Sucrose phosphate glutamate (SPG) was evaluated as a transport medium for specimens submitted to the virology laboratory. The recovery rate in SPG and minimum essential medium (MEM) was compared at different temperatures and incubation times using herpes viruses. The results showed a successful recovery of viruses from clinical specimens and that SPG is equal to or better than MEM for maintaining herpes virus stability during transport. (Key words: Herpes simplex virus; Viral transport) Am J Clin Pathol 1984; 81: 762–764

SUCROSE PHOSPHATE GLUTAMATE (SPG), the recommended transport medium for Chlamydia trachomatis, was tested with the herpes virus group and with clinical viral specimens to determine if it might be practical and effective as a transport medium for both viral and chlamydial cultures. The herpes viruses have been isolated previously from clinical specimens using a variety of transport media.1-5,7,10

In the current study, SPG was pretested with stock herpes virus suspensions and compared with Eagle’s minimum essential medium (MEM) to which 2% fetal bovine serum (FBS) was added. The SPG medium also was tested with patient herpes virus isolates and used in transporting clinical specimens from the 43 hospitals and clinics of Southern California Kaiser-Permanente to the regional virology laboratory, an average distance of 73 miles (range 8–131 miles).

Materials and Methods

The SPG medium was prepared according to Schachter’s formulation with the addition of 10 μg/mL of gentamicin sulfate (Sigma, St. Louis, MO). Eagle’s MEM with HEPES buffer was prepared from powder (Flow, McLean, VA) with the addition of 15 μg/mL gentamicin sulfate and 2% FBS (Irvine Scientific, Irvine, CA). The antibiotic mixture used to treat clinical specimens consisted of MEM with 10% FBS and 1,000 μg/mL vancomycin hydrochloride (Lilly, Indianapolis, IN), 100 μg/mL gentamicin sulfate, and 200 μg/mL amphotericin-B (Squibb, Princeton, NJ).

Cell cultures of human foreskin, human embryonic lung fibroblasts, and VERO cells (Flow and Bartels Immunodiagnostics, Bellevue, WA) were received weekly in tubes and vials. These cultures were maintained by changing the medium, Eagle’s basal medium (BME) with 2% FBS, every five to seven days.

The following stock viruses were obtained from the American Type Culture Collection (Rockville, MD): herpes simplex virus type 1 (HSV-1), MacIntyre strain; herpes simplex virus type 2 (HSV-2), MS strain; cytomegalovirus (CMV), Davis strain; and varicella zoster virus (VZV), Ellen strain. These stock cultures were inoculated into human fibroblasts, incubated at 36°C, and then harvested when they reached 2 to 3+ cytopathic effect (CPE). After being scraped from the glass, the infected cells were diluted to 100 TCID50 with MEM supplemented with 10% glycerol and 10% FBS. The diluted suspensions were then frozen in aliquots at −70°C.

Schachter’s chlamydial culture method was used for the isolation of Chlamydia trachomatis in McCoy cells treated with cycloheximide (Bartels).

The clinical specimens received from the hospitals and clinics were obtained primarily from obstetric or immunodeficient patients. All specimens were submitted in 2 mL of SPG on wet ice. Cotton and plastic swabs were used to collect the specimens, with the exception of endocervical and nasopharyngeal specimens, which were collected with calcium alginate swabs. The transport time ranged from three hours to four days; the median interval between sampling and inoculation was 24 hours. After receipt at the regional laboratory, the specimens were

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Address reprint requests to: Dr. A. L. Warford, Virology Department, Kaiser-Permanente Laboratory, 10407 Magnolia Blvd., N. Hollywood, California 91601.
treated with antibiotic mixture and refrigerated at 1 to 4°C until inoculation. The daily inoculation procedure consisted of adding 0.3 mL treated specimen to cell cultures containing 2 mL of BME with 2% FBS. The BME medium was changed first after 12 to 18 hours and then every five to seven days thereafter for a 28-day period. Only inoculated tubes and vials showing CPE, toxicity, or contamination were subpassaged.

Isolates were confirmed as follows: HSV with immunoperoxidase-conjugated antisera (DAKO, Santa Barbara, CA), CMV and VZV by typical CPE in methylene blue stained coverslip cultures, and adenovirus with fluorescent antiserum conjugate (M. A. Bioproducts, Walkersville, MD). The picornavirus isolates were referred to the four Southern California county public health laboratories and the UCLA Clinical Virology Laboratory for typing.

Five lots of SPG were prepared and tested during the year as follows: stock viral suspensions and new clinical isolates were diluted into two tubes of SPG for incubation at either 4° or 23°C and two tubes of MEM for incubation at either 4° or 23°C. These suspensions were incubated at their respective temperatures for three days. Tenfold serial dilutions of the virus suspensions in the two transport media were prepared within 30 minutes, at day 1 and at day 3. These titrations were inoculated in duplicate into human fibroblasts and incubated for eight days for HSV or 28 days for CMV and VZV.

**Results**

The purpose of the current study was to determine if SPG could be used for transport of viral as well as chla-
Table 1. Isolates from Clinical Specimens Transported in SPG on Wet Ice*

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. Isolates</th>
<th>No. Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex virus</td>
<td>1,649</td>
<td>8,700</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>253</td>
<td></td>
</tr>
<tr>
<td>Varicella zoster virus</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Echovirus (Types 6, 11, 13, 15, 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coxsackievirus (Types A9, A16, B2, B3, B4)</td>
<td>124</td>
<td>1,878</td>
</tr>
</tbody>
</table>

* Specimen sources: conjunctiva, rectum, skin, throat, nasopharynx, bronchia) washings, spinal fluid, urine, tissues (brain, lung, liver, intestines), and various urogenital sites.

mydial culture specimens, thereby eliminating the confusion of two different transport media. Figure 1 shows the results of stock virus suspensions that were incubated to simulate different transport conditions. The results with VZV stock suspensions are not shown, since they were not reproducible. Figure 2 shows the results of patient virus isolates incubated in SPG and MEM at 4°C and 23°C for three days. Both figures show only small differences in loss of titer between SPG and MEM storage after 24 hours for both stock viruses and patient isolates. However, after three days the loss of titer in CMV and HSV-2 was less (P value less than 0.1, t-test) in SPG as compared with MEM at 4°C. No significant difference in stability was noted with HSV-1 between SPG or MEM storage after three days. At 23°C the differences between SPG and MEM storage with HSV-2 and CMV also were not significant.

The stock CMV strain was found to be more stable at 23°C than at 4°C, as has been previously reported for this same virus strain. However, the rate of isolation of CMV from urine specimens stored at 4°C for a week was reported to be higher than from urine stored at room temperature. Therefore, wet ice transport was recommended for all viral specimens.

Viruses and chlamydiae isolated from 10,578 clinical specimens received in 1982-1983 are shown in Table 1. No attempt was made to isolate the hemadsorbing viruses or respiratory syncytial virus.

The simultaneous collection and submission of cytology smears and viral specimens was encouraged to assess the adequacy of the viral transport procedure for herpes. Of 1921 Papanicolaou-stained smears collected in parallel with cultures, 37 were positive for herpes by smear only, while 208 were positive by culture only.

Discussion

The stability of the herpes virus group in SPG was found to be equal to or better than MEM for all three viruses tested after three days of incubation at 4°C or 23°C. Therefore, the collection of a cervical specimen in SPG could be used to diagnose infections by Herpes simplex and Chlamydia trachomatis, which would be particularly desirable in obstetric patients. When SPG was used as the transport medium for 10,578 specimens, 1,960 viruses and 124 Chlamydia trachomatis isolates were recovered successfully. A comparison trial to MEM was not performed.

In addition to being an effective transport medium for herpes viruses, SPG is an inexpensive, autoclavable, and highly stable transport medium that is also suitable for the transport of other viruses.

References