Bright fluorescing material representing viral antigen concentrated primarily in the zone between the central nuclear mass and the nuclear membrane. The central mass also fluoresces. This distribution of viral antigen was typical of the late phase of viral effect.
monolayer where they displayed none of the morphologic patterns already described. Instead the entire cell fluoresced intensely green with both acridine orange and immunofluorescent techniques. They were easily detached by gentle washing and probably soon detached spontaneously and were lysed in the culture medium.

Rounding and detachment of cells continued steadily and nearly all the cells were gone from the coverslip by 168 hours in the primary cell cultures and by 192 hours in the kidney cell line.

Phase contrast microscopy of cultures at various phases of infection did not reveal additional morphologic alterations other than those already described. The reticulated nuclear pattern seen after fixation was not seen in living cells. Instead the living cells had only an enlarged nucleus with diffusely distributed contents. The darkened nuclear membrane, rarefied zone and distinctly demarcated central mass were easily demonstrated in unfixed cells.

Treatment of control cells with deoxyribonuclease and ribonuclease followed by staining with acridine orange confirmed the specificity of this stain. Ribonuclease treatment of infected cells consistently removed all red fluorescing material, the intranuclear material seen in the late part of the early phase and in the middle phase being especially sensitive to this treatment. Deoxyribonuclease removed or markedly diminished the yellow-green fluorescence in all cells. In virus infected cultures bright green fluorescing clumps of material were found even after deoxyribonuclease treatment in cells with distinct central masses. This
fluorescing material was present near the nuclear membrane or irregularly scattered through the central mass. Pretreatment with pepsin followed by deoxyribonuclease removed all remaining green fluorescence.

**Discussion**

There are several reports on sequential cellular changes caused by human adenoviruses (2, 7, 8, 10). Various morphologic patterns have been described and to some extent have provided another method of grouping adenoviruses (7). The general features of the lesions produced by this porcine adenovirus in swine kidney cells resemble but do not duplicate those produced by the human adenoviruses. Many features of the alterations reported here differ from those reported for human adenoviruses. The porcine adenovirus cytopathic effect progresses slowly in comparison with many of the human infections. This may be solely an effect of virus dose, since 100 infectious human adenovirus particles per cell are sometimes used to initiate infection compared with one infectious particle per 500-750 cells used here. The formation of a few large acidophilic, intranuclear granules which subsequently coalesce to form a dense central mass was not a characteristic feature of the porcine adenovirus effect, although an occasional cell with large granules was seen. No cellular crystals such as those described for human adenovirus type 5 (3) were seen in our material and no "toxic" substance producing early cell degeneration (21) was present. The distribution of viral antigen near the periphery of the nucleus
was typical of the late stages of the porcine adenoviral effect and is also reported in the studies of human adenoviruses (6).

The lesions reported here resemble more closely those reported for some other animal adenoviruses. The large central mass with a slightly reticulated or finely granular pattern resembles some stages described in the development of the canine hepatitis virus (1) and some other porcine adenoviruses (4, 16). The early presence of cytoplasmic as well as nuclear antigen in some cells has also been reported for canine hepatitis virus (19). These may represent cytoplasmic sites of antigen synthesis, an idea which conflicts with most reports on the cellular sites of adenovirus synthesis but which is supported by recent biochemical findings (22).

The early appearance of deranged nuclear chromatin and the increased green nuclear fluorescence after acridine orange correlates well with morphologic and biochemical evidence indicating that viral DNA synthesis occurs early in infected cells (1, 5, 8, 11). The accumulations of RNA seen so frequently slightly later in the development of the cellular lesions may represent the labile nuclear RNA described by Mayor (18).

We also found, as did Mayor (18), that DNA susceptible to DNase only after pepsin pretreatment appeared later and in small amounts, suggesting that mature virus particles are formed after viral DNA, viral coded RNA and antigen synthesis have all occurred. It also appeared that only a relatively small amount of mature virus was assembled from the large quantities of DNA and antigen present
in the cell. There is ample biochemical evidence in other adeno-
virus-cell systems to support these findings (11, 20).

The slight differences observed in the development of lesions
in primary porcine kidney cells and porcine kidney cell line were
not considered significant. The slightly delayed cytopathic effect
as seen by light microscopy in the cell line may be explained by
the fact that there were more cells per coverslip than in the
primary kidney cell cultures. The earlier appearance and relative
predominance of cells with distinctly demarcated central masses in
the cell line may be explained by suggesting that the primary kidney
cells tend to become round and detach from the glass more rapidly
and therefore are lost for observation. On the other hand, since
the ring between the central mass and the nuclear membrane con-
sistently contained large quantities of antigen it may be that
these cells synthesize and/or accumulate antigen more readily than
primary swine kidney cells.

The results of this study of a pathogenic porcine adenovirus
are compatible with the biochemical and morphologic data reported
for human adenovirus-cell systems. The cytochemical and immuno-
fluorescence methods employed here provided data which correlated
well with the results of a parallel study of the ultrastructural
alterations produced by this virus in porcine kidney cells (Koestner,
Kasza, Kindig, and Shadduck—unpublished data). The established
swine kidney cell line was useful in studying this virus because of
its uniform growth characteristics, morphology and response to the
virus.
Summary

The sequential effects of a pathogenic porcine adenovirus upon primary and established porcine kidney cell lines were described. Changes in distribution, type and relative amount of nucleic acids in these cells were studied using acridine orange fluorochrome. Presence and distribution of antigen was detected by immunofluorescence techniques. Convalescent porcine globulin was found to be better for this purpose than globulin from hyperimmune rabbits. Using these techniques the effects of this virus were divided into three phases. The early phase consisted of derangement of nuclear chromatin and an increase in the relative amount of nuclear RNA. Little to no antigen was detected in this stage, which began about 12-18 hours after infection and lasted 6-12 hours. The middle phase began 24-36 hours after infection. Cells with a reticulated intranuclear pattern were typical of this stage. These cells had numerous fine threads of chromatin with clumps of antigen between them. By 120 hours in the cell line and 134 hours in the primary cultures cells with alterations typical of the late phase predominated. These cells had a dense or finely granular central mass separated from a thickened nuclear membrane by a clear space. The central mass stained intensely for DNA and there were clumps of DNA in the clear zone. Viral antigen was irregularly distributed through the central mass but was prominent in the clear zone between the central mass and the nuclear membrane. By 168 hours in the primary cultures and 192 hours in the established cell line nearly all the cells had rounded and detached from the glass. No
significant differences were observed between the effects of this virus on primary porcine kidney cells and on the established porcine kidney cell line. The alterations described here are compatible with cytochemical and biochemical data obtained in other adenovirus-cell systems.
CHAPTER II

PORCINE ADENOVIRUS INFECTION: DISTRIBUTION OF THE VIRUS
AFTER INFECTION OF EXPERIMENTAL ANIMALS

Introduction

Previous reports on this porcine adenovirus have dealt with its isolation from the brain of a pig with signs and lesions of encephalitis (9) and its effect on porcine kidney cell cultures (see Chapter I). The increasing number of reports (1, 3, 6, 7) of the isolation of porcine adenoviruses from many parts of the world indicate that these agents are widely distributed in the swine population. The fact that these viruses are frequently isolated from primary kidney cell cultures prepared from kidneys of apparently normal animals (12, 14) suggests that latent infections with these agents are not uncommon. There are no reports of experimental infections of pigs with swine adenoviruses.

The objectives of this study were:

(1) To determine the presence of this porcine adenovirus in a selected number of tissues collected from experimentally infected pigs.

(2) To provide virologic data to assist in the interpretation of the lesions produced by the virus in pigs.

(3) To contribute to the knowledge of the animal host range of the virus.
Materials and Methods

Virus

The virus stock used was in its ninth or tenth passage in primary swine kidney cell cultures. The virus suspensions were frozen and thawed five times prior to inoculation and the suspensions used for intracerebral inoculation were centrifuged at 1000 g for 10 minutes to remove some of the cell debris. The titers of the viral inocula were $10^{3.2}$ to $10^{3.7}$ TCID$_{50}$/ml as indicated by the cytopathic effect in primary swine kidney cultures 14 days after inoculation. Control inocula were prepared in an identical manner using uninfected cell cultures.

Experimental swine

A total of 21 pigs were inoculated with the porcine adenovirus and an additional 10 pigs were used as controls. Each litter was divided into an infected and a control group and these groups were housed separately in flexible-film isolators. Eight germfree and 13 pathogen-free pigs were used. They are treated here as one group since no differences between them could be detected. The techniques used to raise these animals and the microbiologic criteria used to distinguish germfree and pathogen-free pigs have been described (13). All animals were colostrum-deprived and all were inoculated within 48 hours after birth.

Inoculation of pigs

Pigs were exposed by one of four routes of inoculation. Intranasal inoculation (8 infected and 3 control pigs) was performed
by holding the pigs with their heads upright and slowly dripping 3 ml of undiluted inoculum into the external nares. The entire dose appeared to be retained. One of the 8 pigs was exposed by contact to the intranasally inoculated pigs. Intracerebral inoculation (7 infected and 4 control pigs) was performed by injecting 1 ml of undiluted inoculum (after centrifugation) into the left frontal lobe through a 23 ga needle. Pigs inoculated orally (3 infected and 2 control pigs) were held vertically and 5 ml of undiluted inoculum was administered slowly into the side of the mouth which was held closed around the syringe (13). Intraperitoneal inoculation (3 infected and 1 control pig) was performed by injecting 5 ml of undiluted inoculum into the peritoneal cavity 1 cm posterior and lateral to the umbilicus.

Clinical and post-mortem examinations

All pigs were examined at least twice daily for signs of disease. This included determining rectal temperature and physically handling the animals with particular attention to signs of neurologic disorders. At necropsy tissues were observed for gross lesions and representative sections of all organ systems were collected for histologic examination. The results of these investigations are reported elsewhere (see Chapter III).

Tissue collection

Animals were killed by electrocution and serum and selected tissues were immediately collected aseptically. Cortex, cerebellum, medulla, and ileum were collected from 5 pigs (G193 through G197)
inoculated intracerebrally. Cortex, cerebellum, medulla, nasal mucosa, lung, tonsil, and ileum were collected from 7 pigs (H994, H996, H997, H998, H1008, H1009, and H1011) inoculated intranasally and from one pig (H993) exposed to the virus by contact with inoculated pigs. Cortex, cerebellum, medulla, lumbar spinal cord, heart, lung, liver, spleen, kidney, adrenal, tonsil, mesenteric lymph node, ileum, colon, whole blood, cerebrospinal fluid, and nasal, pharyngeal and rectal swabs (collected weekly) were collected from 3 pigs (K566, K567, and K568) inoculated intracerebrally, 3 pigs (K1398, K1399, and K1400) inoculated orally and from 3 pigs (K1401, K1402, and K1403) inoculated intraperitoneally. The same tissues were collected from the litter-mate controls of each group.

**Host range studies**

The following animals and routes of inoculation were used. Chicken embryos were inoculated by one of three routes: intracerebrally (10 infected and 10 controls), on the chorioallantoic membrane (6 infected and 3 controls), or into the yolk sac (6 infected and 3 controls). One week after inoculation the tissue into which the virus had been inoculated was collected, suspended in Hanks' balanced salt solution (10% suspension) and reinoculated by the same route into another group of embryos. One week later the procedure was repeated. Mature rhesus monkeys (2 infected and one control) were each inoculated intrathalamically, intraspinally, and intramuscularly. Newborn mice (10 infected and 10 controls) were inoculated intracerebrally. One week later the brains and spinal
cords were collected and 10% tissue suspensions were prepared and reinoculated intracerebrally into another group of newborn mice. One week later the procedure was repeated. Weanling mice, rabbits, and guinea pigs (10 infected and 10 controls for each species) were each inoculated intracerebrally and intraspinally. Weanling Sprague-Dawley and cotton rats (5 infected and 5 controls for each strain) were inoculated intracerebrally and intraspinally. Brain, chorioallantoic membrane, and yolk sac from the eggs inoculated by these routes respectively, and central nervous system, spleen, and intestine from all other animals were prepared as 10% suspensions and passed three times in established swine kidney cell cultures to detect the presence of the virus. Details of the methods of inoculation, serial passage of the virus in the animals and virus isolation have been described (15).

**Virus isolation**

Ten per cent tissue suspensions (weight-volume) were prepared using Hanks balanced salt solution with antibiotics and sodium bicarbonate buffer as previously described (10). Two-tenths ml of each tissue suspension was inoculated into each of five Leighton tubes containing confluent monolayers of either primary or established porcine kidney cells. These cultures are equally suitable for detecting the virus (Shadduck and Kasza—unpublished observation). Incubation was carried out for one hour at 37 C after which 2.0 ml of maintenance medium was added. The maintenance medium was prepared as previously described (10) except that 2% hypogammaglobulinemic calf serum was used instead of bovine serum. Cultures were incubated
for 14 days. Slides were removed on the seventh and tenth post-
inoculation days, stained with May-Grünwald Giemsa stain and
examined for cytologic evidence of virus infection. At 14 days
postinoculation the cells were scraped or shaken from the tubes,
collected and stored for the next passage. Subsequent inoculations
were carried out exactly as above. All specimens were passed at
least three times in cell cultures before being considered negative.
The identity of all isolates was confirmed using the immuno-
fluorescence techniques previously described (see page 4).

Results

The presence of the virus in the tissues of experimentally
inoculated pigs is shown in Table 1. Virus was isolated from one
or more tissues of 19 of the 21 inoculated pigs. The lungs of 7 of
the 17 pigs tested contained virus as did the nasal mucosa of 6 of
the 8 pigs tested. In the nervous system the virus was isolated
from the cerebral cortex of 11 of 21 pigs tested, twice from cere-
bellum and once from lumbar spinal cord, but it was never isolated
from medulla or cerebrospinal fluid. Virus was present in the ileum
of 14 of 21 pigs and in the colon of 3 pigs while the virus was
present in the tonsils of 10 of 17 pigs and the mesenteric lymph
nodes of 7 of 9 pigs. In addition virus was isolated from kidneys
(4 of 9 pigs) and liver and spleen (once each from 9 pigs). Virus
was not isolated from heart, whole blood, or swabs from infected
pigs nor was it isolated from the tissues or swabs from control pigs.

The virus was isolated most consistently and from the
greatest variety of tissues from the pigs inoculated intranasally.
TABLE 1. Distribution of porcine adenovirus after inoculation of experimental pigs

<table>
<thead>
<tr>
<th>Animal</th>
<th>Route of Inoculation</th>
<th>Days Post-inoc.</th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Nasal Mucosa</th>
<th>Lung</th>
<th>Kidney</th>
<th>Ponsal Mesenteric Lymph Node</th>
<th>Ileum</th>
<th>Colon</th>
<th>Other Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(H993)</td>
<td>i.n.</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2(H998)</td>
<td>i.n.</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3(H1008)</td>
<td>i.n.</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(H994)</td>
<td>i.n.</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5(H1009)</td>
<td>i.n.</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6(H996)</td>
<td>i.n.</td>
<td>13</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7(H1011)</td>
<td>i.n.</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8(H997)</td>
<td>i.n.</td>
<td>48</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>No. pos/No. tested</td>
<td>7/8</td>
<td>6/8</td>
<td>7/8</td>
<td>8/8</td>
<td>7/8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9(GL97)</td>
<td>i.e.</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10(K567)</td>
<td>i.e.</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11(K568)</td>
<td>i.e.</td>
<td>14</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>cord</td>
<td></td>
</tr>
<tr>
<td>12(GL93)</td>
<td>i.e.</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13(GL94)</td>
<td>i.e.</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14(GL96)</td>
<td>i.e.</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15(K566)</td>
<td>i.e.</td>
<td>28</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>No. pos/No. tested</td>
<td>3/7</td>
<td>1/7</td>
<td>0/3</td>
<td>2/3</td>
<td>2/3</td>
<td>3/3</td>
<td>5/7</td>
<td>2/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16(K1400)</td>
<td>i.o.</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>liver</td>
</tr>
<tr>
<td>17(K1399)</td>
<td>i.o.</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18(K1398)</td>
<td>i.o.</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>No. pos/No. tested</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3</td>
<td>0/3</td>
<td>2/3</td>
<td>1/3</td>
<td>1/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19(K1403)</td>
<td>i.p.</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>spleen</td>
</tr>
<tr>
<td>20(K1402)</td>
<td>i.p.</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21(K1401)</td>
<td>i.p.</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>No. pos/No. tested</td>
<td>1/3</td>
<td>1/3</td>
<td>0/3</td>
<td>1/3</td>
<td>0/3</td>
<td>2/3</td>
<td>1/3</td>
<td>0/3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = positive virus isolation; - = no virus isolated; blank space = not tested; i.n. = intranasal; i.c. = intracerebral; i.o. = intraoral; i.p. = intraperitoneal; cord = lumbar spinal cord.
Thirty-five of the 40 tissue samples tested contained virus. In 5 of the 8 animals virus was present in all 5 of the tissues examined and virus was never absent from more than 2 of these 5 tissues. In all but one of these pigs virus was found in the brain. Likewise, it was present in lung or nasal mucosa or both in all but one pig. Virus was always found in the tonsils of these animals and absent in only one sample of ileum. Virus was present in all 5 tissues tested from a pig exposed by contact to the intranasally inoculated pigs.

In pigs inoculated by other routes the virus was usually present in 4 or 5 of the tissues tested. In the pigs inoculated orally or intraperitoneally virus was most often detected in tissues collected from pigs killed on the seventh postinoculation day. In pigs inoculated intracerebrally virus was found most often in tissues of the pigs killed at 7 and 28 days after inoculation.

The virus remained for several weeks in the tissues of inoculated pigs. It was detected in all 5 tissues examined from a pig 48 days after intranasal inoculation and was present 28 days after intracerebral or oral inoculation. Virus was not found in the tissues of a pig 28 days after intraperitoneal inoculation.

Virus was only rarely isolated from tissue suspensions on the first passage in kidney cell culture and occasionally none was detected until the third passage. In most specimens the cytopathic effect was minimal in the first positive culture and one or two more passages were required before the characteristic cytopathic effect appeared with complete destruction of the monolayer within 168 hours.
For these reasons titration of the virus in original tissue suspensions was not attempted.

In the host range study virus was detected in the brains of the embryos inoculated intracerebrally and in the chorioallantoic membrane of the embryos inoculated by this route. Virus was not found in the chicken embryos inoculated with tissue suspensions prepared from previously infected embryos nor was it found in tissues of any of the other animals studied.

Discussion

The high frequency with which virus was isolated from a variety of tissues following intranasal inoculation indicates that this route is the best of those used for viral propagation and/or dissemination. The necessity for several subcultures to detect the presence of the virus in many of the tissues made it difficult to identify the site or sites of primary viral multiplication. The data indicate, however, that respiratory and lymphoid tissue are among the important tissues for virus replication. The relatively long time during which upper respiratory tissue and lymphoid tissue of the oropharynx are directly exposed to the virus after intranasal inoculation may be important for the propagation and dissemination of the virus. Intranasal exposure is the best route tested for the production of lesions (Table 2) and may be one of the important natural routes of infection. The presence of the virus in all 5 of the tissues examined from a pig which was exposed by contact for 5 days to pigs inoculated intranasally shows that direct inoculation is not required for infection.
It is also significant that the virus was, with one exception, present in the brain of all pigs inoculated intranasally. This was not true for other routes of inoculation. Only one animal inoculated intraperitoneally and none of the animals inoculated orally had virus in the nervous tissue. It is apparent that intranasal exposure often results in cerebral infection and this may be important in naturally occurring infections.

The role of the brain as a site of viral multiplication is not clear. In these experiments virus was isolated from the brain as early as 5 days after intranasal contact exposure or intracerebral injection of the virus. The virus was present in other organs from 7 to 28 days after it had been injected intracerebrally, but was not as frequently detected after intracerebral as after intranasal inoculation. It could not be determined whether the virus left the brain soon after intracerebral injection and multiplied in other sites, such as lymphoid tissue or intestine, and then returned to the brain or whether the primary early site of viral replication was the brain with subsequent infection of other organs. It is of interest that respiratory tissue was not infected after intracerebral inoculation.

Oral and intraperitoneal inoculation of the virus resulted in early dissemination and the virus was detected in brain, lymphoid and intestinal tissues of pigs 7 days after inoculation by these routes. In this respect lymphoid tissue and intestine cannot be ignored as possible sites of viral replication. In addition to being infected early after oral and intraperitoneal inoculation they
nearly always contained virus after intranasal inoculation and were also infected following intracerebral inoculation. The fact that these tissues were not regularly infected later than 7 days after inoculation except when respiratory tissue or brain still contained virus suggests that other sites of viral replication may be required to maintain the infection of lymphoid tissue and intestine.

The presence of the virus in the kidneys of 4 animals 14 to 28 days after inoculation re-emphasizes the known importance of "latent" adenoviruses as potential contaminants of primary porcine kidney cell cultures (12, 14). The incidence of kidney infections might have been much higher if the kidneys of all animals, especially those exposed intranasally, had been examined.

The prolonged presence of the virus in the tissues of inoculated pigs is also of interest. The fact that the virus can be isolated from brain, lung, and other tissues for as long as 48 days after inoculation raises questions concerning the tissue sites in which the virus survives, the role of humoral antibody in the maintenance of infection, the possibility that these animals may serve as sources of infection to other pigs and, most importantly, the role of this virus during the course of other natural or experimental diseases of swine. Experiments are now in progress in an attempt to answer some of these questions.

The host range study emphasized again the host specificity of adenoviruses (5). The few isolations which occurred from the chick embryos probably represented only survival of the virus since
it is known that the virus can survive up to 6 days at 37 °C and up to 10 days at 25 °C (9). The failure to isolate the virus beyond the first passage in these tissues also suggests survival rather than multiplication. The results of this host range study agree with the results of Haig et al. (6) using another porcine adenovirus.

Many of the reports on human and animal adenovirus infections indicate that respiratory tissue, intestine, and lymphoid tissues are good sources of these viruses in either natural or experimental infections (2, 3, 4, 11). Multiple passages are also often required for the isolation of adenoviruses. Brain has rarely been found to be infected with adenoviruses, even in those cases where it has been examined. The canine hepatitis virus can be isolated from the brains of infected dogs and foxes but histologic evidence suggests that the vascular tissue and not the nervous tissue is the source of the virus (8). Vascular tissue is not affected by the porcine adenovirus studied here (see Chapter III). Human adenoviruses have occasionally been isolated from patients with meningitis and encephalitis. It appears that the porcine adenovirus studied here is unique among the adenoviruses in its propensity for brain.

Summary

The presence of a porcine adenovirus in various tissues of pigs following several different methods of experimental infection has been determined. The virus was regularly isolated from brain,
respiratory tissue, lymphoid tissue, and intestine following intranasal inoculation. Intranasal inoculation resulted in the widest dissemination of virus of any of the routes tested. The virus was isolated from these same tissues but with decreasing frequency after intracerebral, oral, and intraperitoneal inoculation. Kidney, spleen and liver occasionally contained virus. The virus persisted for many weeks in several experimental pigs and was isolated from several tissues as late as 48 days after inoculation. The regular and repeated isolation of virus from the brain suggested that this porcine adenovirus may be unique among the adenoviruses in its propensity for brain. Following inoculation of chick embryos, rhesus monkeys, and several species of laboratory rodents, the virus was isolated only from chorioallantoic membrane and brain of inoculated chicken embryos. It could not be detected beyond one passage in these tissues.
CHAPTER III

THE LESIONS OF PORCINE ADENOVIRUS INFECTION IN GERMFREE AND PATHOGEN-FREE PIGS

Introduction

In 1966, Kasza (11) reported the isolation of an adenovirus from the brain of a pig with signs and lesions of encephalitis. The virus was serologically unrelated to several types of human and animal adenoviruses (11). The effects of this virus on porcine kidney cell cultures were described (see Chapter I) and the presence of the virus in selected tissues of pigs after experimental infection was determined (Table 1). In spite of an increasing number of reports of the isolation of porcine adenoviruses (2, 5, 6, 7, 14, 17) there have been no descriptions of the lesions produced by these viruses in experimentally or naturally infected pigs. The isolation of our virus from a pig with encephalitis suggested that the virus might be the cause of the disease.

The objectives of this investigation were:

(1) To determine the pathogenicity of the adenovirus for germfree and pathogen-free pigs.

(2) To study the nature and distribution of the lesions produced by the virus after various routes of inoculation and correlate the microscopic changes with the presence of the virus.
Materials and Methods

Inoculum

The virus stock was in the ninth and tenth passage in primary swine kidney cell cultures. The virus suspensions were frozen and thawed five times prior to inoculation. The suspensions used for intracerebral inoculation were centrifuged at 1000 g for 10 minutes to remove some of the cell debris. The titers of the viral inocula were $10^{3.2}$ to $10^{3.7}$ TCID$_{50}$/ml as indicated by the cytopathic effect in primary swine kidney cultures 14 days after inoculation. All control inocula were prepared from uninfected cell cultures using identical procedures.

Experimental swine

The porcine adenovirus was given to 25 pigs and an additional 12 pigs were used as controls. Each litter was divided into infected and control groups and these groups were housed separately in flexible-film isolators. Eight germfree and 17 pathogen-free pigs were used and are treated here as one group since no differences in reaction to infection were detected. The techniques used to raise these animals and the microbiologic criteria used to distinguish germfree and pathogen-free pigs have been described (16). All animals were colostrum-deprived and all were inoculated within 48 hours of birth.

Inoculation of pigs

Pigs were exposed by one of five routes of inoculation. Intranasal inoculation (8 infected, 3 controls) was performed by
holding the pigs with their heads upright and slowly dripping 3 ml of undiluted inoculum into the external nares. The entire dose appeared to be retained. One (H993) of the 8 pigs was exposed by contact with intranasally inoculated pigs and was killed 5 days after the other animals in the isolator had been inoculated. For intratracheal inoculation (4 infected, 2 controls) the pigs were held with their heads upright and necks extended. A 25 ga needle attached to a plastic catheter and syringe was inserted into the trachea on the ventral midline just posterior to the larynx. Proper positioning of the needle was easily determined, since the excited squealing of the pigs produced sufficient positive pressure through the inoculation apparatus to force the plunger of the syringe firmly into the operator's hand. Three ml of inoculum were injected during 1 to 2 minutes. The rate was determined by the severity of the dyspnea occurring during inoculation. After the animals were released they exhibited moderate dyspnea and cyanosis for another 5 to 10 minutes. Intracerebral inoculation (7 infected, 4 controls) was performed by injecting one ml of undiluted inoculum (after centrifugation) into the left frontal lobe through a 23 ga needle. Pigs inoculated orally (3 infected, 2 controls) were held vertically and 5 ml of undiluted inoculum was administered slowly into the side of the mouth which was held closed around the syringe. Intraperitoneal inoculation (3 infected, 1 control) was performed by injecting 5 ml of undiluted inoculum into the peritoneal cavity one cm posterior and lateral to the umbilicus.
Clinical examination

All pigs were examined at least twice daily for signs of disease. This included determining rectal temperatures and physically handling the animals with particular attention to signs of neurologic disorders.

Postmortem examination

Infected pigs were removed from the isolators and killed at the following postinoculation days (p.i.d.): intranasal inoculation—5 (H998), 7 (H1008), 10 (H994 and H1009), 13 (H996), 14 (H1011), and 48 (H997) p.i.d.; intratracheal inoculation—5 (K2317), 7 (K2316), 14 (K2315), and 21 (K2314) p.i.d.; intracerebral inoculation—5 (G197), 10 (K567), 14 (K568), 19 (G193, G194, and G196), and 28 (K566) p.i.d.; oral inoculation—7 (K1400), 14 (K1399), and 28 (K1398) p.i.d.; intraperitoneal inoculation—7 (K1403), 14 (K1402), and 28 (K1401) p.i.d. Control pigs were killed at selected intervals from 5 to 28 days after inoculation.

All animals were killed by electrocution and samples of tissues for virus reisolation were collected at once. Specimens of the following tissues were collected in liquid nitrogen for immunofluorescence: cerebral cortex, cerebellum, medulla, lumbar spinal cord, heart, lung, liver, spleen, kidney, adrenal, tonsil, mesenteric lymph node, ileum, and colon. These tissues were collected from 4 pigs inoculated intratracheally (K2314 through K2317); 3 pigs inoculated intracerebrally (K566, K567, and K568); 3 pigs inoculated orally (K1398, K1399, and K1400); and 3 pigs inoculated
intraperitoneally (K1401, K1402, and K1403). Representative portions of all organ systems from all animals were fixed in 10% buffered formalin for histologic examination. Bouin's, Zenker's, and Carnoy's fixatives were also used on selected tissues. Eyes were fixed in 6% buffered gluteraldehyde.

**Immunofluorescence**

Sections of fresh-frozen tissues were cut on a freezing microtome. A direct immunofluorescence technique using labeled convalescent pig globulin was employed as described previously (see page 4). Known positive tissue culture cells were used as positive controls. Tissue sections were also overlayed with control or convalescent unlabeled pig globulin before being incubated with the labeled globulin. Decreased fluorescence was observed following the use of convalescent but not control unlabeled globulin. Sections of tissue from control animals were similarly examined.

**Results**

**Clinical course**

Regardless of the route of inoculation, the clinical course of the disease was usually mild. The body temperatures of all the pigs fluctuated between 100 F and 104 F and there was no significant difference between control and infected pigs. No signs of encephalitis were observed in any of the animals. The only consistent clinical sign was a mild diarrhea observed in all pigs inoculated orally. Pigs inoculated by other routes did not show clinical illness except for one pig which was removed from the isolator 5 days
after intratracheal inoculation. The pig was killed in extremis after a 6-hour clinical illness consisting of increasingly severe dyspnea, cyanosis, and prostration. Its temperature was normal 2 hours before electrocution.

Lesions

Lesions following intranasal inoculation.—Seven pigs were inoculated intranasally and one was housed with the group but not inoculated. The animals were necropsied on the fifth, seventh, tenth (2 animals), thirteenth, fourteenth, and forty-eighth days following inoculation. Control animals were killed on the seventh and fourteenth days.

The lung was the organ most consistently and severely affected in all pigs of this group killed after the fifth p.i.d. Grossly, small purple-red areas of atelectasis were observed, particularly in the dorsal portions of the diaphragmatic lobes and near the hilus. Lung lesions were prominent in the pig killed at 7 days and involved the diaphragmatic lobes of both lungs. The lungs of the 2 pigs killed at 10 days were more severely involved and had large areas of atelectasis in the dorsal and hilar areas of both diaphragmatic lobes and in the ventral portions of the apical and diaphragmatic lobes. The lungs of pigs studied 13 and 14 days after inoculation had only small areas of atelectasis which were barely visible grossly.

Microscopically, interstitial pneumonia involving several lobules was seen in the severely affected portions of the lungs, particularly of the pigs killed at 10 days (Fig. 8). In addition
many smaller, focal areas of interstitial pneumonia were present, the foci being limited to the immediate vicinity of the small bronchi and bronchioles. Such foci were present in the less severely affected areas of the lungs of the 2 pigs examined on the tenth p.i.d., as well as in the lungs of pigs killed at the seventh and fourteenth p.i.d. (Fig. 9).

Except for extent, distribution, and severity, the lesions were identical in all animals. They consisted of thickened alveolar septae, primarily due to proliferation of septal cells. These cells were irregularly shaped, had poorly defined borders and contained large, vesicular nuclei with prominent nucleoli. Lesser numbers of lymphocytes, plasma cells and histiocytes were present. Many degenerated cells with fragmented cytoplasm and pyknotic nuclei were caught in alveolar septae or clumped within the alveolar lumens (Fig. 10).

Several septal cells contained distinct intranuclear inclusion bodies. They occurred in the thickened interalveolar septae and were recognized by their increased size, prominent, basophilic nuclear membrane and homogeneous, slightly purple, intranuclear central mass separated from the nuclear membrane by a distinct clear zone (Fig. 10). In addition, there were many septal cells which had prominent, deeply basophilic nuclear membranes and dark, homogeneous nuclear contents, suggestive of adenoviral inclusion bodies.

Bronchioles and small bronchi in the affected areas contained clumps of cells with pyknotic nuclei. A few of their lining
Fig. 8. Interstitial pneumonia in a pig (H1008) 7 days after intranasal inoculation. The lesion involves the entire lobule and portions of adjacent lobules and is typical of lesions in pigs 10 days after inoculation. Pyknotic cells are present in bronchioles and alveoli. H & E., X50.

Fig. 9. Focal interstitial pneumonia in a pig (K1403) 7 days after intraperitoneal inoculation. The interalveolar septae around the blood vessels and bronchiole are thickened. There are pyknotic cells in the alveolar spaces. H & E., X125.
Fig. 10. Interstitial pneumonia in a pig (H1008) 10 days after intranasal inoculation. Numerous immature septal cells are present in the thickened interalveolar septae and an intranuclear inclusion body (arrow) is present in one of the cells. The alveoli contain many pyknotic cells. H & E., X500.

Fig. 11. Focal interstitial nephritis in a pig (H996) 13 days after intranasal inoculation. Collecting tubules are surrounded by plasma cells, lymphocytes and histiocytes. One cell contains an intranuclear mass suggestive of an inclusion body (arrow). Many tubular epithelial cells have degenerated and there are several pyknotic cells in the lumen of one tubule. A cell with a large, densely basophilic inclusion body is present in the epithelium of another tubule (double arrow). H & E., X500.
epithelial cells had darkened nuclei with thickened nuclear membranes suggestive of inclusion bodies. Hyperplasia was minimal or absent.

Control pigs often had small areas of atelectasis, especially in the ventral portions of the apical and cardiac lobes, and the interalveolar septae in these areas were 2 to 4 cells thick. Degenerated cells were not seen in the septae or alveolar lumens and no inclusion bodies were present.

Lesions were also observed in the kidneys, thyroids, and lymph nodes. The kidneys of the 4 pigs examined on the tenth, thirteenth, and fourteenth p.i.d. following intranasal inoculation had small, focal peritubular infiltrates of lymphocytes, plasma cells and histiocytes, particularly along the collecting tubules of the renal medulla. Several tubules contained cellular casts of degenerated cells with pyknotic nuclei similar to those present in the alveoli of the lung. Cells with enlarged deeply basophilic nuclei and prominent nuclear membranes suggestive of adenoviral inclusions were found among tubular epithelial cells. More definitely demarcated, basophilic, intranuclear inclusion bodies separated by a clear zone from a prominent nuclear membrane were found in intertubular reticular cells and occasionally within the tubular lining epithelium (Fig. 11).

Although foci of infiltrating cells were found in the kidneys of control pigs they were confined to the cortex and perivascular areas of the medulla and consisted of hematopoietic cells. There were no lesions involving the tubular epithelium in the control pigs.
Lesions in the thyroid were observed microscopically in the same 4 pigs in which renal lesions occurred. These animals were the only ones in the entire study in which the thyroids were affected. The lesions were widely disseminated throughout the gland but were small and focal. They consisted of an increase in the nuclear basophilia of the parafollicular cells and a very mild lymphocytic and plasma cell infiltration. Many degenerated cells with pyknotic nuclei were seen within the follicles (Fig. 12). In addition, the nuclei of some follicular lining cells had the increased size and homogeneous basophilic appearance associated with adenoviral inclusions in other organs. Some parafollicular cells had central intranuclear inclusion bodies surrounded by a clear zone beneath the nuclear membrane. These lesions were similar in many respects to those seen in the lungs and kidneys of these animals.

Lesions were also found frequently in bronchial and mesenteric lymph nodes and less often in other lymph nodes of the 4 pigs necropsied on the seventh, tenth, thirteenth, and fourteenth p.i.d. They were less frequently observed in the pig examined on the fifth p.i.d. and were absent from the pig killed on the forty-eighth p.i.d. The lymphatic follicles of the affected animals contained an unusually high number of immature reticular cells with large vesicular nuclei, sparse thread-like chromatin and prominent eosinophilic nucleoli. Occasional cells were seen in which the nuclear membrane was densely basophilic due to a marked chromatin margination. A homogeneous, moderately basophilic, intranuclear inclusion body occupied the majority of the nucleus and
Fig. 12. Thyroiditis in a pig (H996) 13 days after intranasal inoculation. Numerous reserve cells along with histiocytes are present between the follicles. Several follicular epithelial cells are degenerating and some are present in the follicular lumens. H & E., X500.

Fig. 13. Bronchial lymph node of a pig (H994) 10 days after intranasal inoculation. Numerous reticular cells with prominent nucleoli are present in this follicle and one cell is in mitosis. One cell (arrow) contains a large, intranuclear inclusion body which is separated from the thickened nuclear membrane. H & E., X1250.
there was a distinct clear zone between the inclusion and the
cellular membrane (Fig. 13). In other cells a densely basophilic
nuclear membrane tightly surrounded a large, slightly basophilic,
homogeneous nuclear mass, suggestive of a viral inclusion. No
such changes were seen in tissues from control pigs.

Lesions following intratracheal inoculation.—Four infected
and 2 control pigs were inoculated intratracheally. Infected pigs
were necropsied on the fifth, seventh, fourteenth, and twenty-first
p.i.d. and control pigs on the ninth and twenty-first p.i.d. The
pig examined on the fifth postinoculation day was killed in extremis
after a short clinical illness consisting of dyspnea, cyanosis, and
prostration. At necropsy most of the left lung and large portions
of the diaphragmatic lobe of the right lung were atelectic. Micro-
scopically, the lesions were identical to the lung lesions which
occurred after intranasal inoculation except that they were more
widespread and more severe. Many lobules had severe interstitial
pneumonia and alveoli were almost completely filled with pyknotic
cells. Interalveolar septae were 15-20 cells thick and inclusion
bodies were numerous. Lung lesions were also present in the pig
killed on the seventh p.i.d. but were not so severe. They closely
resembled the lung lesions found at 13 and 14 days after intranasal
inoculation. The pigs necropsied on the fourteenth and twenty-first
days after intratracheal inoculation were free of pulmonary lesions.

Kidney lesions were identical to those already described in
the pig killed on the seventh p.i.d. Bronchial, mesenteric, and
rarely other lymph nodes of pigs killed at the fifth, seventh, and
fourteenth p.i.d. contained cells like those described in the lymph nodes of intranasally inoculated pigs. No lesions were observed in the thyroid and other organs. All control pigs were free of lesions.

Using immunofluorescence, viral antigen was found widely distributed in lung sections of the pig killed 5 days after intratracheal inoculation. Antigen was present in the nuclei of cells in the thickened interalveolar septae and it was usually found in a broad band just beneath the nuclear membrane. The pyknotic cells in the alveolar spaces also fluoresced but with variable intensity and regularity. Antigen was occasionally found in lung sections of the pig killed at the seventh p.i.d. Viral antigen was rarely observed in sections of lymph nodes and then in only a few cells. It was not found in the kidney or other organs and in control tissues.

Lesions following intracerebral inoculation.—Seven pigs were inoculated intracerebrally with the virus and were necropsied on the fifth, seventh, fourteenth, nineteenth (3 pigs), and twenty-eighth p.i.d. Four control pigs were used and necropsied on the fifth, fourteenth, nineteenth, and twenty-eighth p.i.d. No lesions were found in the brains of the pigs examined 5 and 7 days after inoculation except for an area of necrosis corresponding to the site of needle penetration and this was also found in the control animals.

Lesions were present in the brain of the pig examined 14 days after intracerebral inoculation and were progressively more severe in the pigs at the nineteenth and twenty-eighth p.i.d. The
brain lesions were the most consistent and severe lesions in these pigs. They were present in the gray matter of both hemispheres of the cerebral cortex and were most prominent in the deeper layers of the gray matter. The lesions were most frequent and severe in the vicinity of the inoculation site and gradually diminished in frequency and intensity as the tips of the occipital and olfactory lobes were reached. The hemisphere opposite the site of inoculation was consistently but mildly affected.

The lesions consisted of focal accumulations of glial cells and were usually no more than 100 microns in diameter. The predominant cells in such lesions were microglia, which frequently had rod-shaped or indented nuclei. Local proliferating astrocytes with round nuclei and clumped chromatin were also present within the lesions. Toward the periphery the glial cells were more loosely arranged and finally only a few scattered cells were present. Degenerative changes in the neuropil were occasionally observed in the centers of the nodules where the cells were grouped closely together (Fig. 14).

Inclusion bodies were not demonstrated with certainty, although structures suggestive of inclusions were seen in several neurons in affected areas as homogeneous, basophilic intranuclear masses framed by prominent basophilic nuclear membranes with attached clumps of chromatin. Occasionally neurons with condensed cytoplasm and large, intensely basophilic nuclei were present in the center of glial nodules (Fig. 14). These nuclear changes closely resembled those seen in lungs, kidneys, thyroids, and lymph
Fig. 14. Glial nodule in the cerebral cortex of a pig (K568) 14 days after intracerebral inoculation. The nodule consists of microglia and a few astrocytes. Some degeneration of the neuropil is evident. The neuron in the center of the nodule has a homogeneous, deeply basophilic nucleus suggestive of a viral inclusion body. H & E., X500.

Fig. 15. Cerebral meningitis in a pig (K566) 28 days after intracerebral inoculation. Numerous perithelial cells and lymphocytes are present in the meninges near blood vessels. H & E., X500.
nodes of animals inoculated by other routes and are suggestive of inclusion bodies.

Perivascular cuffs, 3 to 8 cells thick, were consistently present in the vicinity of the glial nodules. They were comprised of activated perithelial cells and infiltrating lymphocytes. There were areas of meningitis in the vicinity of the cortical lesions and the meningeal blood vessels were surrounded by cuffs of cells similar to those in the brain (Fig. 15).

No encephalitis or meningitis was present in any of the brains of control pigs.

In addition to the brain, lesions were also found in the kidneys of the pig killed on the twenty-eighth p.i.d. and the lymph nodes of this animal as well as of the pigs killed on the fourteenth and nineteenth p.i.d. These lesions were identical to those of pigs inoculated by other routes.

Because of the focal nature of the lesions in the brain and kidney, the blocks of frozen tissue sectioned for immunofluorescence did not contain lesions. No fluorescence was observed in tissues without lesions. No lesions were found in control pigs.

**Lesions following oral or intraperitoneal inoculation.**—Three pigs were inoculated by each of these routes and were necropsied on the seventh, fourteenth, and twenty-eighth p.i.d. Three animals were used as controls and were killed on the seventh, fourteenth, and twenty-eighth p.i.d. Mild lung lesions appeared in pigs inoculated orally or intraperitoneally and killed 7 days after inoculation (Fig. 9). They had the same microscopic appearance as those
described in other pigs but were much smaller and occurred only in a few sections. Inclusion bodies were infrequent. Mesenteric lymph nodes from these pigs and from pigs examined at 14 days contained a few cells with nuclear changes suggestive of inclusion bodies but no distinct inclusions were found. Lesions were not found in other tissues of pigs infected by these routes and no lesions were present in controls.

Viral antigen was occasionally demonstrated in reticular cells in the lymph nodes. The samples of the other tissues did not contain lesions, and no viral antigen was demonstrated in them.

Frequency of lesions in different tissues.---These data are summarized in Table 2. Lymph nodes of 18 of the 25 infected pigs between 5 and 28 days after inoculation contained lesions. They were found after each of the 5 routes of inoculation but they occurred earlier (5 days) after intranasal and intratracheal inoculation than they did after intracerebral inoculation (14 days).

Lung lesions were found in 9 of 25 infected pigs and were present in pigs inoculated by every route except intracerebral. They were found earliest after intratracheal inoculation (5 days) and were present longest (14 days) after intranasal inoculation. With one exception, they were most severe after intranasal inoculation.

Kidney lesions were present in 6 pigs inoculated intranasally, intratracheally, or intracerebrally. They were found later (28 days) after intracerebral inoculation than after intranasal or intratracheal inoculation (7 to 14 days), but they were more frequent and
more severe after intranasal inoculation. Cerebral lesions were found only in the 5 pigs inoculated intracerebrally.

**TABLE 2. Frequency of lesions in tissues of pigs inoculated with porcine adenovirus**

<table>
<thead>
<tr>
<th>Route of Inoculation</th>
<th>Number of Animals</th>
<th>Lymph Nodes</th>
<th>Lung</th>
<th>Kidney</th>
<th>Brain</th>
<th>Thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intranasal</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Intratracheal</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intracerebral</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Introral</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>25</strong></td>
<td><strong>18</strong></td>
<td><strong>9</strong></td>
<td><strong>6</strong></td>
<td><strong>5</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

Four of the 25 infected pigs had thyroid lesions and these occurred 7 to 14 days after intranasal inoculation.

**Inclusion bodies.**—Intranuclear inclusion bodies were difficult to distinguish from the large nucleoli of the immature cells making up the bulk of the lesions. The lung was the organ in which inclusion bodies were most frequently found and they were present in direct proportion to the severity of the lesion. Carnoy's and Bouin's fixatives made the inclusions more obvious. Cells with distinct intranuclear inclusion bodies were found in all affected organs except brain. By immunofluorescence, viral antigen was found in such cells and was mainly localized in the clear zone between central mass and nuclear membrane, much as it was in the latter stages of inclusion body formation in swine kidney cell cultures (Fig. 7).
Discussion

While a number of porcine adenoviruses have recently been isolated (2, 5, 6, 7, 14, 17), none of them have been shown to be pathogenic for pigs. The isolation of our porcine adenovirus from the brain of a pig with both signs and lesions of encephalitis suggested that this virus might be pathogenic for pigs. The results reported here confirm this observation. The virus produced lesions in newborn germfree and pathogen-free pigs when inoculated by 5 different routes.

The lesions produced by the porcine adenovirus were fundamentally the same regardless of the tissue in which they occurred. Parenchymatous cells of the organs involved, such as epithelial cells of the lung, kidney, and thyroid, cerebral neurons or reticular cells of the lymph nodes were always affected and typical cellular lesions were observed. They included the formation of characteristic intranuclear inclusion bodies and death of the affected cells. The reactive cells were primarily immature reticular cells, histiocytes, microglia, and perithelial cells. Lymphocytes and plasma cells were also a constant feature of the lesions. The virus appears to attack parenchymatous cells of affected organs and result in cell death. The reactive cells are cells of the lymphoreticular system.

Intranasal inoculation was found the most effective of the routes used for producing lesions. Virus inoculated by this route disseminates widely throughout the body and produces lesions in several tissues. The lesions were present in more tissues and, with
one exception, were more severe than those in pigs inoculated by other routes. These findings correlate well with the results of the virus isolation study (Table 1) in which the virus was found more frequently from more tissues after intranasal inoculation than any other route tested. Both lesions and virus were regularly detected in respiratory and lymphoid tissue. These data indicate that the intranasal route may be an important natural route of infection.

Both oral and intratracheal inoculation resulted in lesions which were almost always less severe than the lesions following intranasal inoculation. This suggests that the exposure of both upper and lower respiratory tract tissue and the relatively long exposure of the lymphoid tissue of the oropharynx, which probably occurs after intranasal inoculation, is important in the pathogenesis of the disease. On the other hand, the occurrence of mild lung lesions after intraperitoneal inoculation shows that direct exposure of respiratory tissue is not required for the production of lesions.

The severe pulmonary lesions found in one pig 5 days after intratracheal inoculation and the minimal lung lesions present in another pig at 7 days postinoculation might indicate that pulmonary lesions develop and regress more rapidly after intratracheal than after intranasal inoculation. It is more likely, however, that the clinically ill pig, which had been small and weak since birth, was more susceptible to the virus. There is ample evidence in human patients to show that debilitating diseases or immunosuppressive therapy can increase the severity of adenovirus infections (19).
The pulmonary lesions which occurred in pigs after infection with the porcine adenovirus were similar in many respects to those found in pigs after intratracheal inoculation of human adenoviruses (10). Hyperplastic bronchiolar epithelium, intranuclear changes suggestive of inclusion bodies and pyknotic cells in the bronchiolar lumens have been emphasized as prominent features of these lesions. In our pigs similar bronchiolar lesions were sometimes seen but they were always very mild. On the other hand, the interstitial pneumonia produced by the porcine adenovirus was more severe than that described following inoculation of human adenoviruses. The distribution of the lesions in the lung, the time of appearance after inoculation, and the histologic features of the interstitial pneumonia following inoculation of the pig adenovirus were much like those described in pigs after human adenovirus inoculation.

The interstitial pneumonia found in calves after intranasal inoculation of a bovine adenovirus (4) and in people with fatal adenoviral infections (3, 12) were similar to that found in our pigs but the bronchial and bronchiolar lesions in calves and man were much more severe.

Several stages in the formation of the typical intranuclear inclusion bodies have been described. Cells with small eosinophilic granules scattered between threads of chromatin and larger diffuse intranuclear granular masses are said to be changes representative of adenoviral infection (10, 13, 15). Some of these intranuclear changes were also seen in the material studied here, but histologic evidence should not be considered sufficient for the association of
these changes with a virus, since intranuclear structures similar to those reported were sometimes seen in our controls. Cells with large homogeneous intranuclear masses or large basophilic masses clearly demarcated from the nuclear membrane were considered to be virus-infected cells since they were never found in controls and they were shown to contain viral antigen by immunofluorescence.

Lesions in lymph nodes and kidneys similar to those found in this experiment have been produced by adenoviruses of other species. Lymph node lesions have been found in pigs after inoculation with human adenoviruses (10), calves after inoculation with a bovine adenovirus (4) and in human patients with adenovirus pneumonitis and lymphadenitis (3, 12, 18, 19). Kidney lesions are sometimes present in infected human patients (3) and intranuclear inclusion bodies were seen in chicken kidneys after inoculation with CELO virus (an avian adenovirus) (13). No significant lesions were seen in the kidneys of calves inoculated with a bovine adenovirus (4). The kidneys of pigs inoculated with human adenoviruses were not examined (10).

Thyroid lesions have not been previously shown to be caused by adenoviral infections of man or animals. The similarity of the thyroid lesions to those in other tissues, the presence of intranuclear inclusion bodies and the absence of lesions in controls indicated that the changes were caused by the adenovirus. It is interesting that this virus also produces lesions and inclusion bodies in an established canine thyroid carcinoma cell line (11).
Encephalitis was observed only after intracerebral inoculation of the virus. The nature of the lesions and the type of nuclear changes indicated that neurons were among the cells attacked by the virus. The absence of cerebral lesions in the pigs examined 5 and 7 days after intracerebral inoculation and the increasing severity of the lesions found 14, 19, and 28 days after inoculation suggest the brain lesions develop more slowly than lesions in other organs. The virus may replicate more slowly or less efficiently in brain tissue or, alternatively, the virus may leave the brain and replicate in extracerebral sites before returning to the brain and attacking cells. This could also account for relatively late appearance of cerebral lesions. The virologic data (Table 1) and the presence of lesions in the hemisphere opposite the inoculation site support the suggestion that virus leaves the brain, is disseminated through the body, and subsequently re-enters the brain.

Although brain lesions were not present when the virus was not inoculated intracerebrally, virus was isolated from the brain as late as 48 days after intranasal inoculation (Table 1). The reasons for the absence of lesions in the presence of the virus are not known, but injury to the brain or blood-brain barrier may be required before the virus can produce lesions. Finding the most severe lesions in the vicinity of an injury such as the site of intracerebral inoculation provide some support for this suggestion. Adenovirus encephalitis in man has almost always been described in debilitated patients or in infants (3, 19), some of whom died with severe adenoviral lesions in other organs.
Other reports of adenoviral cerebral lesions include cerebral vasculitis due to canine hepatitis virus (9) and brief comments about meningitis and necrosis of the choroid plexus or encephalitis after intracerebral inoculation of monkeys with human (8) and bovine (1) adenoviruses.

Many features of the disease produced by this porcine adenovirus in germfree and pathogen-free pigs closely resemble adenoviral diseases in man. Of particular interest are the similarities of lesions in lung and lymph nodes, the long period of survival of the virus in various organs, and the production of encephalitis by intracerebral inoculation.

The germfree pig inoculated with this porcine adenovirus provides a precise experimental tool for the study of many poorly understood features of human adenoviral diseases. In addition further study of the thyroid lesions produced by this virus may result in a better understanding of the action of viruses on the highly specialized cells of endocrine organs.

**Summary**

A porcine adenovirus isolated from the brain of a pig with signs and lesions of encephalitis was shown to be pathogenic for germfree and pathogen-free pigs. A total of 25 pigs were inoculated with the virus by the intranasal, intratracheal, intracerebral, oral, and intraperitoneal routes. Lesions were produced by all routes but the most effective method was intranasal inoculation. These animals developed an interstitial pneumonia which involved entire lobules and was especially prominent near the hilus. Distinct intranuclear
inclusion bodies were seen. Lesions and inclusion bodies were also found in the kidney, thyroid, and lymph nodes. Viral antigen was demonstrated in the lesions by immunofluorescence. Intracerebral inoculation resulted in encephalitis of the gray matter of the cerebral cortex. The lesions consisted of glial nodules and shrunken neurons with intranuclear changes suggestive of inclusion bodies. Many features of the disease were similar to adenoviral diseases in man and other animal species. This porcine adenovirus in the germfree pig provides a model for the investigation of poorly understood features of spontaneous adenovirus infections.
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