the blood and with each organ tested. When various organs from MTV-inoculated BALB/c mice were assayed prior to the appearance of MTV activity in their blood, activity was localized primarily in the spleen.

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Preservation of Infectious Cytomegalovirus* (33355)

RICHARD J. HILDEBRANT, JOHN L. SEVER, AND BLANCHE ANDERSON

Department of Obstetrics and Gynecology, University of Florida College of Medicine; and the Section of Infectious Disease Perinatal Research Branch, National Institutes of Neurological Disease and Blindness, Bethesda, Maryland 20014

Since human cytomegalovirus was first isolated by Smith, Rowe, and Weller in 1956, problems have been encountered in maintaining this infectious agent owing to its lability under variety of experimental conditions (1–3). In the past the strains have either been maintained in serial passage in human embryonic fibroblasts (1, 4) or frozen (with some stabilizing additive) at −70° for relatively short periods of time (5, 6). The purpose of this paper is to report long term stability under rather simple conditions of liquid nitrogen storage.

Materials and Methods. Six strains of cytomegalovirus (obtained through the courtesy of Doctor Robert M. McAlister) were utilized in this study. These strains were initially maintained in serial passage in roller tubes of WI-38 tissue and subsequent stocks of seed virus were made again employing WI-38 tissue. The medium consisted of basal medium Eagle with Earle's balanced salts and 2% calf serum.

The method of infection for seed virus was as follows: cells and supernate from roller tubes in which serial passage had been accomplished were inoculated into 32-oz bottles of WI-38 tissue containing 40 ml of the above media; after 48 hr the media was changed; at 5 days or longer, depending on the strain and the development of full cytopathic effects, the cells were scraped from the bottle and suspended in the media. Initially the virus cell supernate combination was simply placed in "Cryules"[1] in 1-ml aliquots

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[1] Wheaton Glass Company.

TABLE I. Preservation of Stock Virus in Liquid Nitrogen.

Strain	Passage level	Date frozen	Titer (log_{10}/ml)	Date thawed	Titer (log_{10}/ml)	Interval (months)
Ribardiere	104	2–16–65	5.9	3–15–68	—[a]	37
Heermange	27	2–23–65	5.9	3–15–68	5.6	37
Kerr	42	6–11–65	5.0	3–15–68	5.0	33
AD 169	277	9–23–65	5.8	3–15–68	6.1	30
Hammond	35	3–31–65	6.0	3–15–68	5.7	36
Davis	42	3–31–65	6.1	3–15–68	5.8	36

[a] Virus present but of very low titer.

and quick-frozen in liquid nitrogen. Subsequently, the cell supernate suspension was sonicated using a Heat System's sonicator for two 20-sec intervals at approximately 5 A of power, the cell supernate suspension being placed in a plastic tube in an ice alcohol bath for cooling. The specimens were titered at the time of initial freezing using a method described by Goodheart *et al.* (4). At varying intervals, thereafter, ampuls were removed from the liquid nitrogen for subsequent titrations. During this interval the samples were shipped from Bethesda, Maryland to Gainesville, Florida in a BT 2 liquid nitrogen container.[2]

Urine specimens were obtained from infants and children either by strapping with a sterile plastic bag or having patients void into a sterile container. Specimens were immediately refrigerated and then transferred to the laboratory within 8 hr. Upon receipt in the laboratory, well-mixed urine specimens were inoculated in 0.1 ml aliquots into each of 4 roller tubes of WI-38 tissue culture. One-ml aliquots (not sonicated) were placed into several sterile "Cryules" and quick-frozen in liquid nitrogen. At varying intervals after freezing, the "Cryules" were thawed in a 37° water bath and inoculated into roller tubes as above. The roller tubes were observed twice weekly for cytopathic effect and the media was changed twice weekly. When cytopathic effect was observed, cells and supernate were passed into fresh tissue for identification and maintenance of serial propagation. The isolates were identified by their characteristic cytopathic effect, presence of typical inclusion bodies by May-Günwald Giemsa staining and neutralization with known positive cytomegalovirus serum antibody.

Results and Discussion. Table I shows the virus titrations and the intervals from initial freezing to final titration. It was found that without sonication difficulty was encountered obtaining reproducible titers. Following sonication the distribution of infected cells was more uniform and titrations were more reproducible. The marked reduction in titer of the Ribardiere strain is unexplained.

In their paper regarding detection of viral isolation Rapp *et al.* alluded to storage of cytomegalovirus in liquid nitrogen, but supplied no data as to its stability under these storage conditions (7). Freezing in liquid ni-

TABLE II. Preservation in Liquid Nitrogen of Specimens for Isolation.

Urine sample	Date of initial inoculation	Latent phase[a] (days)	Date of subsequent inoculation	Latent phase (days)	Interval (months)
Mathews	8–21–67	10	1–11–68	11	4.5
Austin	9– 6–67	13	1–11–68	11	4
Dukes	11– 9–67	4	1–11–68	8	2

[a] Interval between inoculation and observation of cytopathic effect.

[2] Linde Division, Union Carbide Corporation.

trogen without any stabilizer, coupled with sonication, seems to produce relatively stable storage conditions so that one can anticipate the quantity of virus employed for prospective testing. Previously, one had to interpolate a decline in infectivity secondary to inactivation of —70° in the presence of stabilizers such as sorbitol.

Cytomegalovirus was isolated from three fresh urine specimens (Table II). All three specimens yielded virus from aliquots frozen for periods up to 4.5 months. The interval between inoculation and observance of cytopathic effect in both the fresh and frozen samples was similar, indicating that quantities of virus in the urines remained relatively constant over the interval tested.

Summary. The relative stability of cytomegalovirus stored in liquid nitrogen for a period of 3 years is reported.

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Studies on Experimental Bacteremia and Pyelonephritis in the Marmoset (*Callithrix jacchus*)* (33356)

HEONIR ROCHA, EDSON DA SILVA TELES, AND EDILSON BRITO
(Introduced by D. Kaye)

Department of Medicine and Department of Pathology, Faculty of Medicine, University of Bahia, Salvador, Bahia, Brazil

There have been few reports concerned with the use of marmosets in experimental infections (1–4). These animals have been studied in relation to maintenance and care in the laboratory (5, 6), for use in the isolation of viruses (7), and for naturally occurring parasitic infections (8). Aspects of normal pathology (9), as well as base line microbiologic studies (1) also have been documented.

The present study was undertaken to determine the tissue distribution and fate of *Escherichia coli* after infection by the intravenous route or inoculation into the urinary bladder, and to evaluate the susceptibility of these animals to urinary tract infection.

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Materials and Methods. Animals. Normal marmosets (*Callithrix jacchus*) of both sexes were used in this study. Animals were housed in groups of two or three and fed water and a diet consisting of bread and fresh fruits (banana, papaya, pineapple) *ad libitum*. A total of 50 animals were used. Forty were inoculated with bacteria, 20 in the femoral vein and 20 into the lumen of the urinary bladder. Ten served as normal controls.

Bacteria. A strain of *E. coli* and a strain of *Proteus mirabilis* recovered from urine specimens of patients with urinary tract infection were employed. Stock cultures were maintained by storing aliquots of an 18-hr culture in trypticase soy broth (Biological Baltimore Laboratories) at —20°.

Inocula were prepared by subculturing an aliquot of the stock culture in trypticase soy broth at 37° for 4 hr. One-half ml of a 1:10 dilution of the culture in saline solution was