Studies on Transmissible Gastroenteritis of Swine II. Selected Characteristics of a Cytopathogenic Virus Common to Five Isolates from Transmissible Gastroenteritis

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SUMMARY

Five cell culture isolates from transmissible gastroenteritis (TGE) of swine have been studied. There is a cytopathogenic virus common to all of these isolates. Some of the characteristics of this virus, such as its size, approximately 100 mμ, its relative sensitivity to ether, lability at pH 2, pH 3, and pH 10, and its heat lability suggest that it may be a member of the myxovirus class.

Concurrent research in this laboratory indicates that this cytopathogenic virus is not the only virus involved in the etiology of TGE, but it appears to be associated with many of the outbreaks of TGE which have been studied by this laboratory.

The successful passage of transmissible gastroenteritis (TGE) virus in cell culture as reported by Lee (1), Harada, Kumagai and Sasahara (2), McClurkin (3), and Cartwright (4) has made it possible to compare the growth of 5 isolates from widely separated geographic areas in two different cell culture systems. The comparison was done by propagating the isolates in primary swine kidney cell cultures (SK), and in a continuous culture of swine testis cell cultures (ST).

Research in progress at this laboratory indicates that there is a noncytopathic virus which will grow in SK cells and in a continuous culture of swine fetal kidney cells, and produce signs of TGE in 5-day-old specific pathogen free (SPF) pigs. However, it is of considerable interest that there is a common cytopathogenic virus present in several isolates of TGE, and that specific antibodies are present in convalescent sera from experimental and most field cases of the disease which will neutralize this virus. Pigs that have been exposed to the cell-passaged viruses may be resistant to TGE when exposed to virulent virus-bearing tissue.

This paper deals with the partial characterization of the cytopathogenic virus common to 5 isolates from TGE, and suggests that it may be a member of the myxovirus group.

Materials and Methods

Cell Cultures:

Swine kidney cells were prepared and used as described by McClurkin (3). The continuous ST cell culture was started in this laboratory from trypsinized testicular tissue from swine fetuses. They were seeded in Earle's balanced salt solution with 0.5% lactalbumin hydrolysate (LAH) and 10% pig serum. After a period of adjustment the cells grew well and could be removed from the glass with the aid of either versene or trypsin, and subcultured at weekly intervals. Prior to inoculation the medium was replaced with Eagle's basal medium containing 0.5% LAH without serum.

Virus Isolates:

Five virus isolates were used. Tissue virus from New York[1], Purdue, and Iowa isolates were prepared for inoculation of cell cultures as described by McClurkin (3).

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1. Kindly provided by Dr. Ben Sheffy of the Veterinary Virus Research Institute, Cornell University, Ithaca, N.Y.

The Japanese[2] isolate was received as the lyophilized 25th passage in SK cells. The Illinois isolate[3] was received as a frozen suspension in primary dog kidney cells.

The Purdue and Iowa isolates were inoculated into ST cells after 31 (Purdue) and 26 (Iowa) consecutive passages at 3- or 4-day intervals in SK cells. The New York virus was inoculated into ST cells after 5 consecutive passages at 3- or 4-day intervals in SK cells. The Illinois isolate was inoculated directly into ST cells. The Japanese isolate was passed from the 25th to the 28th passage at 3-day intervals in SK cells, then inoculated into ST cells.

The virus characterization studies reported in this paper were done after 10 to 13 consecutive passages at 1- and 2-day intervals in ST cells. The virus-cell suspension, removed from the glass by freezing and thawing, was then treated with sonic vibration[4] for 30 seconds at a setting of 3 to break up the cellular debris and aggregates of virus.

Determination of Pathogenicity and Immunogenicity of the ST Cell Culture Virus for Hysterectomy-derived Pigs:

All pigs used in these tests were maintained and fed as described by McClurkin (3). Various passage levels of the virus growing in ST cells were administered to the pigs by the oral route. The pigs were observed for signs of disease and, when indicated, a serum sample was taken from the survivors 10 to 14 days' postexposure to determine the presence of neutralizing antibody.

The resistance of the surviving pigs was determined by oral administration of virulent tissue-virus prepared from the Purdue isolate (3).

Preparation and Staining of Virus Infected Cells for Studies with Acridine Orange and Fluorescent Antibody:

SK and ST cells were seeded on coverslips in Leighton tubes. The cells were inoculated with a 10^{-2} dilution of the virus isolates and fixed 36 to 48 hours after inoculation. Those cells to be studied by means of fluorescent antibody were fixed in acetone for 5 minutes at room temperature and stained with fluorescent antibody prepared from antiserum against the Purdue isolate. (3).

The cells to be studied with acridine orange were fixed in alcohol-formalin-acetic acid mixture in a ratio 8:1:1 using 37% ethanol and stained according to the method described by Dart and Turner (5).

Determination of the Rate of Virus Reproduction in ST Cells:

Three ml. of each of the virus isolates were inoculated onto ST cells growing in 30 ml. of medium on 16 oz. Blake bottles from which the medium was removed. The bottles were rotated for 5 minutes at room temperature. The inoculum was then removed, and the cells washed twice with phosphate buffered saline, new medium added, and the cells were incubated at 37 C.

As incubation was in progress, 1 ml. of medium was removed from each bottle at 3-hour intervals, for a period of 33 hours, then at 48 and 72 hours. All samples were held at —65 C until assayed for virus content in ST cells.

Virus Assay and In vivo and In vitro Serum Neutralization:

The Purdue immune serum used in these tests was prepared from a pig which received an oral exposure to the ST cell-cultured Purdue isolate. Following recovery from a mild disease the pig was given one subcutaneous injection of 20 ml. of cell cultured virus at 30 days of age and one intravenous injection of 20 ml. of cell cultured virus at 55 days of age. The pig was bled for serum at 65 days of age.

The Purdue convalescent serum was prepared from pigs which had recovered from an experimental disease following the oral administration of virulent Purdue tissue-virus.

The ID_{50} for each virus isolate was determined by the method of Reed and Muench (6), in the presence and absence of a 1:640 final dilution of Purdue immune serum. The virus and virus-serum mixtures were held at 37 C for 60 min. before inoculation into ST cells to test for infectivity. The infectivity endpoints in the presence and absence of immune serum were compared and the quantity of each virus isolate neutralized was expressed as the reciprocal of log 10. Serum neutralization tests were also

2. Kindly provided by Dr. E. O. Haelterman of Purdue University, Lafayette, Indiana.

3. Kindly provided by Diamond Laboratories, Des Moines, Iowa, through Dr. C. E. Phillips of the Animal Inspection and Quarantine Division, Agricultural Research Service, National Animal Disease Laboratory, Ames, Iowa. This virus was cultured in primary dog kidney from sucrose gradient fractions of TGE-infected intestinal tissue prepared by Dr. M. Ristic of the University of Illinois.

4. Sonifier, Model L.S. 75, S.N. 7209 Bronson Instruments, Inc., Stanford, Connecticut.

carried out using a 10^{-1} dilution of each virus isolate and Purdue convalescent serum in 2-fold final dilutions from 1:10 to 1:320.

In vivo serum neutralization was attempted using 2 ml. of a 10^{-1} dilution of virulent tissue-virus of Purdue origin and 2 ml. of Purdue immune serum in final dilutions of 1:5 and 1:20, and 2 ml. of a 10^{-3} dilution of the tissue-virus with 2 ml. of a 1:5 final dilution of the serum. The 10^{-1} virus and serum dilutions were mixed in equal quantities and fed to 2 pigs after incubation at 37 C for one hour. The 10^{-3} virus and serum dilutions were mixed in equal quantities and fed to 2 pigs after incubation at 37 C for 2 hours. Two pigs, used as virus controls, received 2 ml. of a 10^{-1} dilution of the tissue virus, and a third pig received 2 ml. of a 10^{-3} dilution of the virus.

Plaque Formation and Plaque Reduction Studies:

ST cells were seeded on 60 mm × 15 mm Falcon[5] plastic Petri dishes and maintained at 37 C in 5% CO_2 atmosphere. When the cells were sheeted out the medium was removed and 0.2 ml. of a virus dilution in 0.2 ml. of diluent or 0.4 ml. of specified dilutions of a virus-antiserum mixture was put on the plates. Each inoculum was removed after an absorption period of 5 to 10 minutes at room temperature, and the cells overlaid with 8 ml. of 1% Ionagar No. 2[6] containing Eagle's medium and 2% SPF calf serum. Thirty-six hours after inoculation 5 ml. of a 0.01% neutral red solution in 2% agar was added to agar overlay, and after 12 hours further incubation the plates were observed for the formation of plaques.

Virus Filtration Studies:

The 5 virus isolates were passed through 100 mμ and 50 mμ Millipore filters by means of positive pressure. The filtrate, undiluted, and diluted 10^{-1} was inoculated into ST and SK cells.

Viability Tests of the Cell Culture Virus at 27 C, 37 C and 56 C:

Pathogenicity of virus held for 8 days at 27 C was determined by administration to pigs. Virus held at 27 C for 8 days and at 37 C for 3 days and 6 days was titrated in SK cells to determine infectivity. Virus held at 56 C for 30 minutes was inoculated into SK and ST cells to determine infectivity, and virus held at 56 C for 10 minutes was titrated in SK cells.

Viability Tests of Cell Culture Viruses after Treatment with Molar Magnesium Chloride at 37 C and 50 C:

Undiluted viruses were suspended in equal quantities of 2 M/$MgCl_2$ and held at 37 C for 24 hours and at 50 C for 1 hour. The virus — M/$MgCl_2$ mixtures were then diluted to 10^{-1} and inoculated into SK cells. Purdue virus, held at 37 C for 24 hours in M/$MgCl_2$, was diluted to 10^{-1} and inoculated into SK cells and fed to susceptible pigs to determine pathogenicity.

Measurement of the Stability of Cell Cultured Viruses in Organic Solvents:

Stability of infectivity in the presence of diethyl ether was measured by adding ether to the viruses in a concentration of 20% by volume. The mixture was then placed on a shaker at 4 C for 20 hours. Excess ether was removed by shell freezing the mixture and allowing it to thaw at room temperature while being subjected to negative pressure.

The stability of infectivity in the presence of chloroform was measured by adding chloroform to the virus in a concentration of 4.8% by volume, shaking at 4 C for 10 minutes, and then centrifuging the mixture for 10 minutes at 2000 rpm at 4 C.

The solvent-treated virus was titrated in SK cells and the infectivity endpoints compared with the virus titer before treatment. Pathogenicity of the undiluted Purdue virus, treated with ether for 24 and 48 hours at 4 C, was determined by feeding to susceptible pigs.

Measurement of Stability of the ST Cell-Cultured Virus in Acid and Alkaline Media:

Acetic acid (1/N) was used to lower the pH of 10 ml. of virus to pH 6, 4, 3 and 2. Two ml. of virus was removed at each pH and incubated at 37 C for 1 hour. The pH of each sample was then adjusted to 7 by addition of 7.5% sodium carbonate.

Seven and one-half per cent sodium bicarbonate was used to raise the pH of 10 ml. of virus to pH 8, 9, and 10. Two ml. of virus was removed at each pH and incubated at 37 C for 1 hour. The pH of each sample was then adjusted to 7 by the addition of 1/N acetic acid.

The endpoint of infectivity for the treat-

5. Falcon Plastics, Division of B-D Laboratories, Inc., 5500 West 83rd St., Los Angeles 45, California.

6. Manufactured by "Oxford" Division of OXO Limited, London. Distributed by Consolidated Laboratories, Inc., Box 324, Chicago Heights, Ill.

TABLE I. Results Following Oral Exposure of Pigs to the Cytopathic Virus Propagated in Swine Testis Cell Culture

Isolate	Cell passage	Pig No.	Signs of TGE[1]	Clinical outcome	Neutralizing antibodies in serum[2] at 2 to 3 weeks of age	Reation to exposure to virulent tissue-virus[3]
Japanese	1	1	Vomiting and diarrhea	Died		
Japanese	9	2	Vomiting and diarrhea	Recovered	Not done	None
		3	Diarrhea	Recovered	Not done	None
		4	Diarrhea	Recovered	Not done	None
Japanese	13	5	Loss of weight	Recovered	1:160	None
		6	Diarrhea	Died		
		7	Vomiting and diarrhea	Recovered	1:320	None
Purdue	4	8	Diarrhea	Died		
		9	Diarrhea	Died		
Purdue	6	10	Vomiting and diarrhea	Recovered	Not done	None
		11	Diarrhea	Recovered	Not done	None
		12	Diarrhea	Recovered	Not done	None
Purdue	8	13	Loss of weight	Recovered	1:640[4])	Not exposed, held for
		14	Loss of weight	Recovered	1:640)	antiserum production
		15	Loss of weight	Recovered	1:640)	
		16	Loss of weight	Recovered	1:640)	None
		17	Loss of weight	Recovered	1:640)	None
Purdue	16	18	Diarrhea	Recovered	1:80	Diarrhea, died
		19	Vomiting and diarrhea	Died		
		20	Vomiting and diarrhea	Died		
Iowa	11	21	Diarrhea	Recovered	1:160	None
		22	Diarrhea	Recovered	1:320	Diarrhea, died
		23	Diarrhea	Recovered	1:20	Diarrhea, died
Illinois	2	24	Diarrhea	Recovered	1:160	None
		25	Diarrhea	Recovered	1:160	None
Illinois	13	26	Diarrhea	Died		
		27	Diarrhea	Died		
		28	Diarrhea	Died		
New York	11	29	Diarrhea	Died		
		30	Vomiting and diarrhea	Died		
		31	Diarrhea	Recovered	1:320	None

[1]All pigs showed a loss of weight and condition even when no frank diarrhea or vomiting was observed. Those pigs which developed vomiting and diarrhea showed a greater loss of weight and condition.

[2]Reciprocal of the final dilution of serum which would neutralize 10^4 $TCID_{50}$ doses of ST cell-cultured virus from the Purdue isolate.

[3]All pigs were exposed to virulent tissue-virus of the Purdue isolate.

[4]This group bled for serum at 3 weeks of age.

ed virus was determined by titration in SK cells and compared with the endpoint of untreated virus in SK cells.

Determination of Viability of Purdue and Iowa Isolates after Lyophilization:

A 1:10 suspension of tissue-virus from a pig which received cell-passaged virus was prepared in virus diluent (3), shell frozen in 1 ml. quantities by means of dry ice and alcohol, and lyophilized. Cell-passaged virus in SK cells (3), without the addition of a stabilizer, was also shell frozen and lyophilized.

The pathogenicity of the lyophilized viruses, after 1 month of storage at 4 C, was determined by feeding to pigs. The infectivity of the lyophilized viruses was measured after 8 months of storage at 27 C and 4 C by titration in SK cells.

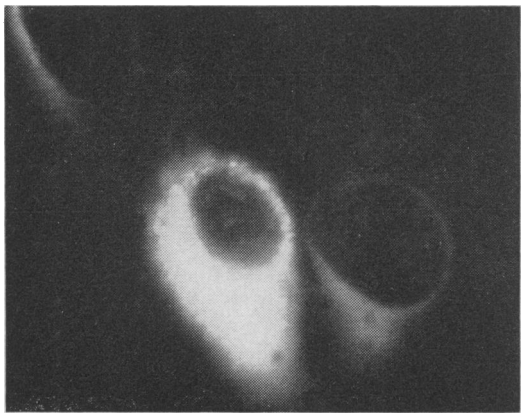

Fig. 1. The Illinois isolate growing on ST cells stained with fluorescent antibody. Prepared from Purdue immune serum. X1000.

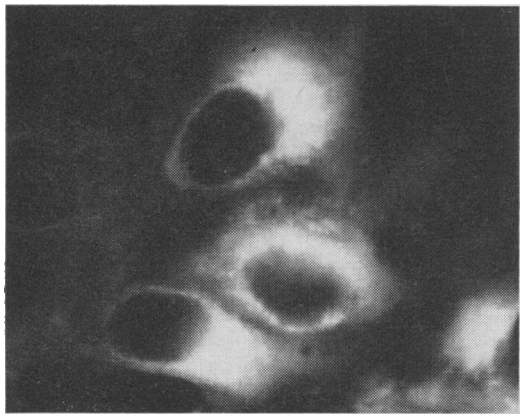

Fig. 2. The Japanese isolate growing on ST cells stained with fluorescent antibody. Prepared from Purdue immune serum. X1000.

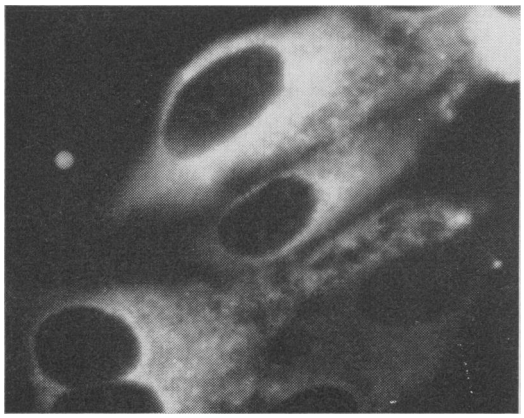

Fig. 3. The New York isolate growing on ST cells stained with fluorescent antibody. Prepared from Purdue immune serum. X1000.

Results

Pathogenicity and Immunogenicity of ST Cell-Cultured Virus Isolates for Hysterectomy-Derived Pigs:

The results of infecting 31 pigs with different cell passages of the 5 isolates, as shown in Table 1, indicated that all of the isolates could produce frank clinical signs of TGE, and that they also stimulated a serum antibody similar to that produced in the field disease. In most instances, they stimulated resistance in test pigs to further signs of TGE when subsequently exposed to virulent Purdue tissue-virus.

Comparison of the Cytopathology and Immunofluorescence of the Five Virus Isolates on SK and ST Cells:

The formation of clusters of large round cells that appeared to lie on top of the cell sheet, as described by McClurkin (3), was a very prominent feature of the early infection and beginning cytopathic effect (CPE) in all 5 isolates propagated in SK cells. The incubation period required for the appearance of the round cells and subsequent CPE varied with the quantity of the virus used for inoculum. When a high concentration of virus was used, the round cells appeared in the upper one-half of the cell sheet after 48 hours' incubation. At this stage, they could be shaken from the surface of the cell sheet leaving what appeared to be normal cells underneath.

After 96 hours' incubation, plaques appeared in the cell sheet. This process continued slowly, but even after 7 days' incubation the entire cell sheet was not completely removed from the glass.

The manifestations of virus infection in the ST cells were different from those in the SK cells in that the formation of clusters of round cells was not seen and the destruction of the cell sheet began at the butt of the tube within 18 hours of inoculation. Cell destruction continued until all ST cells were gone from the glass 72 to 96 hours after inoculation. When ST-cell-passed virus was inoculated onto SK cells, a typical reaction for SK cells, as previously described, was observed.

Examination of fluorescent antibody (FA)-stained SK and ST cells by means of ultraviolet light indicated the presence of a fluorescent antigen in the cytoplasm of some of the cells of the infected cell sheets from all of the 5 isolates of the cytopathic

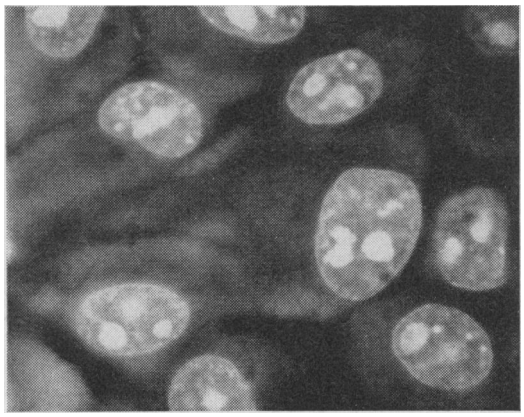

Fig. 4. Normal ST cells stained with acridine orange. X1000.

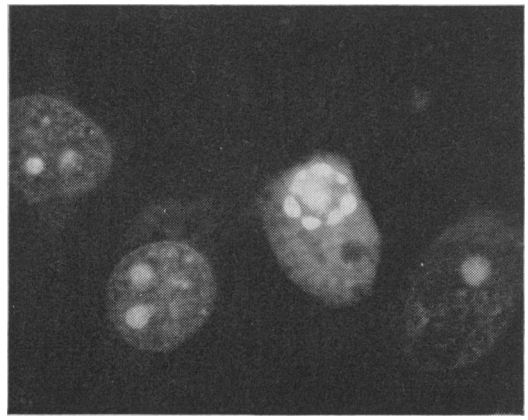

Fig. 5. ST cells infected with the Purdue isolate and stained with acridine orange. X1000.

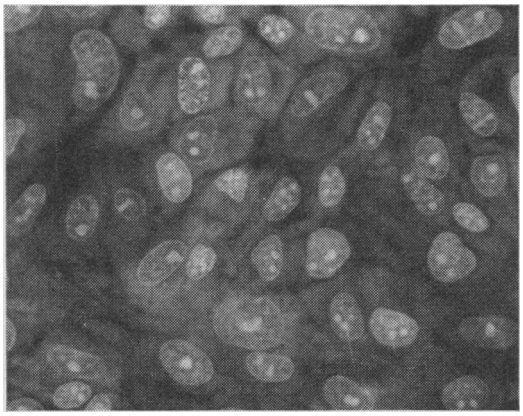

Fig. 6. Normal SK cells stained with acridine orange. X400.

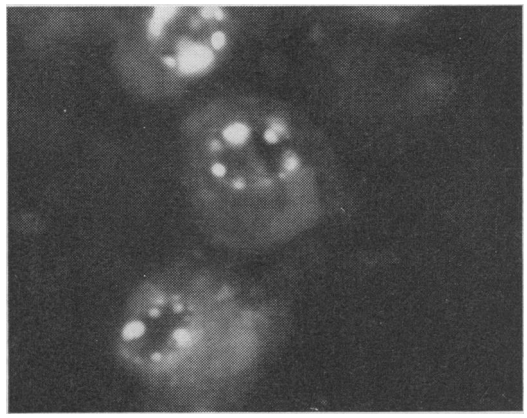

Fig. 7. SK cells infected with the Japanese isolate and stained with acridine orange. X1000.

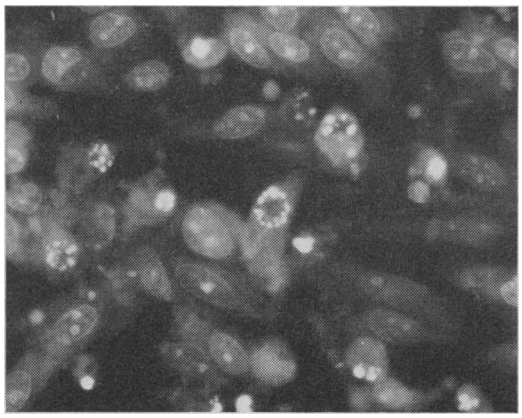

Fig. 8. SK cells infected with the Illinois isolate and stained with acridine orange. X400.

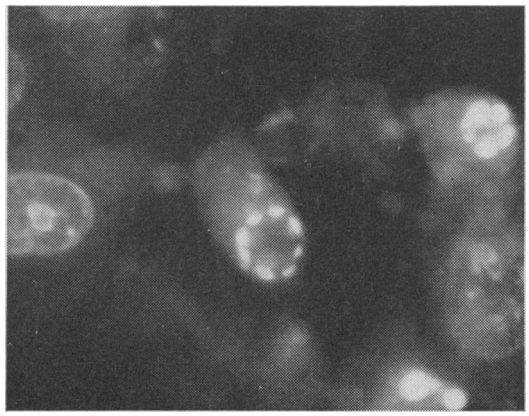

Fig. 9. SK cells infected with the New York isolate and stained with acridine organe. X1000.

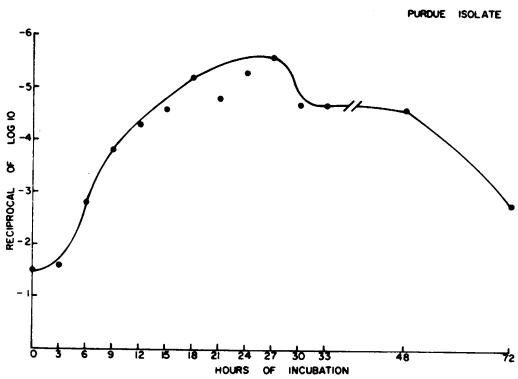

Fig. 10. The growth curve of the Purdue isolate growing on ST cells. Each dot on the curve represents the ID_{50} at that particular hour.

virus. The FA results in ST cells with 3 of the isolates are shown (Figs. 1, 2, 3).

The immunofluorescence in the cytoplasm could be demonstrated before CPE was recognized and disappeared as CPE progressed, suggesting that only a certain stage of viral replication will fluoresce. Most of the clusters of round cells were lost during the process of acetone fixation, but the few that remained on the stained coverslips did not show fluorescence.

Examination of acridine orange-stained SK and ST cells by means of ultraviolet light did not reveal cytoplasmic inclusions or any definite change in the cytoplasm of the infected cells. However, the nuclei of the cells infected with each of the 5 isolates were somewhat shrunken, with marked clumping and margination of the chromatin material (Figs. 4, 5, 6, 7, 8, 9).

The Rate of Virus Reproduction:

The growth curve of the Purdue isolate in ST cells is shown in Fig. 10. The growth curves of the other isolates were identical in that there was a 3-hour latency period following inoculation in which no increase in virus titer could be demonstrated. From the 3rd to the 12th hour of incubation there was a sharp rise in virus titer which reached a maximum between the 21st and 27th hour of postinoculation. After 27 hours, the virus titer began to decline. There was a marked rate of decline after 48 hours, however, and preliminary growth curve studies with the Purdue isolate revealed that the virus titer did not drop below 10^1 ID_{50}/ml. up to 120 hours' incubation.

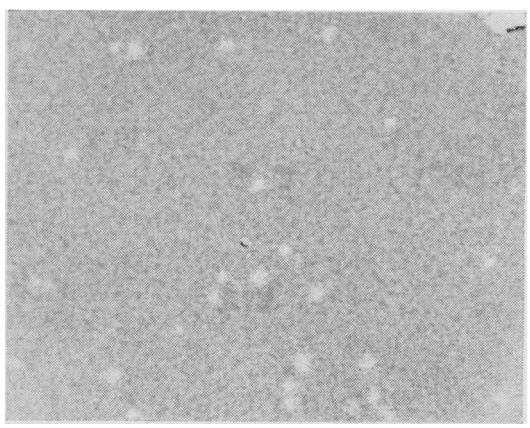

Fig. 11. Plaques produced by the New York isolate growing on ST cells.

Development and Character of Plaques Produced by the 5 Virus Isolates on ST Cells and Plaque Reduction by Antiserum Prepared from the Purdue Isolate:

At dilutions of 10^{-4} and 10^{-5} all of the isolates produced plaques of approximately 1 mm in diameter with irregular margins (Fig. 11) within 36 hours of inoculation.

Critical plaque titrations and plaque reduction studies were not carried out, but it was found that at a 10^{-4} dilution from 20 to 60 plaques were produced. At this dilution of the viruses, a 100% reduction in plaque formation resulted from virus neutralization by using Purdue immune serum at a 1:640 final dilution or Purdue convalescent serum at a 1:160 final dilution.

Virus Assay and Serum Neutralization of the 5 Virus Isolates:

After 10 or more passages in ST cells the virus titer of the Purdue isolate was $10^{5.5}$ ID_{50}/ml., Iowa isolate $10^{5.4}$, Japanese isolate $10^{4.8}$, Illinois isolate $10^{4.5}$ and the New York isolate $10^{4.8}$.

A constant quantity of Purdue immune serum (1:640 final dilution) neutralized the following ID_{50}'s/ml. quantities of virus: Purdue isolate $10^{5.2}$, Iowa isolate $10^{5.18}$, Japanese isolate $10^{4.8}$, Illinois isolate $10^{4.5}$, New York isolate $10^{4.8}$.

At a final dilution of 1:160 the Purdue convalescent serum neutralized the following ID_{50}'s/ml. quantities of virus: Purdue isolate $10^{4.5}$, Iowa isolate $10^{4.4}$, Japanese isolate $10^{3.8}$, Illinois isolate $10^{3.5}$, New York isolate $10^{4.8}$. The differences in the ID_{50} of virus isolates neutralized by the immune serum were no greater than would be expected from different samples of the same isolate.

TABLE II. Loss of Infectivity of the ST Cell-Cultured Virus Isolates in Acid and Alkaline Media as Determined by Inoculation into SK Cells

Virus isolate	pH 3	pH 4	pH 5	pH 6	pH 8	pH 9	pH 10
Purdue	2[1]	0	0	0	0	2	4
Iowa	2	0	0	0	0	1	4
Japanese	2	1	0	0	1	2	4
Illinois	3	2	0	0	0	1	4
New York	3	0	0	0	0	3	4

[1]Loss of infectivity titer expressed as the reciprocal of $\log_{10}$.

In Vivo Serum Neutralization Using Virulent Tissue-Virus of Purdue Origin and Immune Serum Prepared from Purdue ST Cell-Culture Virus:

All the pigs fed the virus-serum mixtures developed vomiting and diarrhea. One of the two virus control pigs which received the 10^{-1} dilution died and 3 of the 4 pigs which received the virus-serum mixture died. The virus control pig which received the 10^{-3} virus dilution developed frank signs of TGE and recovered, as did the 2 pigs receiving the 10^{-3} virus-serum mixture. This indicated that there is yet a second virus present in the virus-bearing tissue which is not neutralized by the ST cell-cultured virus antiserum.

Virus Filtration:

Following filtration through 100 mμ Millipore filters, infectivity for SK and ST cells was demonstrated in the undiluted filtrates of the 5 isolates, but not at dilutions of 10^{-1}. This represented a loss of approximately 10^4 ID_{50}/ml. tissue culture doses of virus. No infectivity could be demonstrated after filtration through a 50 mμ Millipore filter.

Viability of the Cell Culture Virus at 27 C, 37 C and 56 C:

Virus held at 27 C for 8 days was administered to pigs and inoculated into SK cells. The pigs developed signs of TGE and the cells developed CPE, thus indicating that the virus was still pathogenic and infective. However, the recovered pigs developed signs of TGE again when they were exposed to virulent virus-bearing tissue of Purdue origin.

Virus held at 37 C for 3 days and titrated in SK cells showed a loss in the infectivity titer of 3 logs, but after 6 days at 37 C it was infective in the undiluted virus only, showing a loss of more than 4 logs.

Virus held at 56 C for 10 minutes and titrated in SK and ST cells showed a loss in infectivity titer of 4 logs, but after 30 minutes at 56 C, no infectivity could be demonstrated even with undiluted virus.

Stability of the 5 Virus Isolates in Molar Magnesium Chloride at 37 C and 50 C:

After suspension of the Purdue virus in $M/MgCl_2$ for 24 hours at 37 C, no infectivity for SK cells and no pathogenicity or immunogenicity for pigs could be demonstrated. Infectivity for SK cells could not be demonstrated with any of the 5 virus isolates after treatment with $M/MgCl_2$ for 1 hour at 50 C.

Resistance of Cell Culture Virus to Organic Solvents:

Under the conditions of the experiments described, the 5 isolates were not completely inactivated by ether or chloroform. However, infectivity for SK cells was demonstrated only in the undiluted virus. This represented a loss of at least 10^4 ID_{50}/ml. tissue culture doses of infective virus.

After treatment with ether for 24 hours at 4 C, the Purdue isolate was pathogenic for pigs, but after 48 hours' ether treatment, the virus lost both its pathogenicity and immunogenicity.

Stability of the ST Cell-Cultured Virus in Acid and Alkaline Media as Determined by Infectivity for SK Cells:

The 5 virus isolates were inactivated at pH 2. The effect of the pH ranging from pH 3 to 10 is shown in Table 2. The viruses were quite stable at pH ranging from pH 4 to 8.

Viability of the Lyophilized Purdue and Iowa Isolates:

Pathogenicity was demonstrated by feeding the reconstituted lyophilized tissue-virus and cell culture virus from each isolate to 2 pigs after 1-month storage at 4 C. All pigs developed signs of TGE and died

indicating the virus could withstand the process of lyophilization.

Infectivity for SK cells was not demonstrated beyond a 10^{-1} dilution of the reconstituted tissue-virus or cell-culture virus held for 8 months at 4 C; thus a loss of approximately 10^3 cell culture doses of virus occurred. Infectivity for SK cells was demonstrated only in the undiluted reconstituted lyophilized cell-culture virus held for 8 months at 27 C, representing a loss of approximately 10^4 cell culture doses of virus.

Discussion

The facts that the 5 virus isolates studied produced signs of TGE in susceptible pigs, and that they stimulated a significant degree of resistance to a lethal challenge inoculum suggest that they may be closely associated with the natural disease. However, there is a non-cytopathic virus which grows in cell culture and can produce signs of TGE, with ensuing death or resistance to further infection, and suggests there is yet a second virus which is responsible for the over-all etiology of TGE. The noncytopathic virus does not produce a serological response to the cytopathic virus, nor does the antiserum against the cytopathic virus remove the disease producing ability from the noncytopathic virus culture. Furthermore, inability to isolate the cytopathic virus from all field cases gives further support to the probability that the cytopathic virus is not the cause of the over-all disease of TGE.

The nature of the cytopathic virus and the conditions under which it produces CPE are not completely understood. For example, if the cytopathic virus grown on SK cells is seeded on SK monolayers in 1- or 2-liter diphtheria toxin bottles, no observable CPE is produced. Yet these culture supernates produce typical CPE when passed into smaller bottles or tubes, and they produce signs of TGE in pigs at a dilution of 10^{-4}. Furthermore, the Illinois isolate, received in frozen dog kidney cultures, was not cytopathic for dog kidney cells nor was it cytophatic for SK cells, but it was cytopathic for ST cells. After it was grown on ST cultures, it became cytopathic for SK cells, but not for dog kidney cells.

Studies designed to characterize the noncytopathic virus involved in TGE, and to assess the role that the cytopathic and the noncytopathic virus play in TGE will be the subject of further investigations.

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