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Sensitivity of BHK-21 Cells Supplemented with Diethylaminoethyl-Dextran for Detection of Street Rabies Virus in Saliva Samples

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A tissue culture system for detecting rabies virus from saliva samples of suspected animals was developed and compared to suckling mouse inoculation. Swab samples were obtained from the mouth of the animal heads received for rabies diagnosis; these swabs were submerged in maintenance medium. The maintenance medium was inoculated intracerebrally into suckling mice and onto BHK-21 cells with diethylaminoethyl (DEAE)-dextran (BHK/DEAE) and without (BHK). Rabies immunofluorescence was performed on the brain of the mice dying during the observation period and also on both tissue culture systems every day after infection. The BHK-DEAE system detected 28 positive samples obtained from 48 rabid animals and the BHK system detected 18. By suckling mouse inoculation only 11 of the same positive samples were detected. A total of 90 samples was studied by the three methods. Rabies virus was detected by the tissue culture methods earlier than by suckling mouse inoculation. The BHK-DEAE method was an economic and fast method for rabies virus detection in saliva samples, which could be used for ecological and pathogenesis studies, as well for rabies diagnosis before the death of the suspected animal.

The immunofluorescence (IF) test has a sensitivity comparable to that of the mouse inoculation technique for detecting rabies virus in brain and salivary glands of rabid animals (1, 5, 11, 18). However, it is difficult to detect rabies antigen by IF in saliva samples. The mouse inoculation test must be used for this type of specimen, although the processing of a large number of samples using this procedure for ecologic or pathogenesis studies is costly and time consuming. Another technique for rabies virus isolation from saliva samples is urgently required.

Tissue culture-adapted strains of street and fixed rabies virus have been propagated in a number of different cell systems (17). However, the low efficiency of cell infection and the slow development of the virus within the cells have been major obstacles in the use of these systems for isolating street rabies virus.

Kaplan et al. (7) have shown that the infectivity of fixed rabies virus was enhanced in a number of cell lines by the addition of diethylaminoethyl (DEAE)-dextran to the virus inoculum.

It was postulated that DEAE-dextran would increase the sensitivity of BHK-21 C_{13} cells (14) for street rabies virus isolation. The present report deals with the results obtained using this system for isolation of rabies virus from saliva samples obtained from suspected rabid animals.

MATERIALS AND METHODS

Cells. BHK-21 cells were grown on 60-mm disposable petri dishes containing three cover slips (18 by 18 mm). Each petri dish was seeded with 2×10^6 cells in 5 ml of the growth medium described by MacPherson and Stoker (10) and incubated at 37 C. Under these conditions the monolayer was confluent in 48 h.

Samples. Using cotton swabs, saliva samples were obtained from the mouths of animal heads received at the laboratory for rabies diagnosis. The swabs were introduced into 3 ml of BHK-21 maintenance medium containing 0.3% bovine albumin fraction V instead of calf serum. When needed, bacterial contamination was eliminated by membrane filtration (porosity 0.45 μm). One infected dog brain sample in a 20% suspension prepared in the same medium was included in the study.

The samples were stored at −60 C until processed.

Inoculation procedures. The swabs, after thawing, were pressed against the wall of the container to release most of the medium before proceeding to inoculate the different systems.

Suckling mice were inoculated by the intracerebral route with 0.01 ml of the sample and kept under observation for 4 weeks. The rest of the medium was split in two samples and DEAE-dextran (Pharmacia

Fine Chemicals AB, Uppsala, Sweden) at a final concentration of 50 μg/ml was added to one of these.

Three petri dishes were inoculated with each sample. To do this, the growth medium was removed from the dish and the monolayer was rinsed twice with Hanks salt solution and inoculated with 0.5 ml of the sample. The inoculum was incubated 1 h at 37 C, the monolayer was rinsed with Hanks salt solution, and 5 ml of maintenance medium was added. The dishes were incubated at 33 C in a 4% CO_2 atmosphere.

Starting 1 day after inoculation, a cover slip was obtained daily for 6 to 8 days from each sample for staining by IF (5). The IF-stained cover slips were observed under an ultraviolet light microscope. The reagents and equipment utilized for the IF technique are described elsewhere (9). The number of cells showing rabies antigen were counted and a sample was considered positive when one or more cells showed rabies antigen.

The brains of all mice dying during the observation period were also studied by IF.

RESULTS

One dog brain sample and 89 saliva samples from dogs, cats, bats, monkeys, and rabbits were processed by the three isolation systems. Forty-eight of these animals had been shown to be rabies positive by IF and mouse inoculation of the respective brains.

Of the 48 rabies-positive animals, 28 saliva specimens were positive by the BHK cell plus DEAE-dextran (BHK-DEAE) system, whereas the BHK cell without DEAE (BHK) system yielded 18 positive specimens. By suckling mouse inoculation only 11 of the same samples were positive.

Both tissue culture systems became positive 1 to 7 days after inoculation, whereas the mice developed rabies symptoms 6 to 22 days after inoculation (Fig. 1). More infected cells were found in the BHK cells when the inoculum was supplemented with DEAE-dextran than in the cells inoculated without this compound.

DISCUSSION

Since Galtier (6) introduced rabbits as experimental animals for the diagnosis of rabies in 1879, investigators have been searching for more sensitive systems for the isolation of this virus. Webster and Dawson introduced Swiss albino mice for this purpose (16), and Casals demonstrated that the sensitivity for rabies virus of suckling mice was greater than that of older mice (4). A system more sensitive than suckling mouse inoculation for isolating rabies virus from saliva samples is presented here. The inoculation of BHK cells with saliva samples supplemented with DEAE-dextran followed by IF staining has allowed us to recover two and one-half times more positive samples than were detected by suckling mouse inoculation (28:11). Moreover, the tissue culture system was less expensive and more rapid for detecting the virus in this type of sample.

The daily staining and reading of IF preparations from each sample under study represents a large volume of work. Based on our results, it could be assumed that inoculating a single petri dish with the specimen and staining one cover slip, for example, on day 5 postinfection would have allowed us to detect 96% (27 out of 28) of the positive samples for the BHK-DEAE system (Fig. 1). This assumption is presently under study at our laboratory.

Rabies antigen can be detected by IF in suckling mouse brains several days before the animals show any rabies symptom (2, 13). Thus, the use of this method can accelerate the diagnosis of positive saliva samples in mice. Since this procedure would not detect samples lacking enough rabies virus to kill the mice, the difference of sensitivity in favor of the tissue culture systems presented here would still be valid.

The higher sensitivity of the tissue culture systems as compared to suckling mouse inocula-

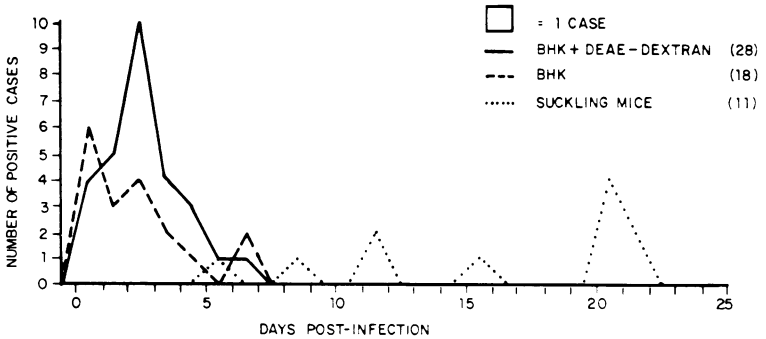

FIG. 1. *Sensivity and time required for the detection of rabies virus in different systems.*

tion could be explained by the fact that approximately ten tissue culture infective doses are needed to obtain one mouse lethal dose (3). Furthermore, Mitchell et al. (12) using a tissue culture amplification procedure could detect residual rabies virus in six lots of inactivated vaccine which had been negative in the regular safety test in mice.

Kaplan et al. (7) enhanced the infectivity of fixed rabies virus for a number of cell lines using DEAE-dextran; in the present study this enhancement was demonstrated for street rabies virus in BHK cells.

Vaughn et al. (15) found virus in saliva by mouse inoculation from 46% of experimentally infected dogs (25 out of 54) in samples obtained on the day of death of these animals. Using the BHK-DEAE system, we detected virus in 58% of the saliva samples taken from heads of naturally infected animals (28 out of 48). Twenty-two percent of the saliva samples from rabid animals (11 out of 48) were positive by suckling mouse inoculation. This difference in sensitivity of the mouse inoculation test could be accounted for by the dilution made in our study when the saliva samples were collected.

The inoculation of BHK cells with saliva samples supplemented with DEAE-dextran represents an inexpensive and rapid method for rabies virus detection which could be employed for ecological and pathogenesis studies; it could also be used to speed up rabies diagnosis of animals kept under observation. It should be borne in mind that, as occurs with other methods, a negative result would not rule out the disease (8).

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