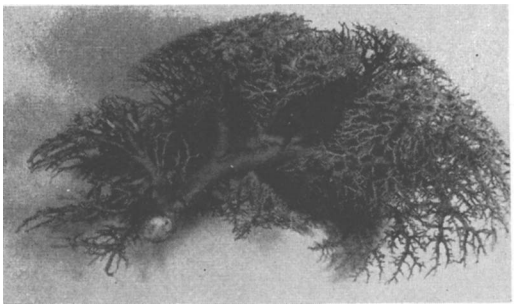

FIG. 3. Cast of portal system. (#25)

following needle puncture of the liver, with its attendant local hemorrhage. Some specimens showed no detectable changes at all.

Discussion. The proposed method for portal venography was easily performed and yielded excellent results. There was clear visualization of the entire portal system within the liver save a small section obscured by the sinusoidal filling at site of injection. The effects of the procedure upon the dog liver were studied both acutely and at intervals up to 12 days after the injections and showed minimal focal changes attributable to the procedure. Investigations are in progress in attempt to use this method of study in human patients.

Summary. A new method for portal venography by retrograde hepatic vein flushing was described. Application for the study of human cases is in progress.

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Susceptibility of Primary Cultures of Feline Renal Cells to Selected Viruses. (26396)

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Several workers have reported on the susceptibility of feline renal cell cultures to feline viruses(1-4). ECHO 10, ECMO(5), and herpes simplex(6) viruses have also been shown to be cytopathogenic in such cultures. Other human and bovine enteroviruses have been reported to be noninfective for feline renal cells(5). Since cultures of feline renal cells are a major cell type in this laboratory, their viral spectrum warranted an investigation. Twenty-four animal and human viruses were investigated for their ability to produce a cytopathogenic effect (CPE) with subsequent multiplication in primary cultures of feline renal cells. This paper reports our results.

Materials and methods. Primary cultures of feline renal cells were prepared by the trypsin digestion method described by Madin *et al.*(7). The growth medium was 0.5% lactalbumin hydrolysate in modified Hank's balanced salt solution with an addition of 10% lamb serum. The medium used at time of inoculation was lactalbumin hydrolysate containing 2% lamb serum. All media contained 500 units of penicillin and 500 μg of streptomycin per milliliter. Each of 4 renal culture tubes was inoculated with 0.1 ml of undiluted virus. When 70 to 80% of the cells were showing evidence of degeneration, 0.1 ml of fluid and cells was passed. In the absence of CPE, 0.1 ml of fluid and cells was transferred at 5- to 10-day intervals to new cell cultures. In all instances when CPE was absent the fluid was tested for virus by inoculation to susceptible cell cultures following 2 or more blind passages. No special attempts were made to adapt these viruses to

TABLE I. Susceptibility of Feline Renal Cell Cultures to Virus Infection.

Virus	Source	Primary inoculations CPE	Primary inoculations Days	Succeeding passage No.	Succeeding passage CPE	Succeeding passage Titer*
GROUP 1†						
Vesicular stomatitis (Ind.)	Allantoic fluid	+	1	10	+	4.3
" " (N.J.)	" "	+	1	10	+	3.5
Infectious bovine rhinotracheitis	Bovine kidney	+	2	10	+	6.5
Equine abortion	Hamster liver	+	4	11	+	5.5
ECHO 10	Monkey kidney	+	4	6	+	5.0
Vaccinia	FL cells	+	1	10	+	4.6
Herpes	Herpetic vesicle	+	1	10	+	6.5
Sendai	Allantoic fluid	+	1	12	+	5.0
Adenovirus 4	KB cells	+	2	7	+	3.0
GROUP 2‡						
Parainfluenza III (SF-4)	Bovine kidney	—		3 blind passages		
Canine hepatitis	Dog "	+	2	Lost at 3		
Coxsackie B-1 through B-5	KB cells	+	1	" " 3 to 5		
Adenovirus 3	HeLa cells	+	2	" " 2		
" 7	KB cells	+	4	" " 2		
GROUP 3§						
Newcastle disease	Allantoic fluid	—		3 blind passages		
Poliovirus types I, II, III	Monkey kidney	—		3 " "		
Coxsackie A-9	KB cells	—		2 " "		
ECHO 4	Monkey kidney	—		2 " "		

* Log $TCID_{50}$/0.1 ml of inoculum in feline renal cells.
† Group 1—Viruses that propagated readily, with CPE.
‡ Group 2—Viruses which produced CPE for 1 or more passages without multiplication.
§ Group 3—Viruses which did not elicit CPE nor multiply.

this tissue culture system.

*Viruses.** The viruses studied are listed in Table I.

Virus titration. Serial 10-fold dilutions of tissue culture fluid containing the virus were prepared, using nutrient fluid as the diluent. One-tenth milliliter of each dilution was inoculated into each of 4 feline renal culture tubes. The end point was calculated by the Reed and Muench method and expressed as the 50% tissue culture infective dose ($TCID_{50}$) per 0.1 ml of inoculum.

The viruses which induced a cytopathic change with subsequent multiplication were each identified following 6 or more passages by either the complement fixation, hemagglutination inhibition, or serum neutralization test.

Coverslip preparations were stained with hematoxylin and eosin following fixation in Bouin's fluid.

Results are presented in Table I. The viruses were placed in 3 groups, based upon response elicited in the cell cultures. The first of these includes the viruses that propagated readily with a cytopathogenic effect. Viruses in this group are infectious bovine rhinotracheitis (IBR), vesicular stomatitis (VS) (New Jersey and Indiana), equine abortion (EAV), vaccinia, herpes, Sendai, ECHO 10, and adenovirus type 4. Group 2 includes those viruses which produced a cytopathogenic effect for 1 or more passes but did not multiply. These are canine hepatitis, Coxsackie B-1 through B-5, parainfluenza III (SF-4), and adeno 3 and 7. Newcastle dis-

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Major Crandell is now at 3790th Epidemiological Laboratory, Lackland AFB, San Antonio, Texas.

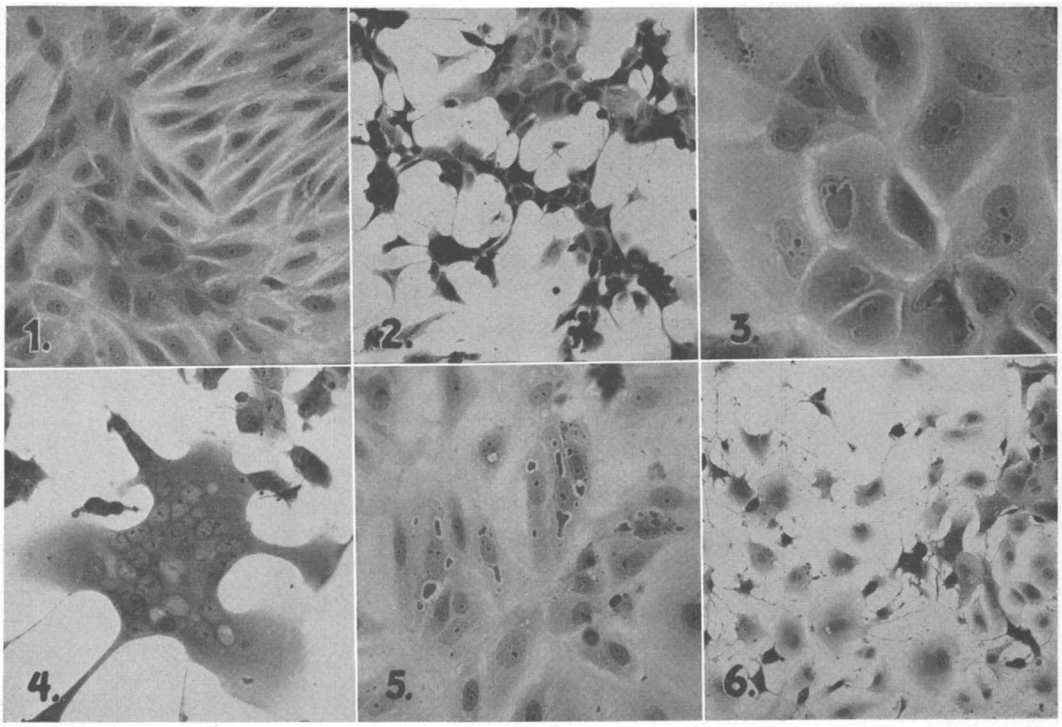

FIG. 1. Uninoculated feline renal cell culture. H & E, ×177. AFIP Neg. No. 60-3481.

FIG. 2. Cytopathic change in culture of feline renal cells infected with virus of infectious bovine rhinotracheitis. 48 hr. H & E, ×67. AFIP Neg. No. 60-3485.

FIG. 3. Intranuclear inclusion bodies in culture of feline renal cells infected with equine abortion virus. 72 hr. H & E, ×267. AFIP Neg. No. 60-3487.

FIG. 4. Syncytium in culture of feline renal cells infected with vaccinia virus. 48 hr. H & E, ×167. AFIP Neg. No. 60-3486.

FIG. 5. Cytoplasmic inclusion bodies in cultures of feline renal cells infected with adeno 4. 60 hr. H & E, ×177. AFIP Neg. No. 60-3482.

FIG. 6. Cytopathic change in culture of feline renal cells infected with Sendai virus. H & E, ×67. AFIP Neg. No. 60-3484.

ease virus (NDV), polioviruses I, II, and III, ECHO 4, and Coxsackie A-9 did not elicit a cytopathogenic change nor multiply; these comprise Group 3.

Fig. 1 shows a control culture of feline renal cells.

The cytopathogenicity with the viruses of IBR (Fig. 2), vaccinia, Sendai, equine abortion, adeno 4, VS (Ind. and N.J.), ECHO 10, and herpes simplex was very distinct. In stained preparations intranuclear inclusion bodies were demonstrated in cultures infected with IBR, EAV (Fig. 3), and herpes simplex. The cytopathogenicity of herpes simplex has been previously described in feline renal cells(6), but this is the first report, to our knowledge, of a primary isolation of this virus in these cells.

Formation of syncytia was a prominent feature in the early passages of vaccinia (Fig. 4). The cytopathic effects of VS (Ind. and N.J.), and ECHO 10 were characteristically rounding and shrinking of the cells. The presence of cytoplasmic inclusion bodies in cells infected with adeno 4 (Fig. 5) was in sharp contrast to the intranuclear inclusion bodies observed in feline cells infected with adeno 3 and 7. Cytopathogenic changes in the cultures induced by the Sendai virus were more diffusely spread throughout the culture than with the other viruses in Group 1. The cells were stellate in shape, with numerous protoplasmic bands joining adjacent cells (Fig. 6).

With all the viruses in Group 1, the titers of the fluids from the final passage indicated that the viruses had multiplied. With the exception of the VS viruses, the titers ob-

tained in feline renal cells appeared to be constant between the 5th and 10th passage for those tested. Both strains of VS virus multiplied initially with a titer of 10^{-5} but decreased considerably between the 5th and 20th serial passage.

Although the viruses in Group 2 elicited an early CPE in the cultures, they failed to multiply. During the early passages, intranuclear inclusion bodies were demonstrated in cultures infected with the viruses of canine hepatitis and with adeno 3 and 7. This behavior of these 3 viruses is another similarity between canine hepatitis virus and the adenovirus group. Although parainfluenza III (SF-4) virus did not elicit a demonstrable cytopathogenic change in the unstained cultures, both cytoplasmic and intranuclear inclusions were observed in stained preparations on the first passage.

The viruses in Group 3 did not produce a cytopathogenic effect in the feline renal cells, and infective virus could not be demonstrated when the fluid was transferred to susceptible cell cultures.

Our results with the human enteroviruses agree with previously reported data(5), except that cytopathic changes were present in early passages with Coxsackie B-1 through B-5.

Summary. The susceptibility of primary feline renal cell cultures to 24 viruses is reported. Of these, infectious bovine rhinotracheitis, vesicular stomatitis, equine abortion, vaccinia, herpes, Sendai, ECHO 10, and adenovirus 4 were shown to produce marked cytopathic effects with multiplication. Canine hepatitis virus, Coxsackie B-1 through B-5, adeno 3 and 7, and parainfluenza III (SF-4) were cytopathogenic for one or more passages but did not multiply. The polioviruses, Newcastle disease, ECHO 4, and Coxsackie A-9 had no cytopathogenic effect on the cell, and there was no evidence of viral multiplication.

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Acid Hematein Staining of Uterine Neoplastic Tissues.* (26397)

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A cytochemical method for staining phospholipids with acid hematein has been developed(1,2). Using this method to examine normal and neoplastic tissues of mice, rats, and human beings, Hori noted that non-neoplastic cells generally contain acid-hematein-positive substance in varying amounts, whereas solid tumors do not(3,4,5). The reaction also becomes less intensive in liver cells of rats during azo dye carcinogenesis (6) and of mice bearing transplanted tumors (7). The present paper deals with the reaction manifested by non-neoplastic and neoplastic cells of the human uterus.

Materials and methods. Uterine tissue specimens were obtained from 18 patients by the conventional biopsy method. They included 2 non-neoplastic cases, 12 squamous cell carcinomas, and 4 myomas. After being fixed with a formol-calcium or formol-calcium-cadmium solution for 6 hours, a portion of each tissue specimen was subjected to the acid hematein test as described in Hori's re-

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