Separation of a Soluble Antigen and Infectious Particles of Bovine Viral Diarrhea Viruses and their Relationship to Hog Cholera

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SUMMARY

A soluble antigen present in infectious tissue culture fluids was separated from the infective virus particle by ultracentrifugation of two serologically related strains of bovine viral diarrhea viruses, NADL-MD and Oregon C24V.

Neutralizing antibodies against the two viruses were absent in four hog cholera antisera, but present in significant titer in the commercially prepared antiserum. Precipitin tests utilizing the agar double diffusion technique formed a single line of identity between the concentrated soluble antigen of both viruses and NADL-MD and hog cholera antisera. No lines were observed using concentrated virus pellet and noninfected BEK cell antigens or control SPF calf and swine sera.

Introduction

Tissues from animals clinically infected with bovine mucosal disease were differentiated from normal tissues in agar double diffusion (1). Also, using tissues from infected animals as antigens, a serological relationship between a mucosal disease and hog cholera was observed (2, 3).

This is a report of the separation by ultracentrifugation of a soluble antigen and infectious particles of mucosal-viral diarrhea viruses and of their relationship with homologous and hog cholera antisera in agar double diffusion.

Materials and Methods

Cell Cultures. Primary cell cultures from bovine embryonic kidney (BEK) were started in Hank's balanced salt solution containing 10% calf serum. The medium was changed after three days to Earle's balanced salt solution with 10% calf serum. Eagle's basal medium (EBM) containing 2% bovine fetal serum was used for maintenance.

Viruses and Antisera. Two cytopathogenic strains of mucosal-viral diarrhea disease viruses were used in this study; the prototype virus, Oregon C24V (4) and a serologically related virus, NADL-MD, isolated by the authors. NADL-MD antiserum was prepared in a specific pathogen-free (SPF) calf. Five hyperimmune hog cholera antisera were used in this study; one prepared against standard hog cholera virus in a SPF pig,[1] three antisera prepared against a variant, lapinized, and standard hog cholera virus,[2] and one commercially prepared antiserum. Phenol was removed from the commercial antiserum by dialyzing it against several changes of phosphate buffered saline.

Preparation of Antigens. Five-liter tissue culture bottles (6″ x 14″ cell surface area) of five-day-old BEK cell cultures were washed with warm phosphate-buffered saline solution. Then, five ml. of EBM containing 100 $TCID_{50}$ of virus were added to each bottle and allowed to adsorb for one hour at room temperature. After adsorption, an amount of maintenance medium just sufficient to cover the cell layer was added (approximately 165 ml.). The cultures were incubated at 37 deg. C and observed daily for evidence of cytopathic effect (CPE). NADL-MD virus cultures showing complete CPE in 72 hours were harvested following freezing and thawing. C24V virus cultures were harvested in six days with approximately 75% cell destruction. Tissue culture fluids were separated from cellular debris by centri-

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1. Kindly furnished by Dr. A. W. McClurkin. National Animal Disease Lab., Ames, Iowa.
2. Kindly furnished by Dr. J. P. Torrey, National Animal Disease Lab., Ames, Iowa.

fugation for 30 minutes at 6500g in a refrigerated centrifuge.

Separation and Concentration of Antigens. Clarified tissue culture fluids were centrifuged for 2½ hours at 98,500g in a Spinco Model L preparatory ultracentrifuge after the method used for separation of soluble antigens and infectious particles of rinderpest and canine distemper viruses (5). Following centrifugation, the supernatant fluid was carefully decanted and the virus pellet resuspended in EBM to 1% the original volume. Supernatant fluids were concentrated by pressure dialysis at 4 deg. C using ⅝ inch cellulose casing (Visking Company). After the volume of fluid was reduced to approximately 15 - 20 ml., the casing was sealed and powdered Carbowax 20 M, a high molecular weight polyethylene glycol (Union Carbide) was applied to the casing surface (6) to further concentrate the supernatant fluid to 1% the original volume.

Control Antigens and Sera. Control antigens for the agar double diffusion test were prepared by concentrating noninfected BEK cell culture fluid after the procedure previously described. Nonimmune SPF calf and swine sera were controls.

TABLE I — Infectivity Assay of Ultracentrifuged Tissue Culture Fluids

	NADL-MD Virus	C24V Virus
Clarified Tissue Culture Fluid	6.5*	6.3
Ultracentrifuged Supernatant Fluid	3.2	3.3
Virus Pellet Concentrate**	8.2	8.2

*Titers in neg. Log_{10} $TCID_{50}$/ml.
**Resuspended to 1% original volume

TABLE 2 — Serum Neutralization Titers of Various Antisera Against 100 $TCID_{50}$ of Viral Diarrhea Viruses in Primary Bovine Embryonic Kidney Cell Cultures

	Neutralization Titers	
Antisera	NADL-MD Virus	C24V Virus
NADL-MD	3000*	128*
Hog Cholera (SPF derived)	< 4	< 4
Hog Cholera #2875	< 4	< 4
Hog Cholera #7085	< 4	< 4
Hog Cholera #9197	< 4	< 4
Commercial Hog Cholera	512	128

*Reciprocal of final serum dilution

Infectivity Assay. Clarified tissue culture fluid, ultracentrifuged supernatant fluid, and virus pellet concentrate were titrated for infectivity in BEK cell culture tubes by inoculating ten-fold dilutions into 5 culture tubes per dilution. Cultures were incubated at 37 deg. C and observed daily for evidence of CPE. End points were calculated by the method of Reed and Muench (7).

Serum Neutralization Tests. Two-fold dilutions of heat inactivated (56 deg. C for 30 minutes) NADL-MD and hog cholera antisera were mixed with equivalent amounts of EBM containing approximately 100 $TCID_{50}$ per 0.1 ml. of NADL-MD virus in one series and C24V virus in the second. Mixtures were incubated at 37 deg. C for 30 minutes, and 0.2 ml. of each dilution was inoculated into each of five BEK cell culture tubes. Fifty per cent neutralization end points were calculated by the method of Reed and Muench (7).

Agar Double Diffusion. Clean glass slides (1″ x 3″) were covered to the depth of approximately 3 mm. with melted agar composed of 1% Noble agar (Difco) in deionized water containing 1:10,000 parts Merthiolate (Eli Lilly Company) and adjusted to pH 7.2 by addition of M/15 Na_2HPO_4 solution. After the agar solidified, wells 3.5 mm. apart, were cut with a number one cork borer. Agar slides were incubated in a moist chamber at room temperature and observed daily for one week.

Results

Following ultracentrifugation, 0.1% or less of infective virus particles remained in the supernatant fluids (Table 1).

Serum neutralization titers of NADL-MD antiserum were 3,000 against the homologous virus and 128 for C24V virus. No neutralizing antibodies were demonstrated in 1:2 dilutions of hog cholera antisera tested except in the commercially prepared antiserum a significant titer was obtained against NADL-MD and C24V viruses (Table 2).

In agar double diffusion slides, a single line of identity formed between NADL-MD and C24V concentrated supernatant

fluid antigens and NADL-MD and all five hog cholera antisera. No precipitin lines were present using either concentrated virus pellet or noninfected cell antigens (Fig. 1 & 2). Control SPF calf and swine sera produced no observable precipitin lines.

Discussion

Cross reactions in the agar double diffusion test between bovine viral diarrhea antigens prepared from infectious tissue culture fluids and hog cholera antisera are due to a soluble component. A sedimentable component obtained after ultracentrifugation produced no lines of identity between the homologous antiserum and hog cholera antisera although it contained over 99.9 per cent of the infectious virus (Fig. 1 & 2). The apparent relationship between hog cholera and bovine viral diarrhea is therefore dependent upon the common soluble antigen in the same way that rinderpest and canine distemper are related (5).

The presence of neutralizing antibodies against bovine viral diarrhea viruses in some hog cholera antisera (8) is probably due to earlier infection with the former agent and not through actual crossing. Hog cholera antiserum prepared in an SPF pig and individual sera from other hyperimmune pigs failed to neutralize bovine viral diarrhea viruses (Table 2). However, such sera produced excellent precipitin lines in the agar double diffusion test (Fig. 2). A commercial hog cholera antiserum, which probably represented a serum pool from several different pigs, had unexpectedly high neutralizing antibody titers against the same bovine viral diarrhea viruses (Table 2). It also produced precipitin lines in the agar double diffusion test.

The role of soluble antigen in conferring protection against hog cholera virus has not been determined. Presumably the accelerated antibody response described previously (9) may be in operation. An animal previously exposed to the soluble antigen may respond quicker with neutralizing antibody since one immune mechanism is already in operation.

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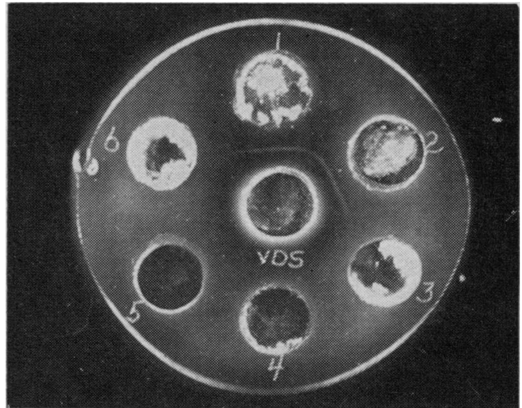
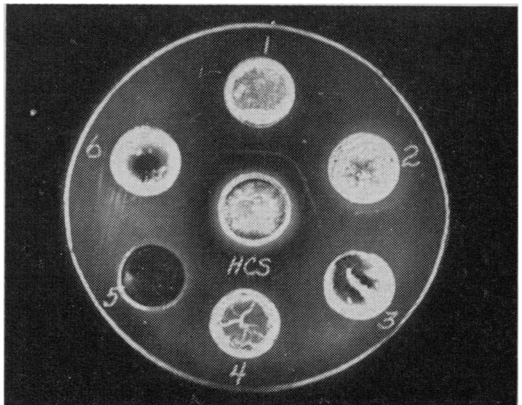


Figure 1: Agar Double Diffusion Slides Center well (VDS) — NADL-MD antiserum Outer wells — (1) NADL-MD ultracentrifuged supernatant fluid concentrate, (2) C24V ultracentrifuged supernatant fluid concentrate, (3) NADL-MD virus pellet concentrate (4) Noninfected BEK cell culture concentrate, (5) SPF calf serum, and, (6) C24V virus pellet concentrate.



Figures 2: Agar Double Diffusion Slides Center well (HCS) — Hog cholera antiserum (#2875) Outer wells — (1) NADL-MD ultracentrifuged supernatant fluid concentrate (2) C24V ultracentrifuged supernatant fluid concentrate, (3) NADL-MD virus pellet concentrate, (4) Non-infected BEK cell culture concentrate, (5) SPF swine serum, and (6) C24V virus pellet concentrate.