Propagation of Hog Cholera Virus in Tissue Culture.* (26215)

JAMES H. GILLESPIE, BEN E. SHEFFY AND JAMES A. BAKER
Veterinary Virus Research Institute, New York State Veterinary College, Cornell University, Ithaca, N. Y.

Cultivation in tissue culture of a cytopathogenic strain of hog cholera virus would be useful for its study since inoculation of pigs has been the only method available. Pig studies are not easily done, and, consequently, our knowledge of hog cholera virus has not kept pace with that available for those viruses that have been cultivated in tissue culture with observable cytopathic effects (CPE).

Hog cholera virus has been propagated in tissue culture(1-10), but either cellular changes were not observed or were not easily recognized, and surety that virus was present required a test in pigs. Other animal viruses, and in particular virus diarrhea virus, also have been observed to behave in this manner; yet, persistent attempts with different strains(11) eventually located some that were cytopathogenic. Also, modifying mediums used for culture of cells proved successful for some viruses(12,13).

This report concerns the culture of a particular strain of hog cholera virus that produces CPE.

Tissue culture method. Primary monolayer cultures were prepared from kidneys of pigs 5 to 16 weeks of age. Growth was initiated with Earle's balanced salt solution (80%), 0.5% lactalbumin hydrolysate (10%), bovine serum (10%), penicillin (500 units per ml), streptomycin (100 mg per ml), and mycostatin (100 units per ml). Excellent cell sheets were produced and were maintained in good condition for at least 2 months by a change of medium twice a week.

At time of virus inoculation, some of the tubes were washed with buffered salt solution and a type of medium used successfully by Karzon for cultivation of distemper virus was substituted(12). This medium consisted of 50% bovine amniotic fluid, 46% Earle's salt solution and either 4% lamb serum, 4% bovine serum, or 4% pig serum. The same antibiotics as used in the original medium were added.

Virus inoculum. The following strains of hog cholera virus were prepared for inoculation: 1. Virulent strain A(14), maintained as fully virulent virus by transfer in swine. A 10% suspension of spleen from an infected pig was used as inoculum. 2. Modified strain A(14), modified in virulence by 17 serial transfers in rabbits. A 10% suspension of spleen from an infected rabbit was used as inoculum. 3. Persistent strain A(15), initially modified in virulence by transfer in rabbits and then reverted to virulence by establishing a persistent viremia in young pigs. A 50% suspension of blood from an infected pig was used as inoculum. 4. Virulent strain I† was inoculated as a 50% suspension of blood from infected swine. 5. Virulent strain II†, furnished as dried material which was reconstituted before inoculation. 6. Virulent strain III,† also furnished as dried material which was reconstituted and then inoculated.

All strains were inoculated in 0.2 ml amounts into each of 5 tubes that contained the different media. Uninoculated tubes were retained for comparison. The tubes were incubated at 37°C for 7 to 10 days before final reading.

Presence of virus. Since the objective sought was a strain of virus that produced CPE, tubes were examined daily for evidence of cellular changes. Whether effects were noted or not, fluids were removed from each group of tubes 7 to 10 days after inoculation, pooled, and 0.2 ml were transferred to other tubes. If no changes were noted after 5

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† Virulent strain I was kindly supplied by Dr. Donald P. Gustafson, Purdue Univ. and virulent strains II and III by Dr. Howard W. Dunne, Pennsylvania State Univ.

transfers, further attempts were discontinued. If cell changes were noted, transfers were continued. Results are presented in Table I. In later passages, Parker 199 (86%), lamb serum (4%), 0.5% lactalbumin hydrolysate (10%), plus antibiotics was used. This also was tried with 4% bovine serum substituted for lamb serum.

Sera were procured from 14 pigs at time of inoculation with modified hog cholera virus and again 21 days afterward. For inoculation, Strain A virus was used for 4 pigs and another strain was given the others. Since some pig sera were toxic to tissue-cultured cells, each serum was diluted 5-fold and then tested for its capacity to neutralize virus. Equal portions of diluted serum and undiluted tissue culture fluid from infected tubes were mixed, placed in the refrigerator for 2 hours and 0.2 ml inoculated into each of 5 tubes. At the same time, tissue culture fluid was titrated for virus. Also, comparative virus titrations were made in pigs. Fluids from tubes also were cultured for PPLO and other bacteria. None were demonstrated.

Results. Only strain A, that had been established as a persistent viremia in pigs, produced CPE and only in the medium that contained amniotic fluid and lamb serum (Table I). Subsequent passages showed this to be a reproducible effect during 26 serial passages (Fig. 1) in this medium, although similar changes were noted when Parker 199 and 4% lamb serum was used. As in initial tests, when bovine serum was substituted for lamb serum, CPE did not develop.

TABLE I. Cultivation of Hog Cholera Virus in Tissue Culture.

Virus strain	Medium for cell maintenance: Earle's medium + serum of			Karzon's medium + serum of		
	Lamb	Bovine	Pig	Lamb	Bovine	Pig
A virulent	—	—	Toxic	—	—	Toxic
A modified	—	—	"	—	—	"
A persistent	—	—	"	+	—	"
Virulent I	—	—	"	—	—	"
" II	—	—	"	—	—	"
" III	—	—	"	—	—	"

— means no CPE; +, CPE found.

Toxic means cells in uninoculated tubes degenerated.

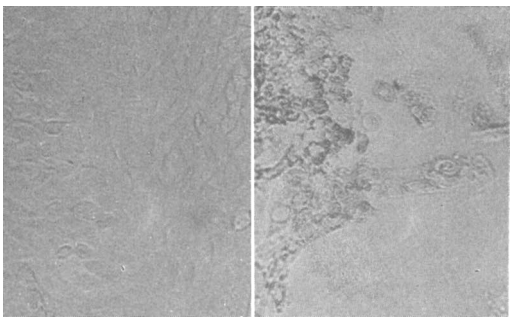

FIG. 1. Uninoculated monolayer of pig kidney cells (left). On right, pig kidney cells infected with hog cholera virus. Note cytopathic effects in the form of cell degeneration and broken cell sheet.

Titrations of tissue culture fluids from inoculated tubes showed approximately 100 $TCID_{50}$. Comparable tests in pigs showed the same endpoints. Tissue-cultured virus from 8, 16 and 32 transfers produced signs of illness and lesions typical of hog cholera. After 3 days temperatures became elevated and the pigs died 9 to 20 days later. Autopsy revealed hemorrhagic lymph nodes and petechiation of kidneys and bladder. Tissue-cultured virus was neutralized by sera from pigs 21 days after inoculation with modified virus but not by sera at time of inoculation.

Discussion. Factors responsible for the CPE produced by this particular strain of hog cholera virus are not shown by these studies. It is important to note that this strain had been modified and reverted from attenuation to virulence in a single pig passage. Furthermore, there were 100 to 1000 times more virus in the inoculum than is ordinarily produced by virulent strains maintained in the usual manner(15). The large amount of virus may have been a factor. Initially, it was thought that the medium was important, and it is, to the extent that lamb serum permitted the virus to be cytopathogenic, while bovine serum did not. Lamb serum, therefore, was an essential constituent whether in combination with amniotic fluid medium or Parker 199. Whatever the reason, a strain of hog cholera virus is now available for study and, since it was neutralizable it should be helpful for serological tests.

Summary. A strain of hog cholera virus has been procured that produced CPE in tissue cultures of pig kidney cells. This tissue-cultured virus produced typical hog cholera when inoculated into susceptible swine and it was neutralized by immune sera.

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Response of Lactic Acid Bacteria to Amino Acid Derivatives. V. L- DL- and D-Valic Acids.* (26216)

MERRILL N. CAMIEN AND MAX S. DUNN
Chemical Laboratory, University of California, Los Angeles

It was recognized as early as 1947(2) that either L- or D-phenyllactic acid, but not D-phenylalanine, is an effective substitute for L-phenylalanine in *Lactobacillus arabinosus* nutrition. Corresponding relationships between the optical forms of phenyllactic acid and phenylalanine were subsequently shown to hold additionally for a variety of other lactic acid bacteria(3). Utilization by *L. arabinosus, Leuconostoc mesenteroides* and *Streptococcus faecalis* of various hydroxy acid analogs in place of the corresponding amino acids was reported by Holden, *et al.* (4), but these authors' data do not include determinations of optical specificity. It was desirable, therefore, to secure more complete information on utilization of optically active hydroxy acids other than phenyllactic acid. The present report concerns utilization of the valine analogs, L-, DL- and D-valic (α-hydroxyisovaleric) acids.

Methods. L- and DL-valic acids were synthesized from L- and DL-valines essentially as described by Baker and Meister(5). D-Valic acid was produced by resolution of DL-valic acid with cinchonine†. Microbiological procedures and medium were the same as those used previously(3) except L-phenylalanine, final concentration 20 mg %, was included in the medium and DL-valine, DL-norleucine and DL-norvaline were omitted. Bacterial cultures have been described(1,6).

Results. Preliminary screening of 10 bacteria revealed that *Lactobacillus arabinosus*

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† DL-Valic acid 10 g, cinchonine 25 g and dry acetone 50 ml were stirred with a magnetic stirrer for 3 hours in a closed flask. Filtration of mixture yielded 13 g of essentially pure D-valic acid-cinchonine salt. The product was washed with cold acetone to remove residual traces of the highly acetone-soluble L-salt. It is of interest that although this procedure was consistently successful for resolution of DL-valic acid, it appeared to be relatively ineffectual in resolving DL-leucic acid.