Cellular Resistance in Tissue Culture, Induced by Noncytopathogenic Strains, to a Cytopathogenic Strain of Virus Diarrhea Virus of Cattle. (27481)

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(Introduced by James A. Baker)
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Initial isolates of virus diarrhea (VD) virus from cattle did not produce cytopathic effects in tissue culture(1,2). They were defined by calf inoculations(3,4). Later, a cytopathogenic strain, designated Oregon C24V, was isolated from the spleen of a dead calf which showed typical lesions of virus diarrhea (5). Also, this strain produced plaques in agar overlay cultures of bovine testicular cells (6). Subsequently, other strains have been isolated; most of them have been cytopathogenic but usual tissue culture methods reveal only those that produce cytopathic effects.

Since studies of noncytopathogenic strains have been confined to calves, it would be helpful if some means other than use of calves could be found. Accordingly, tests were made in agar overlay tissue cultures for cellular resistance to a cytopathogenic strain by previous inoculation with noncytopathogenic viruses.

Materials and methods. Cell cultures. Cell cultures from embryonic bovine kidney cells representing the 2nd through 6th transfers were used. Stock cultures were grown in 16-oz bottles in a combination of Earle's balanced salt solution, 0.5% lactalbumin hydrolysate, antibiotics and 10% of either calf or lamb serum(7). The cells were removed from the glass with a saline-trypsin-versene mixture (STV) that contained 0.05% trypsin and 0.025% versene. The cells were covered with STV, incubated at 36°C and with occasional shaking of the bottles, usually came off the glass within 15 minutes. Cells were sedimented by centrifugation, resuspended in growth medium described above and seeded into 3-oz prescription bottles (furnished by Brockway Glass Co., Brockway, Pa.) each with 8 ml which contained 5×10^5 cells per ml. The bottles were placed in an incubator at 36°C and after a change of growth medium, a confluent sheet satisfactory for plaque assay studies formed after 6-7 days.

Virus. Of the cytopathogenic strains tested, including Oregon C24V, the one designated C60F produced the most clearly defined plaques and was selected for cellular resistance tests. Three noncytopathogenic strains were tested for their ability to induce cellular resistance: New York 1 which had been isolated from a sick calf and subsequently transferred for 91 passages in embryonic bovine kidney cells; Indiana 46 (kindly furnished by Dr. W. R. Pritchard) in spleen from an infected calf and transferred for 21 passages; Saunders strain (furnished by Dr. Frank Ramsay) as infected spleen and transferred for 4 passages.

Virus assay. Tenfold dilutions that ranged from 10^{-4} to 10^{-7} of noncytopathogenic VD virus strains were prepared in H-M medium(7), which consisted of Earle's balanced salt solution, 0.5% lactalbumin hydrolysate, 0.1% yeast extract (Difco), 0.1% bovine albumin (Fraction V, Armour Laboratories) and phenol red. The pH was adjusted with 7.5% sodium bicarbonate. The growth medium was removed from the cell cultures and they were washed twice with H-M. Each of 5 bottles was inoculated with 0.1 ml of a virus dilution. After adsorption for 2 hours at 36°C, 8 ml of H-M medium was added to each bottle. These cultures were incubated at 36°C for 3 days, the medium was removed and the cells again washed twice with H-M. After washing, each culture was inoculated with 0.1 ml of a dilution of C60F which con-

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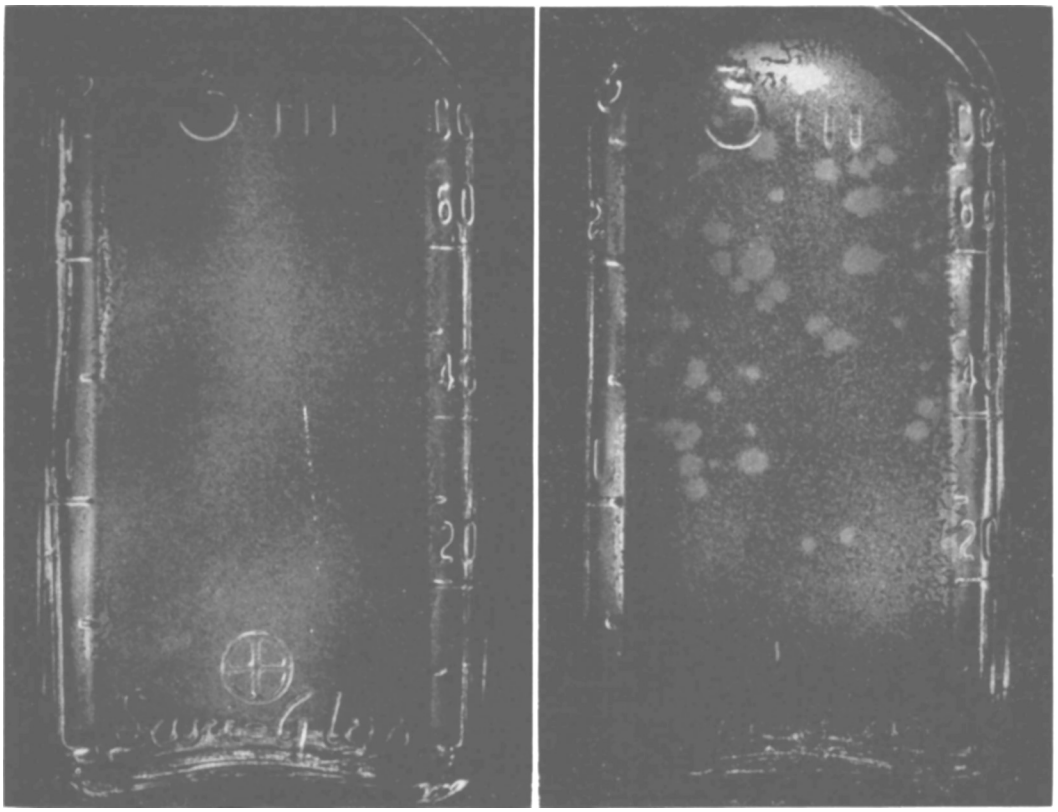

FIG. 1. Cellular resistance induced by an initial inoculation of a noncytopathogenic strain of VD virus to subsequent inoculation of C60F (left photograph); in right photograph, plaque formation produced by C60F is shown.

tained approximately 50 plaque forming units (PFU). Cell cultures which had not been inoculated with noncytopathogenic virus were included as controls. After adsorption for 2 hours, 9-10 ml of an overlay consisting of H-M medium and 1.5% agar (Difco) were placed in each bottle. Plaque counts were usually made 4 days after inoculation but occasionally after 5 days. For more accurate readings, 1 ml of a 1/10,000 dilution of neutral red was placed in each bottle, which were held at 36°C for 2 hours to permit penetration of the dye into the cells. Then the bottles were inverted, incubated at 36°C overnight and plaques of any size were counted the following day. Endpoints were calculated by the Reed and Muench method. The test of each strain was repeated.

Neutralization tests. Serum from a calf obtained at the time of inoculation with strain NY 1 and again 14 days later were used undiluted and also in 5-fold dilutions with H-M medium. Each dilution was mixed with an equivalent amount of H-M medium containing approximately 100 infective units per 0.1 ml of a noncytopathogenic strain of VD virus. Mixtures were incubated at 4°C for 2 hours and 0.2 ml was inoculated into each of 3 bottle cultures which had been washed twice with H-M. After adsorption for 2 hours at 36°C, 8 ml of H-M medium was added to each bottle. They were incubated for 3 days at 36°C and each culture was washed twice with H-M, inoculated with approximately 50 PFU of strain C60F and plaques counted as described above. Each noncytopathogenic strain was tested in this manner.

Results. Production of plaques by the C60F strain in embryonic bovine kidney cell cultures is shown in Fig. 1. Cellular resistance to plaque formation by noncytopathogenic virus is included also.

Endpoints for strain NY 1 were $TCID_{50}$ $10^{6.2}$ per ml and $10^{6.5}$ per ml in the repeated test; for Indiana 46, they were $10^{6.4}$ per ml and $10^{6.6}$ per ml; and for Saunders strain, $10^{5.8}$ per ml and $10^{6.4}$ per ml. Serum which had been obtained from a calf after VD virus infection completely neutralized the noncytopathogenic virus in a 1/2 dilution and no cellular resistance was noted; the 1/10 dilution partially neutralized because plaques were fewer in number and smaller than were found at higher serum dilutions or with undiluted serum obtained at time of inoculation. In these, plaque formation was similar to controls.

Discussion. An accurate and reproducible assay method became available for measurements of noncytopathogenic strains of VD virus when it was found that cell cultures became resistant to a plaque-forming cytopathogenic strain of VD virus if the noncytopathogenic strain had been inoculated at least 3 days earlier. Neutralization of the noncytopathogenic strains by VD antibodies made possible serological comparisons of VD virus strains whether or not they were cytopathogenic. Identification of noncytopathogenic strains, therefore, became possible without the use of calves.

The nature of cellular resistance induced by noncytopathogenic strains to plaque-forming cytopathogenic strains of VD virus has not been shown by these studies. It may be similar to the interference produced in MCN cell cultures by Newcastle disease, mumps or 6-6 viruses to vesicular stomatitis virus(8). More recently, the RIF-induced resistance to Rous sarcoma virus also was attributed to interference because resistance was not established until after 3 days(9). In agreement with these studies, initial tests with VD virus showed complete resistance to 50 PFU after 3 days but not after 2 days.

Summary. Three noncytopathogenic strains of virus diarrhea (VD) virus from cattle induced resistance to a plaque-forming strain of VD virus in agar overlay cell cultures of embryonic bovine kidney cells. Plaque inhibition made possible the measurement of virus concentration of noncytopathogenic VD virus strains. Cellular resistance was neutralized by VD virus antibodies.

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