Isolation of Cytomegalovirus from a Cohort of 100 Infants Throughout the First Year of Life[1] (34345)

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Neonatal infection with cytomegalovirus (CMV) was originally recognized in its highly fatal form as cytomegalic inclusion disease. Necropsy findings from early studies indicated that CMV infection of early childhood was far more prevalent than the cases of severe disease attributed to this cause (1). It has become apparent with time that infection with this virus may result in less severe symptomatology (2–4). Emanuel and Kenny (4) found some neonatal infections to be followed by milder and more varied sequelae than previously reported and suggested that a thorough description of the disease spectrum would depend on prospective rather than retrospective study of newborns. This is a report of the results of a pilot prospective study designed to determine the incidence of CMV infection during the first year of life in 100 children living at home.

Materials and Methods. The study cohort consisted of 100 infants born between June 15 and August 15, 1966, in two Seattle hospitals: University Hospital and the Hospital of the Group Health Cooperative of Puget Sound. The criteria for selection of infants were : (i) they were considered healthy, full-term infants at birth; (ii) they lived within 0.5 hr driving distance from the University of Washington; (iii) their mothers were willing to have the infants examined and cultured. Ten refusals were encountered during recruiting for this study.

Specimen collection. Oropharyngeal and urine specimens were cultured for CMV. The mouth–throat specimens were obtained by rubbing a sterile cotton applicator bilaterally along the buccal mucosa while pressing the external surface of the cheek, and then over the tonsillar area. Urine was collected in a plastic urine collector (Cutter Resiflex Wee Bag).

The collection medium for oropharyngeal specimens consisted of trypticase soy broth (Baltimore Biological Laboratories) containing 0.5% bovine albumin and 200 units/ml of penicillin. Specimens were transported in a cold chest to the laboratory, where they were inoculated into cell culture within 4–6 hr.

Specimens were obtained at five time intervals during the first year of life: from all 100 babies in the hospital on the first or second day of life and again at 4 weeks of age, from 95 at 3 months of age, 82 at 6 months (actual 5–8 months) and 60 at 1 year (actual 11–15 months). Both throat and urine specimens were obtained at the first four time periods. Only urine was routinely collected at the 1 year visit, at which time a blood specimen was also obtained from as many infants as possible. A subsequent follow-up was completed on a portion of the original 100 infants. During the summer of 1968 through February, 1969, at the age of from 2 to 2.5 years, 30 of the infants (including 7 previously shown to shed CMV) were visited and specimens were obtained.

Throat, urine, and blood specimens were

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also collected when possible from members of the families of infants positive for CMV, at the remaining visits to that infant after he was proven to be an excretor. For controls, specimens were obtained from families of nonexcreting infants matched to resemble by size and age distribution the excretors' families.

Laboratory Procedures. The methods used in this laboratory for CMV isolation and identification have been described (4). Human embryonic fibroblasts (U W strain H200) in passages 14–25 were used for isolation. Cultures which developed no foci of cytopathogenic effect in 8 weeks were termed negative and discarded.

Sera were tested for CMV antibody by a microtiter complement-fixation (CF) technique (5). The U W-167 strain, isolated in our laboratory from a case of fatal neonatal inclusion disease was used for preparation of antigen. Two units of antigen (as determined from a "block" titration), 2 units of complement and overnight fixation in the cold were employed. Only strong reactions of 3+ and 4+ were considered positive. All sera were screened on the same day at a dilution of 1:8, and those giving a positive reaction were titrated.

A tube dilution technique was used for the detection of serum neutralization (5). Sera were screened at a dilution of 1:8 against approximately 100 infectious doses of U W-167 virus.

Results. Table I shows age and socioeconomic characteristics of the mothers of the 100 infants studied. The University Hospital serves a heterogeneous group composed of staff and student wives, persons of low income, and unwed mothers. The Group Health Hospital, on the other hand, serves a more homogeneous middle-class clientele.

Fifty-one infants born at University Hospital and 49 born at Group Health Hospital were studied. Two sets of twins were included, both born at University Hospital.

Table II presents the CMV isolation results at five culture periods. The three isolations obtained at the first two periods were from the urine; oropharyngeal specimens were negative. Thereafter, at 3, 6, and 12 months, the prevalence rate of isolation was 10–13% of both the oropharyngeal and urine culture.

TABLE II. Prevalence Rate of Positive CMV Cultures by Time after Birth.[a]

Age	Source	Neg	Pos	% Pos
Birth	Urine	92	1	1
	Throat	98	0	0
4 weeks	Urine	77	2	3
	Throat	78	0	0
3 months	Urine	78	10	11
	Throat	75	8	10
6 months	Urine	58	9	13
	Throat	59	7	11
12 months	Urine	42	5	11

[a] Difference between number of cultures negative or positive and total children examined at time interval was due to contaminated specimens.

Cytomegalovirus was isolated at least one time from 15 children. Complete cultural results on each of these children are presented in Table III. Most of the CMV excretors in

TABLE I. Distribution of 98 Mothers According to Age, Ethnic and Social Characteristics.

Socioeconomic characteristics		University Hospital	Group Health Hospital	Total
Age range (years)		15–41	17–38	15–41
Mean age (years)		23	26	24
Race	Caucasian	43	44	87
	Negro	5	2	7
	Other	1	3	4
Husband's occupation	Unemployed (or unmarried)	11	2	13
	Nonprofessional	27	32	59
	Professional or managerial	11	15	26

TABLE III. Cultural Results and Complement Fixing Antibody Titer for the 15 Infants from Whom CMV was Isolated at Least One Time during the First Year of Life.

Infant no.	Sex	Birth wt (g)	Specimen	Age					
				1–2 days	1 mo	3 mo	6 mo	12 mo	24–30 mo
GU 510	M	2948	Throat	—	—	+	+		+
			Urine	—	+	+	+	+	+
			CF titer					1:32	1:16
GU 531	F	3147	Throat	—	—	+	+		c
			Urine	c	+	+	+	+	+
			CF titer						1:16
GU 535	F	2410	Throat	—	—	c	+		
			Urine	—	—	+	+		
GU 539	M	3487	Throat	—	—	+	+		
			Urine	—	—	+	+		
GU 541	F	2807	Throat	—	—	+	c		+
			Urine	—	c	+	c	+	+
			CF titer					1:64	1:32
GU 553	F	3544	Throat	—	—	+			
			Urine	—	—	c			
GU 554	F	2977	Throat	—	—	+	+		
			Urine	—	—	+	+		
			CF titer					<1:8	
GU 558	M	3204	Throat	—	c	—	c		
			Urine	+	—	—	—	—	
			CF titer					<1:8	
GU 569[t]	M	2863	Throat	—	—	—	+		—
			Urine	—	—	+	+		+
			CF titer					1:16	1:32
GU 570[t]	F	3062	Throat	—	—	—	—		c
			Urine	c	—	c	+		—
			CF titer					1:64	1:32
GU 573[t]	M	2400	Throat	—	—	+	+		
			Urine	—	—	+	+	+	
			CF titer					1:16	
GU 574[t]	M	2810	Throat	—	—	+	+		
			Urine	—	—	+	+	+	
			CF titer					1:16	
GU 577	F	3175	Throat	—	c	c	—		—
			Urine	—	c	—	+	—	—
			CF titer					<1:8	<1:8
GU 596	M	3629	Throat	—	—	—	+		
			Urine	—	—	—	+	—	
GU 601	M	2920	Throat	—	—	+	+		c
			Urine	—	—	+	+		+
			CF titer					1:32	1:16

[a] Abbrev.: t = twin; + = CMV isolated; — = negative for CMV; c = contaminated culture; blank = no specimen, not tested.

this study were detected at the 3-month examination when 9 infants were first found to be excreting virus. One was first found at the first culture, 2 at 1 month, and 3 at 6 months. The first positive culture on 7 of 13 infants was from the urine only. In the other first positives and in all but one subsequent positive (with successful examination of both specimens) both urine and oropharyngeal swabs were positive.

Four of the CMV excretors were members of the two sets of twins. Three first became positive at the 3-month culture and the fourth at the 6-month culture.

At the 2–2.5-year examination of 30 of the children no new CMV shedders were found. Seven of this group had been shown to be CMV shedders sometime during the first year of life. From the 23 originally negative children, 22 oropharyngeal specimens and 20 urine specimens were satisfactorily cultured and found negative for CMV. From the seven originally positive children CMV was again isolated from five. The urine of all five was positive. Two of the oropharyngeal cultures were positive, two contaminated and one negative.

Table III also shows the CMV CF antibody titers at 1 and 2.5-years of age for the 11 children on whom at least one serum was available. Eight of the 11 viral excretors had antibody titers ranging from 1:16 to 1:64. The second titers of the 6 children with two bloods were within one 2-fold dilution of that found at 1 year. Virus was isolated only once from 2 of the 3 infants without CF antibody titer. Sera was available from two of three mothers of these infants; neither had measurable CF antibody. These five sera (3 infants and two mothers without CF antibody) were tested for neutralization antibody. Sera from infant GU 554 from whom virus was isolated repeatedly, and from his mother, neutralized CMV at a 1:8 dilution. The other three sera were negative. None of the 39 children on whom sera were available from whom CMV was not isolated had CF antibody.

A comparison of infants who excreted CMV with those who did not showed no significant difference between excretors and nonexcretors for sex, race, breast feeding, occupational class of father, and maternal age or parity. All excretors were Caucasian. Three fathers of five excretors, including both sets of twins, were physicians. Only one of the three had CMV CF antibody (1:8).

The mean birth weight of excretors (3049 g) was lower than that of nonexcretors (3421 g). This difference was significant at $p = 0.035$ (Student's t distribution). The twins were excluded from these calculations since they would be expected to have lower birth weights. The mean birth weight of excretors including the twins was 2960 g.

At the last examination the general development of all infants appeared to be within normal limits. Although respiratory symptoms and jaundice were observed in several CMV excretors, there were no findings that occurred significantly more frequently in excretors than nonexcretors. Long-term follow-up of shedders for clinical sequelae is being carried out by E. R. Alexander and I. Emanuel. The findings to date are negative.

Family studies. The 13 families of the 15 excretors were visited 1–3 times during the first year to obtain specimens. Two families were lost at 6 months to further follow-up. Specimens were obtained from 15 families of nonexcretors. In all, 114 isolation attempts were completed on 42 persons from the families of the CMV excretors and 108 cultures on 44 members of the families of nonexcretors with similar age distribution. There were about 2 adults to each child studied. A total of 5 CMV isolations were obtained from three persons, the mother and 4-year-old brother of one excretor (GU 535) and the 3-year-old brother of another (GU 510). CMV was not isolated from any member of the control families.

The prevalence of CF antibody in the family members on whom sera were available is shown in Table IV. While no difference was observed between the fathers, more mothers of infants who shed CMV had antibody and at higher titer than mothers of nonshedding infants.

Discussion. Since there was a progressive loss to follow-up culture in the original cohort of 100 babies, it is not possible to give a

TABLE IV. Complement Fixing Antibody Titers for CMV in Family Members of Study Infants.

Family with	Family members	Antibody titer[a]					
		<8	8	16	32	64	Total
Infant excreting CMV	Mothers	2	2	2	2	3	11
	Fathers	7	1				8
	Others[b]			2	1		3
Infant not excreting CMV	Mothers	8	2	3			13
	Fathers	4	1				5
	Siblings	2					2

[a] Expressed as the reciprocal of the highest serum dilution giving complement fixation.
[b] Sibling, grandmother, aunt.

precise incidence rate of CMV infection during the first year of life. However, most first positive cultures had been obtained by 3 months and all by 6 months, and 95 and 82 of the children were cultured at those time periods. Despite additional loss from contaminated cultures, it can be estimated that the incidence rate was from 15 to 18%.

Such an incidence rate during the first year of life is no longer as surprising as it would have been several years ago, since prevalence studies have shown that from 3 to 27% of children are excreting CMV (6–11). The highest prevalence rates were found in children 1 and 2 years of age and from those of lower socioeconomic status. A review of nine serological studies supported the isolation studies by finding from 7 to 50% of children with CMV CF antibodies (8). These prevalence studies utilized sick or institutionalized children and other special groups and might not have predicted the incidence rate we found in relatively representative free living American children. The only incidence study, other than this report, of which we are aware, was carried out in Chinese on Taiwan and showed very high rates in small numbers of children cultured frequently (12, 13).

Several studies found a very low CMV isolation rate from newborns (6, 9, 10, 14). The prospective design of our study showed that most infants are first shown to excrete CMV at 3 months of age. Alexander's findings are similar (12, 13). This may be interpreted that infection occurs after the first month of life rather than *in utero* or at birth. An attractive alternant hypothesis would be that although the virus was transmitted from the mother to the infant at birth, the infection did not become manifest by detectable shedding until after the 1-month culture. This delay in manifestation could be due to slow multiplication of the virus and insensitivity of the isolation technique. The presence of maternal antibody might slow virus growth. An apparent delay of several weeks to months in manifestation of CMV infection by virus isolation and development of CF antibodies has been reported in infection following extracorporeal circulation (15, 16) and after renal transplantation (17).

Although the family investigations were carried out retrospectively after the infants were demonstrated to shed CMV, there was a striking association between positive infants and the CMV CF antibody titer in their mothers, but not fathers, compared to matched controls. Our sample of siblings was too small to draw any conclusions; however there was no sibling in the family to infect six of the shedding infants. This study would suggest the mother as the source of CMV infection of the infant, but a prospective study with mothers and siblings cultured and tested for antibodies prior to delivery will be necessary to determine more precisely the usual source and time of infection.

Most of the infants once positive for CMV continued to excrete virus. An exception was the infant who excreted virus on the second

day of life and was negative on five subsequent cultures during the first year of life. Hildebrandt (14) reported a similar case.

Cytomegalovirus disease in twins has been described (4, 18). Both sets of twins encountered in this study became infected. The possibility that twins run a higher than average risk for cytomegalovirus infection merits investigation.

Summary. A cohort of 100 apparently normal newborn infants was studied five times during the first year of life to determine the incidence of cytomegalovirus infection. Virus was isolated from oropharyngeal or urine specimens at least once from 15 of the infants. Initial isolations were obtained as follows: one infant on hospital day 2, two at one month, nine at 3 months, three at 6 months, and none later. Most children once positive continued to shed the virus. CMV CF antibodies were demonstrated in 8 of 11 virus excretors and none of 39 nonexcretors. One of the 3 excretors without CF antibody had CMV neutralizing antibody and the other 2 had virus isolated only once. Both sets of twins included in the study became infected. Virus isolation was not related to sex, maternal age or parity, breast feeding, or occupation of father. The mean birth weight of babies who subsequently excreted virus (with or without the twins) was significantly lower than that of the nonexcretors. During the follow-up period, the general health and development of the infected infants did not differ from the noninfected infants. Studies of the families of the CMV excretors and matched control families of nonexcreting infants suggested the mothers as the most likely source of the infants' infections.

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