Tissue Explants Used for Enhancement of Herpesvirus Isolations

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Herpesvirus recovery from normal and experimentally infected simian tissues prepared into 10 to 20% homogenates was compared with that obtained by explanting these same tissues. The explant procedure was by far the more sensitive of the two methods. As a result, 8 herpesvirus isolations were obtained by conventional virus isolation procedures in contrast to 74 isolates when explants were made from these same tissue sources. There is also evidence to suggest that the explant method is more sensitive than the use of tissue homogenates for recovery of other viruses as well.

Evidence of viral infections is most frequently obtained by serological or isolation procedures (2, 6, 11). When the specificity of the test antigen is known, serological procedures are generally successful in identifying the infecting viral agent. Current techniques, however, frequently fail to produce a virus isolate from clinical specimens of an infected individual. Newer methodology has enhanced the detection of certain viruses (5, 7, 12), but the frequency of virus recovery is still inadequate and these techniques are not readily adaptable to a wide variety of viruses. It is, therefore, desirable that other methods be applied to enhance the frequency of virus isolation from clinical specimens.

It has been recognized for a long time that uninoculated cell cultures may show spontaneously the cytopathological effects (CPE) of a latent virus infection. Rowe et al. (15) found this when they observed adenovirus CPE in human adenoid explant cultures. It has also been well established that virus isolation may be accomplished only by the use of viable cells, for example, in the recovery of varicella-zoster virus (16). Similarly, Benyesh-Melnick et al. (1) were able to demonstrate that isolates were more readily recovered from cultures prepared from the organs than from cell cultures inoculated with organ suspensions. Measles virus has been successfully isolated from patients with subacute sclerosing panencephalitis only by the cultivation of brain cells and HeLa cells (4) or with BS-C-1 cells (13).

The spontaneous occurrences of simian foamyvirus CPE in kidney cell cultures of a number of monkey species is frequently observed. Other viruses which have appeared in uninoculated monkey kidney cell cultures include picorna-, adeno-, herpes-, myxovo-, papova-, and reoviruses (10). Rogers et al. (14) isolated a number of viruses from a wide variety of chimpanzee organ explant cultures, some of which were maintained in excess of 1 year. These observations suggested to us that a tissue explant method would be more effective in the recovery of viral isolates from routine specimens from clinical and experimentally infected animals than the procedures now employed in most diagnostic virus laboratories.

This report presents comparative data on a modification of the explant technique described by Rogers et al. (14) and routine methods currently used in this laboratory for the isolation of herpesviruses. Uninfected, "normal" and herpesvirus-infected nonhuman primates were employed as sources of organ specimens. A preliminary report of this study was presented at the 12th International Congress of Biological Standardization, Annecy, France, 20–24 September 1971.

MATERIALS AND METHODS

**Explant procedure.** The procedure employed for preparation of tissue explants consisted of mincing with scissors the desired tissue obtained at necropsy, surgery, or biopsy. Each tissue was collected in Hanks balanced salt solution containing antibiotics (BSS), minced as small as possible, and washed three times with BSS. Screw-cap bottles (1 oz, ca. 30
ml) containing cover slips were liberally seeded with the minced tissue and allowed to stand for approximately 0.5 hr at room temperature. After this period, which provided time for the tissue to attach to the glass, 2 ml of growth medium was gently added. Growth medium consisted of Eagle’s minimal essential medium (MEM) in Earle’s saline with 0.12% NaHCO₃, supplemented with 10% fetal calf serum and the following antibiotics: 2 units of bacitracin/ml, 200 units of penicillin/ml, 200 µg of streptomycin/ml, 200 µg of neomycin/ml, and 50 units of nystatin/ml. The bottles, after careful sealing, were incubated at 37°C for 3 to 4 days. At that time, the bottles were very gently removed from the incubator, and another 2 ml of MEM was added. After 1 week, the medium was carefully decanted and frozen or tested, and fresh medium was added to the bottles. With weekly medium changes, many of these explants have been maintained for 40 or more weeks without passage. Testing of the decanted media consisted of inoculating primary baboon kidney or Vero cell cultures, which in our hands have been equally sensitive, and observing for CPE (2, 6, 11).

Routine procedure. The procedures used are standard for this laboratory and have been described in detail elsewhere (6). Essentially, tissue pieces obtained from the same specimen (and at the same time) as used for explants were prepared as 10 to 20% suspensions in BSS, homogenized in a Sorvall Omnimixer, and centrifuged to remove cell debris. Supernatant fluids were inoculated into the same cell systems as employed for testing the fluids removed from the explants.

Virus identification. Isolates were identified by serum neutralization tests adapted to microtiter techniques (3); the sera used were prepared in this laboratory as part of its function as the National Institutes of Health/World Health Organization Simian Virus Reference Center (9). Experimental baboons (Papio cynocephalus) and African green monkeys (Cercopithecus aethiops) were inoculated with a herpesvirus isolated (unpublished data) from baboon lung tissue. Capuchins (Cebus apella) were inoculated with Herpesvirus hominis type 2 (8).

RESULTS

A comparison of virus recovery from the various animals by the explant method and the routine procedure is given in Table 1. Both the baboon and African green monkey series were part of a pathogenesis study involving a new simian herpesvirus (0430). All animals were inoculated with 0.5 ml (10⁴–5 TCID₅₀) of virus suspended in Vero cells by the following routes: African green monkeys, either intravenous or intravenous plus one drop scarified onto the cornea of one eye; baboons, intratracheally. The African green monkeys were sacrificed on days 2, 5, and 14 postinoculation, whereas the baboons were sacrificed on days 2, 5, 8, and 14 postinoculation. The listed tissues were obtained from these animals at death and split into two portions, one for explants and the other for homogenization. Details concerning experimental inoculation of the capuchins have been described elsewhere (8).

Rectal and throat swabs routinely obtained from these animals were processed by procedures standard for this laboratory (6). Two of the African green monkeys were shedding adenovirus (type unidentified) in their stools on the 14th day, and one baboon was found to have herpesvirus (0430) in its throat when sacrificed on the 5th day.

Generally, herpesvirus was detected in the supernatant fluids of the explants within 1

| Animal                                      | No. tested | Tissue* positive | Homogenates/ explants* |
|---------------------------------------------|------------|------------------|------------------------|
| Normal baboons                              | 6          | Intestine        | 0/1                    |
| Experimental baboons (herpesvirus 0430)     | 26         | Trachea          | 1/12                   |
| Experimental African green monkeys (herpesvirus 0430) | 6          | Spleen           | 0/3                    |
| Experimental capuchins (H. hominis, type 2) | 8          | Vagina           | 0/4                    |
|                                            |            | Uterus           | 0/1                    |
|                                            |            | Bladder          | 0/4                    |
|                                            |            | Ovary            | 0/2                    |
|                                            |            | Adrenal          | 0/2                    |
|                                            |            | Lung             | 1/1                    |
|                                            |            | Kidney           | 0/2                    |
|                                            |            | Spleen           | 0/3                    |

* Organs tested included the tonsil, submaxillary gland, brain, thyroid, cervical and mesenteric lymph nodes, thymus, liver, and testes in addition to those listed.

* Number of positive tissue homogenates/number of positive tissue explants.

* Adenovirus.

* Enterovirus.
week after excision. Infection of these explants could be detected by testing of fluids in a number of instances, however, for several weeks after planting. Many of the cultures were maintained for periods up to 40 weeks. Inasmuch as virus was usually detected by assaying the fluid bathing the explants, and since these same explants frequently yielded virus for periods up to 5 weeks, it was assumed that the virus was proliferating within these tissue explants. Residual inoculum was similarly not considered to be a factor because of the dilution factor involved in the preparation and maintenance of these explants.

Although a great many tissues tested were negative for virus isolation, it is obvious many more isolates were made from explant cultures than by conventional methods. The lung was the only organ yielding a significant number of isolates from homogenized tissues, and yet three times as many isolates were made from explant cultures. Cover slips allow for the removal of tissue explants and examination by other methods, including staining for viral inclusions, electron microscopy, and fluorescent-antibody staining. In most instances in which herpesviruses were isolated, the explants grew poorly, if at all. Therefore, viral CPE was not observed. In several instances, herpesvirus isolates were made from explant cultures which grew into monolayers without obvious CPE and survived for many weeks. In these cases, no subsequent viral isolates were made. The adenovirus and enterovirus isolates were recognizable by the CPE they produced in the explant cultures. Virus recovery from the tissues of normal animals is difficult to evaluate because of the essentially negative results; however, explants did yield a virus where the same tissue was negative when examined by routine procedures.

**DISCUSSION**

The technique for using explant cultures is not new and, in fact, is being used extensively in an attempt to isolate oncogenic viruses from human and animal tumors. However, we believe the potential of this technique has not been exploited sufficiently in the routine isolation of herpesviruses and probably other viruses from pathological tissues obtained at necropsy or biopsy. The success of this technique may be due to a variety of factors, including removal of the infected tissue from host defense mechanisms or inhibitors, stimulation of virus replication by cellular growth in the explant, spread of agents by cell to cell transfer, and comparatively gentle treatment of the tissue prior to testing for the presence of the virus. The possibility for replication of herpesvirus in a suitable cell is also greatest in the infected organ, allowing for the production of significant amounts of virus. This is in contrast to the usual practice of inoculating tissue extracts into a variety of cell systems frequently not even related to the test situation. Admittedly, because of the experimental nature of the study, mostly herpesviruses were isolated in this study, but the experience of others, recovery of adenoviruses and enteroviruses, and unpublished results with poxvirus-infected animals in our laboratory emphasize the utility of this technique for isolation of a variety of virus types. The ease with which cultures were established from small bits of tissue in an uncomplicated system suggests that this technique can be readily adapted to material obtained by needle biopsy. A useful by-product of these studies has been the availability of a number of cultures for the propagation and study of viruses.

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