MEDICAL GRAND ROUNDS

Parkland Memorial Hospital

June 18, 1971

CYTOMEGALOVIRUS

James P. Luby, M.D.

INTRODUCTION

The modern era in the investigation of the cytomegalovirus (CMV, salivary gland virus) infections of man began in 1956-1957 when three groups of researchers independently reported isolation of the virus in tissue culture (94,98,112). Reliable serological tests for detection of infection soon followed. Subsequent to these methodological advances, the manifestations of congenital infection were delineated. Recent developments include the establishment of CMV as the most common etiology of post-transfusion mononucleosis (post-perfusion syndrome), a leading cause of heterophile negative infectious mononucleosis and have focused attention on the ubiquity of CMV infection in the immunosuppressed host, particularly in patients undergoing renal transplantation. This review stresses the following: CMV infections are common and may cause human illness in all age groups. Infection is often chronic and it is probable that the virus may persist in a latent form in tissue for the lifetime of the individual. Reinfection may occur. Mechanisms of recovery are presently poorly defined and involve host defenses other than antibody and interferon. "Auto-antibodies" may occur in the course of infection. Therapy of severe illness, particularly due to congenital infection, using anti-DNA drugs [cytosine arabinoside (CA) and 5-iodo-2-deoxyuridine (IDU)] is presently experimental but appears, at least, to be a promising investigational approach.

ETIOLOGY

In man, the term cytomegalovirus actually encompasses a number of different serotypes which can be distinguished by neutralization tests. At present, at least 3 human serotypes are recognized (113). CMV is species specific; animals such as monkeys and certain rodents (mice, guinea pigs) are infected with their own antigenically distinct cytomegaloviruses. CMV is an enveloped DNA virus whose capsid possesses icosahedral symmetry. It is a member of the herpesvirus group. In man, other members of the herpesvirus group include herpes simplex, varicella zoster and Epstein-Barr viruses.

Although, <u>in vivo</u>, CMV commonly involves cells of epithelial origin, <u>in vitro</u> cultivation can only be accomplished in tissue cultures composed of human fibroblasts. The cytopathic effect (CPE) produced in tissue culture is considered specific. After a latent period which is dependent in part upon the size of the virus inoculum and which may be prolonged, focal areas of CPE are produced. Individual cells round up and giant, distorted cells with enlarged nuclei are formed.

The foci of CPE enlarge slowly and develop yellowish-brown pigment. Generalized involvement of the tissue culture monolayers occurs slowly. This corresponds to the fact that mature infectious virus particles are usually cell associated and pass from cell to cell and only infrequently are released into the tissue culture fluid.

On staining with hematoxylin and eosin, large intranuclear inclusions surrounded by a clear area are observed. These intranuclear inclusions usually stain eosinophilic as does the cytoplasmic inclusion body which can often be found next to and indenting the nucleus. The intranuclear inclusion body on light microscopy can sometimes be seen to be granular.

The events in the cellular maturation of herpes simplex virus and CMV are thought to be similar and can be visualized with the aid of the following diagram (Figure 1) (20).

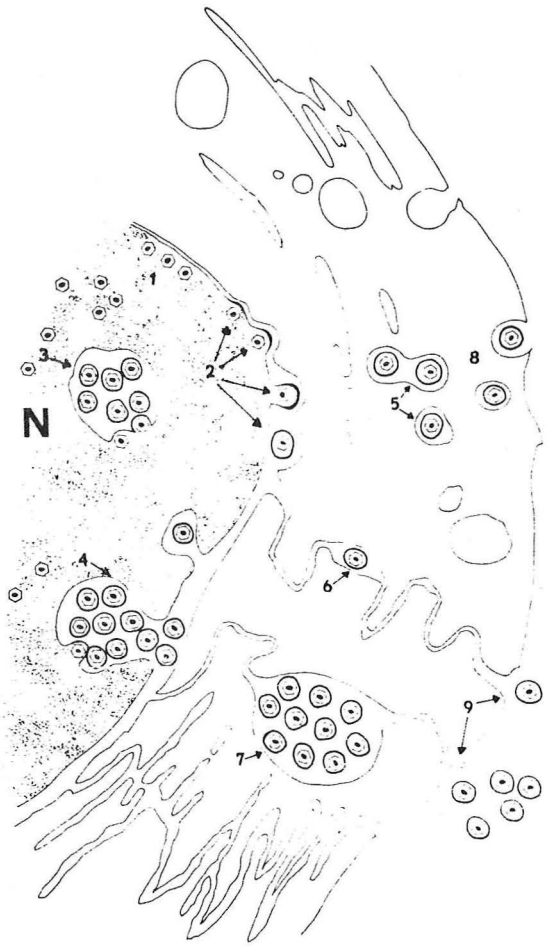


FIGURE 1

Diagram illustrating the process of envelopment and release of herpesvirus from infected cells. The numbers refer to specific steps in the process and are discussed in the text.

1) Virus is assembled in the nucleus and approaches the nuclear membrane. (Diagramatically, the central dark core depicts the DNA portion of the virion. The capsid is shown by the circle surrounding the core.)

2) The inner nuclear membrane becomes thickened and envelops the virus particle. The enveloped virion is free in the perinuclear cisterna.

3) Nucleocapsids may also acquire envelopes by budding into nuclear vacuoles.

4) The apparent nuclear vacuoles may be indentations of the nuclear membrane cut in cross section.

5) The outer lamella of the nuclear membrane wraps around the enveloped nucleocapsid and 8) moves to the cytoplasmic membrane where the vacuole releases the particle outside the cell.

6,7,9) The cisternae of the endoplasmic reticulum may serve as an additional route to the exterior of the cell.

To account for the fact that mature infectious CMV particles are usually cell associated, it has been postulated that lysomal enzymes may alter the virion before exit from the cell can occur (20). Infectivity of CMV virions may be dependent on an intact envelope, although this has not been established for certain. It seems clear that the CMV envelope is derived from host cell nuclear membrane.

CMV infectivity may be destroyed by freezing. This has obvious application to the handling of clinical specimens for virus isolation. To prepare a stock virus suspension for use in tissue culture and plaque reduction neutralization tests, serial passage in human fibroblasts may be necessary. This is followed by sonication of infected cells and inclusion of a preservative such as sorbitol prior to freezing and storage.

Antigen for use in the complement fixation (CF) and indirect hemagglutination (IHA) tests is prepared from infected tissue cultures. The CF antigen consists of a combination of a subviral "soluble" component and the virion (7).

EPIDEMIOLOGY

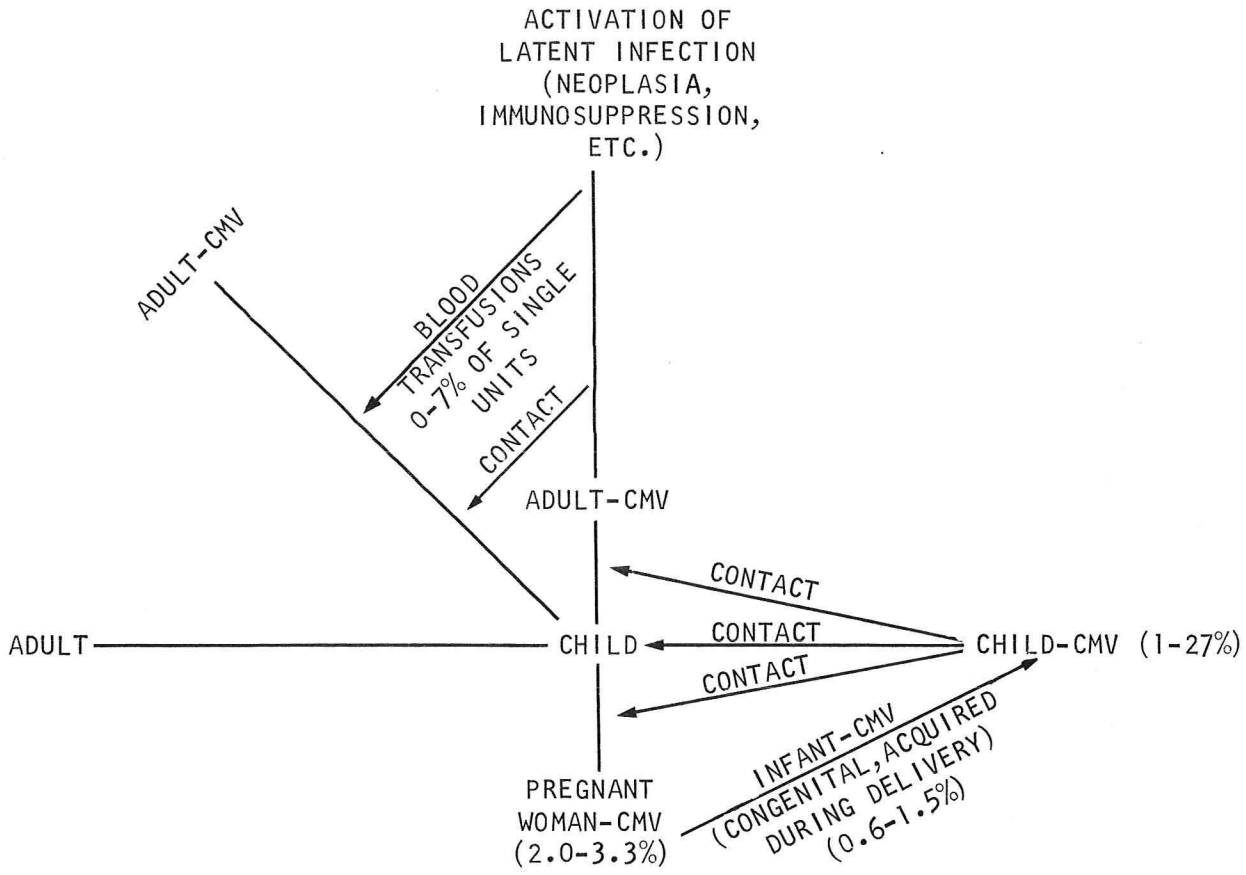
Before discussing the epidemiology of CMV infections, certain definitions are necessary. A chronic viral infection is an infection in which the virus persists in a demonstrable form for an unusually long period following an acute infection. A latent viral infection is one in which the virus is not demonstrable except during sporadic episodes when the virus becomes activated. Examples of latent viral infections in man include other members of the herpesvirus group (herpes simplex, varicella-zoster viruses). CMV infections are often chronic. It is probable that a latent infection also occurs, although this is difficult to document from human case material particularly since reinfection with a different CMV serotype may also occur.

For ease of presentation, an overall scheme of CMV infections is presented (Figure 2).

The most significant pool of circulating CMV is the infected younger child. Surveys document that 1 to 27% of children in the U.S. are actively excreting virus either in saliva or urine (70). The highest prevalence rates are in children 1 and 2 years of age and of lower socioeconomic status. Institutionalized children often show high rates of virus excretion (95). Child-to-child transfer of CMV occurs by contact transmission. Adults may become infected by coming into contact with children excreting CMV. Virus excretion in infected children may be prolonged. There is a documented instance of a child with congenital disease due to CMV who excreted virus in urine for 8 years (47). As a consequence largely of these events, the population gradually and progressively acquires experience with

FIGURE 2

EPIDEMIOLOGY OF CMV INFECTIONS



CMV. Serological surveys, utilizing the CF test, reveal a progressive increase in antibody prevalence with age (Table 1) (104).

TABLE 1

PREVALENCE OF CMV CF ANTIBODIES IN RESIDENTS OF GREATER LONDON

AGE GROUPS (years)	NO. POSITIVE / NO. TESTED	% POSITIVE
0-½	3/9	33
½-5	4/93	4
5-10	15/97	15
10-15	54/257	21
15-25	47/130	36
25-35	62/114	54
35-75	46/85	54

2.0-3.3% of women in the U.S. excrete CMV in urine at some time during pregnancy (46). A higher percentage of women seroconvert during this period. Logically, the source of virus probably stems from either a primary infection (contact with an infected child or less commonly an infected adult) or a reactivation of a latent infection. Since younger, primiparous mothers are at higher risk with respect to delivering an infant with congenital disease, it has been postulated that primary infections during pregnancy are especially hazardous. Recently, Japanese workers have reported that cervical secretions in pregnant women may also be positive for CMV (Table 2) (82).

TABLE 2

RECOVERY OF CMV FROM HEALTHY PREGNANT WOMEN IN JAPAN

SPECIMEN	STAGE OF PREGNANCY	NO. TESTED	NO. POSITIVE	% POSITIVE
Cervical secretions	First trimester	30	0	0
Cervical secretions	Second trimester	62	6	9.6
Cervical secretions	Full term month	61	17	27.8

To account for their findings, these workers postulated that hormonal changes in late pregnancy reactivated a latent infection. This study provides solid evidence that a reactivation phenomenon may occur since it seems improbable that primary infections in pregnancy could account for the observed results. It should be noted, however, that three different groups of women were involved. No attempt should be made to generalize these results to women in other countries until further studies have been performed.

Vertical transmission of CMV occurs by congenital infection (cytomegaloviremia and subsequent placental and fetal involvement) or during delivery or shortly thereafter (contact with infected cervical secretions; milk may rarely be positive for CMV). In newborn nurseries, 5 surveys have established that 0.6-1.5% of all infants are excreting CMV (46). Most of these infants are asymptomatic (100). In 2 large series of newborns where CMV excretion was detected by routine survey, the prevalence of damage to the central nervous system at 12 months follow-up was 3/15 (20%) and 2/26 (7.8%), respectively. Further such studies are clearly needed. A recent study of a cohort of 100 infants born to middle and upper socioeconomic class mothers revealed that 9% of such infants became virus excretors at 3 months of age. It was reasoned that the mothers represented the most likely source of the infants' infections (70).

Cytomegaloviremia may occur in the course of infection and CMV transmission has been shown to occur by blood transfusion. It can be estimated from direct virological examination of single units that between 0/290 (0%) and 2/35 (5.7%) are involved (78). The results of the one study where no units were found to contain CMV can be questioned on methodological grounds.

The incidence of CMV infection, as manifested by virus isolation and/or significant rises in CF antibody between pre- and post-transfusion specimens, after blood transfusion has been measured in several series and can be summarized

(Table 3). It should be noted that 4/5 series relate to patients undergoing open heart surgery with cardiopulmonary bypass.

TABLE 3

INCIDENCE OF CMV INFECTION* AND DISEASE FOLLOWING BLOOD TRANSFUSION

	INCIDENCE OF CMV INFECTION AND DISEASE (% OF PATIENTS)									
	I†	D	I	D	I	D	I	D	I	D
Number of Units Transfused										
1									7	-
3-7									14	-
8-9									29	-
10-14									52	-
≥ 15									23	-
Multiple (unspecified)	44	31	56	56	35	-	25	25	29	-
Total	44	31	56	56	35	-	25	25	20	-
Number of Patients Studied	16		9		152		25		Multiple pts in each category	
Reference	31§		33§		50§		62§		118	

* Virus isolation and/or four-fold increase in CF antibody titer over pre-transfusion levels.

† I = infection; D = disease

§ All patients underwent open heart surgery with cardiopulmonary bypass.

Evidence for the presence of CMV in stored as well as fresh blood has been obtained (Table 4) (118). In blood, CMV is mainly associated with leucocytes. It also has been found in plasma associated with erythrocytes, although the possibility of white blood cell contamination of this layer could not be excluded (2).

Renal transplant recipients often require multiple blood transfusions. They are also placed on potent immunosuppressive drug regimens (azathioprine, prednisone, etc.). In some instances, it seems probable that this immunosuppression may have reactivated a latent infection. CMV infection in these patients is extremely common (Table 5) (17).

TABLE 4

RISK OF CMV SEROCONVERSION IN PATIENTS RECEIVING FRESH* VS STORED† BLOOD

STUDY GROUP	PATIENT WITH ADEQUATE DATA	PATIENTS GIVEN FRESH BLOOD TRANSFUSIONS		PATIENTS GIVEN STORED BLOOD TRANSFUSIONS	
		NO. TESTED	CASES OF SEROCONVERSION	NO. TESTED	CASES OF SEROCONVERSION
Single-unit transfusions	37	3	0 (0%)	34	4 (12%)
Multiple-unit transfusions	38	24	6 (25%)	14	4 (29%)
Transplant recipients	13	5	1 (20%)	8	3 (27%)
Totals	88	32	7 (22%)	56	11 (20%)

* Some blood units $\leq$ 3 days old

† All units > 3 days old

TABLE 5

CMV INFECTION IN RENAL TRANSPLANT RECIPIENTS BY PRE-TRANSPLANT ANTIBODY STATUS

PRE-TRANSPLANT ANTIBODY STATUS	POST-TRANSPLANT CMV INFECTION*			
	PRESENT	ABSENT	TOTAL	
Negative (CF < 1:4)	9	8	17 (53%)	$p > 0.1$
Positive (CF $\geq$ 1:4)	23	13	36 (64%)	
Total	32	21	53 (60%)	

* Four-fold rise in CF antibody titer

Patients with neoplastic disease are also multiply transfused, on steroids and may be hypogammaglobulinemic. A high rate of active CMV infection, as assessed by virus excretion studies, has been reported in such patients (Table 6) (27).

TABLE 6

CMV EXCRETION (URINE AND/OR SPUTUM) IN PATIENTS WITH NEOPLASTIC DISEASE BY STATUS

PATIENT STATUS	VIRUS-POSITIVE PATIENTS (11)		VIRUS-NEGATIVE PATIENTS (21)	
	No.	%	No.	%
Receiving chemotherapy*	7	64	12	57
Receiving steroid therapy*†	7	64	5	24
Hypogammaglobulinemia	4	36	3	14
Terminal disease§	3	27	7	33

* Therapy within 1 month of initial culture

† Statistical significance: p = 0.05 (Fisher Exact Test)

§ Dying within 1 month of initial culture

IMMUNOLOGY

Antibody (IgM, IgG and IgA) against CMV develops in the course of infection. It has repeatedly been demonstrated that prolonged virus excretion, however, can occur in the presence of serum antibody. In part, the explanation for chronicity of infection may be related to the fact that virus transfer occurs from cell to cell and that antibody cannot reach the intracellular space.

CMV is a poor inducer of interferon and is relatively insensitive to its effects. The effective host defense mechanisms in CMV infections most probably involve cell-mediated responses. The mechanisms underlying prolonged virus excretion in congenital infections are not known. A qualitative defect in thymus derived (T) immune cell competence can be postulated but is not proven. Lymphocytes from children with congenital CMV infection have been shown to respond by blastic transformation to phytohemagglutinin. Several of these children also have been successfully vaccinated against smallpox. These considerations point to intact T cell competence but a qualitative defect or specific tolerance to CMV antigen cannot be excluded. In animal experiments, acute infection with murine CMV results in a state of "immunosuppression" (83,84). When Newcastle disease virus (NDV), a potent interferon inducer, was injected into these animals, interferon production was markedly suppressed. Although NDV multiplication occurred in these animals in contrast to controls, the NDV neutralizing antibody response measured 10 days after infection was much lower. This "immunosuppression" also extended to inert antigens, such as sheep red blood cells. It was theorized that a nonlethal virus-induced alteration in immune cell competence occurred during CMV infection and involved both antibody and interferon.

Recently, in acquired CMV infections (heterophile negative infectious mononucleosis, post-transfusion mononucleosis) a variety of "auto-antibodies" have been documented. They appear during the course of infection and disappear as evidence of active infection subsides. These "auto-antibodies" include cold agglutinins, mixed IgM-IgG cryoglobulins, anti-platelet and lymphocytoxic

antibodies. Tests for rheumatoid factor, direct Coombs tests and antinuclear antibody tests become positive (Table 7) (62).

TABLE 7

IMMUNOLOGIC MANIFESTATIONS OF PATIENTS WITH CYTOMEGALOVIRUS INFECTION (POST-TRANSFUSION MONONUCLEOSIS)

PATIENT	ANTINUCLEAR ANTIBODY	RHEUMATOID FACTOR	COLD AGGLUTININS	CRYOGLOBULINS	DIRECT COOMBS' TEST
1	-	-	1:512	-	ND*
2	-	-	-	-	-
3	+	-	-	-	ND
4	-	1:1024	-	+	+
5	-	-	-	-	+
6	-	-	1:2048	+	-
7	-	1:1024	-	-	-
8	-	-	1:32	-	-
9	-	1:128	-	-	-
10	+	-	-	-	ND

* Not done

The origin and pathogenetic significance of these "auto-antibodies" is uncertain.

From a diagnostic viewpoint, the presence of anti-CMV IgM antibody in cord blood or serum of the infant establishes the diagnosis of congenital infection since IgM cannot cross the placenta. The CF test is insensitive in detecting this IgM antibody. An indirect immunofluorescent test using slides of CMV infected tissue culture cells and fluoresceinated anti-human IgM antibody has been developed (43). An indirect hemagglutination (IHA) test has just been reported and detects both IgM and IgG antibody (8,34). Due to ease of performance, the IHA test (with and without 2-mercaptoethanol) may circumvent the need of the indirect immunofluorescent test for the rapid detection of congenital infections.

PATHOLOGY

The pathology of congenital CMV infections reveals typical giant cells with intranuclear and intracytoplasmic inclusions (salivary glands, bile ducts, renal tubular cells, pancreatic acinar cells, etc.). An interstitial inflammatory response is present and can be marked. This inflammatory response is composed chiefly of mononuclear cells. Fibrous tissue proliferation can occur and in hepatic tissue may progress to a picture in children indistinguishable from post-necrotic cirrhosis.

CLINICAL

Although most CMV infections are inapparent, the following syndromes can be produced (Table 8).

TABLE 8

CLINICAL SYNDROMES
CAUSED BY CMV INFECTION

I. Congenital

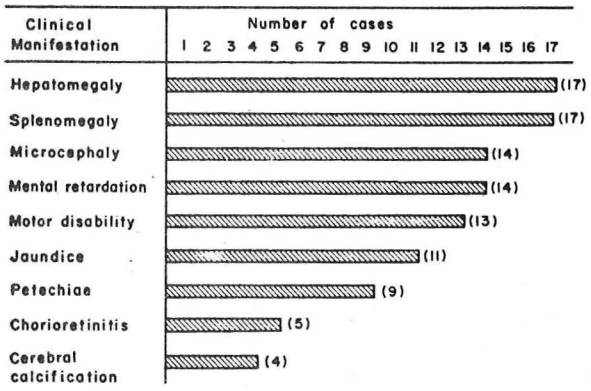
II. Acquired
- A. Heterophile negative infectious mononucleosis
  CMV mononucleosis
- B. Cytomegalovirus hepatitis
- C. Post-transfusion mononucleosis
- D. Generalized disease in the compromised host
- E. Miscellaneous
  1. Acquired hemolytic anemia
  2. Landry-Guillain-Barré syndrome
  3. Pericarditis (myocarditis)

CONGENITAL

The fully developed syndrome of congenital CMV infection is characterized by low birth weight and involvement of multiple organs. Anemia, thrombocytopenia, hepatosplenomegaly, subependymal encephalitis with subsequent development of periventricular calcification and functional derangement and chorioretinitis are common manifestations of disease in the neonate (Figure 3) (114).

FIGURE 3

Clinical Features of 17 Infants With Cytomegalic Inclusion Disease



Pneumonitis and an interstitial nephritis usually without significant functional impairment may be present. The salivary glands are usually involved. Post-mortem examination may reveal changes in the pituitary, adrenal glands and pancreas. The anemia has a hemolytic component but the Coombs' test is negative. Established sequelae include mental/motor retardation, microcephaly, deafness and blindness. Other developmental abnormalities may include indirect inguinal hernias and structural abnormalities of the derivatives of the first embryonic arch (high arched palate, micrognathia, etc.) (119).

Other developmental abnormalities (cerebral, ocular, cardiovascular, gastrointestinal, genitourinary, pulmonary, musculoskeletal and a miscellaneous category) have been associated with congenital CMV infection. It has been argued that the diversity of such abnormalities speaks against their being causally related to the effects of CMV infection (46).

Infants with congenital infections due to toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus may present with a similar clinical picture. These infections have been grouped together in the so-called TORCH complex.

ACQUIRED CMV INFECTIONS

Heterophile negative infectious mononucleosis. Delineated in large part by the Finnish investigators, CMV is the most important cause of heterophile negative infectious mononucleosis. Acute acquired toxoplasmosis may also present as this syndrome, but is less common. Pharyngitis with lymphadenopathy may or may not occur. The absence of pharyngitis and lymphadenopathy in the series of patients compiled by the Finnish workers may reflect in part a selection process. The essential features of this syndrome have been delineated in several reports and can be summarized (Table 9).

TABLE 9

CLINICAL AND LABORATORY FEATURES OF CMV MONONUCLEOSIS*

FEATURE	PER CENT OF PATIENTS		
Fever	100	100	100
Rash	36	13	0
Pharyngitis	55	0	0
Lymphadenopathy	45	0	0
Splenomegaly	36	13	-
Hepatomegaly	27	-	-
Pneumonitis	18	0	0
Abnormal liver function tests	91	100	100
Mononuclear cells > 50	82	100	100
Atypical lymphocytes	45	100	100
"Auto-immune" antibodies‡	-	-	100
Length of fever (range)	≤ 3 months	3-22 days	17-22 days
Age of patients (range)	13-34 yrs	18-66 yrs	24-36 yrs
Number of patients studied	11	8	5
Reference	14	59	64

* All patients had serological evidence of CMV infection

‡ Presence of cold agglutinins, cryoglobulins, positive rheumatoid factor tests, Coombs' test, antinuclear antibody tests (one or more tests positive)

Transient electrocardiographic changes were noted in 2/8 (25%) of patients in one series. Urine cultures may be positive for CMV (3/5 tested). The most sensitive test for the diagnosis of CMV mononucleosis is the serial examination of serum specimens (acute, at onset of illness; and convalescent, 3-4 weeks after onset) for CF antibody titer changes.

Cytomegalovirus hepatitis. Merging with the syndrome of CMV mononucleosis, this agent can produce signs and symptoms primarily related to hepatic dysfunction (107). Hepatomegaly with or without splenomegaly is present. Associated lymphadenopathy is usually absent. A predominance of mononuclear cells with atypical lymphocytes may be found on peripheral blood film. Jaundice may be pronoucced with the serum bilirubin elevated up to 15 mg.%. The SGOT and SGPT are increased but not to the extent seen with viral hepatitis. In two patients, the predominant manifestation was cholestasis with generalized pruritus and alkaline phosphatase levels elevated up to 42 King-Armstrong units. Liver biopsies generally have revealed a picture indistinguishable from viral hepatitis. No cytomegalic cells were observed.

In children, CMV hepatic involvement may become chronic. In some instances, the clinical and pathological features are consistent with post-necrotic cirrhosis (39). No attempt has been made in adults to assess the possibility or lack thereof of CMV infections inducing chronic liver disease.

Post-transfusion mononucleosis. Serologic conversion after multiple transfusions to CMV antigen occurs commonly (Table 3). The incidence of overt clinical illness due to CMV infection may, in part, reflect the rigorousness of the investigator. Originally recognized by Seaman and Starr (97), Finnish workers were the first to establish that CMV was the most important etiology of this syndrome (58). It should be recognized that the agents causing long and short incubation period hepatitis, Epstein-Barr virus and Toxoplasma gondii, can be transmitted by transfusion of blood products. The latter two agents can cause a clinical picture identical to CMV post-transfusion mononucleosis. This syndrome has been predominantly recognized in patients undergoing open heart surgery and on cardiopulmonary bypass. Whether there is something in this procedure itself which predisposes the individual to develop illness once infected is not known. It is well established that patients with serum CF antibody to CMV prior to surgery may develop post-transfusion mononucleosis and laboratory evidence of active CMV infection. The relative contribution of reactivation of a latent infection versus reinfection with a different CMV serotype is uncertain. It is probable, however, that most instances of post-transfusion mononucleosis represent new infections. The clinical and laboratory features in 5 series of cases are presented (Table 10).

The incubation period varies between 1 and 8 weeks. The symptoms and signs present in post-transfusion mononucleosis are comparable to that seen in non-transfusion acquired CMV mononucleosis (Table 9). An acquired hemolytic anemia that usually is direct Coombs' test positive may be present and occasionally is severe enough to require transfusions. This hemolytic anemia usually is transient, appears during the course of post-transfusion mononucleosis, recedes with the disappearance of atypical lymphocytes from the blood and may be responsive to corticosteroid therapy (85). These features tend to distinguish it from the hemolytic anemia that is related to a purely mechanical disruption of erythrocytes and which may occur following insertion of prosthetic valves at open heart surgery. It should be noted that post-transfusion, CMV may induce significant hepatic dysfunction and this feature may predominate.

TABLE 10

CLINICAL AND LABORATORY FEATURES OF CMV POST-TRANSFUSION MONONUCLEOSIS*

FEATURE	PER CENT OF PATIENTS				
Fever	100	100	100	60	100
Rash	20	-	33	0	25
Pharyngitis	0	-	0	0	-
Lymphadenopathy	0	-	0	0	0
Splenomegaly	0	0	-	50	100
Hepatomegaly	80	-	-	70	50
Pneumonitis	-	-	-	0	0
Abnormal liver function tests	-	-	-	60	100
Mononuclear cells > 50	-	-	100	-	
Atypical lymphocytes	-	100	100	60	100
"Auto-immune" antibodies‡	-	-	-	90	
Hemolytic anemia	-	-	-	30	50
Virus isolation:					
Blood	-	40	-	-	100
Urine	60	0	-	100	50
Cardiopulmonary bypass	100	100	67	70	100
Incubation period (range)	1-3 wks	-	3-4 wks	3-8 wks	1-6 wks
Length of fever (range)	1-16 days	-			17-33 days
Age of patients (range)	3-15 yrs	-	15-37 yrs	36-61 yrs	33-62 yrs
No. of patients studied	5	5	3	10	4
Reference	31	33	58	62	69

* Virus isolation (pre-transfusion cultures negative) and/or four-fold rise in CMV CF titer

‡ Presence of cold agglutinins, cryoglobulins, platelet agglutinins, lymphocyte cytotoxic antibodies, positive rheumatoid factor tests, Coombs' test, antinuclear antibody tests (one or more tests positive)

Generalized disease in the compromised host. Since these patients receive blood transfusions, it seems apparent that they can develop a post-transfusion mononucleosis syndrome. The immunosuppression which they receive may mask the hematological response, however. The antibody response may occasionally be blunted. Since many other events may be occurring in these patients, it has been found difficult to ascribe other particular syndromes to the presence of CMV. Nevertheless, the virus may disseminate in the patient with impaired defenses. The organ systems most commonly involved include lung, liver, spleen, lymph nodes, kidney, pancreas, salivary glands, adrenals and gastrointestinal tract. Occasionally the brain, thymus and parathyroid glands may be involved. The gastrointestinal tract lesions can be found from the esophagus to the colon and are ulcerative. Typical cytomegalic cells can be found in the ulcers. The features of CMV pneumonitis in the compromised host are given (Table 11) (91,92).

TABLE 11

CLINICAL AND LABORATORY FEATURES OF CMV PNEUMONITIS IN THE COMPROMISED HOST

SYMPTOMS	Asymptomatic → fever, cough, dyspnea. Pleuritic chest pain absent
PHYSICAL SIGNS	Fever, increased pulse rate. Signs limited in relation to x-ray findings. Cyanosis, tachypnea, scattered rales
CHARACTERISTICS OF SPUTUM	Scanty, mucoid in appearance. Cytomegalic cells rare
X-RAY FEATURES	Multiple small 2 to 4 mm nodular densities scattered throughout both lung fields. Nodules may increase in size. "Interstitial pulmonary edema". Pleural effusion rare
FUNCTIONAL ABNORMALITIES	pO_2 N or ↓*. May respond transiently to O_2 therapy. pCO_2 N or ↓. Diffusion capacity$_{CO}$ ↓ (one patient)
PATHOLOGY	Cytomegalic cells. Variable inflammatory response related to degree of immunosuppression. Chronic interstitial infiltrate. Hyaline membranes focally. Proteinaceous exudate in alveolar spaces.
CLINICAL LABORATORY	Cold agglutinin titer may be elevated
VIROLOGY	Log_{10} $TCID_{50}$ titer/gm wet weight lung tissue from 2.0-6.5. Sputum and urine cultures may be positive
ASSOCIATION WITH OTHER ORGANISMS	Common. Ps. aeruginosa, other gram-negative rods, candida sp., Nocardia asteroides, Mycobacteria tuberculosis, Pneumocystis carinii

* N = normal;
↓ = decreased

Comment: A progressive interstitial pneumonia in the compromised host may be due to CMV.

Since this syndrome may be caused by other agents, notably Pneumocystis carinii, and since therapy of CMV infections is potentially feasible (cytosine arabinoside, IDU) as are pneumocystis infections (pentamidine isethionate), a search should be made for a specific etiology. Viral cultures (sputum, blood, urine) should be obtained. Transtracheal aspiration is sometimes positive for Pneumocystis carinii by GMS stain. Since viral cultures and serological tests for evidence of CMV infection often require an extended period to become positive, specific etiologic diagnosis early in the course of illness, when therapy might be effective, may necessitate lung biopsy.

Miscellaneous syndromes.

Acquired hemolytic anemia. Acquired hemolytic anemia, which may or may not be associated with erythrocyte antibodies, has been documented in the course of CMV infections. The anemia seen in congenital CMV infections has a hemolytic component but is Coombs' test negative. Coombs' test positive hemolytic anemia occurs as a transient phenomenon in the course of post-transfusion mononucleosis. Zuelzer has postulated that acquired hemolytic anemia in childhood may commonly be linked to CMV infection (117). This postulate is based primarily on pathological changes in lymph nodes of these children. Typical cytomegalic cells (Stage III) may be found. More commonly, cells intermediate in development (Stages I and II) are present. Stage I and II cells have irregular intranuclear inclusions, halo formation, strands to the nuclear membrane and cytoplasmic swelling. In several of his patients with these intermediate lesions, CMV was isolated. The pathogenesis of acquired hemolytic anemia due to CMV infection is uncertain. Further studies, particularly emphasizing viral isolation and serology, both in pediatric and adult medicine, need to be performed.

Landry-Guillain-Barré syndrome. Three cases of this syndrome were associated with CMV infection by diagnostic rises in CF titer during the course of illness (63). CMV was isolated from two patients (throat swab, urine). The syndrome was accompanied by manifestations of CMV acquired mononucleosis (pharyngitis, lymphadenopathy, mononuclear cells > 50%, atypical lymphocytosis) in two patients but not in the third. Albuminocytologic dissociation was seen in all three patients. Motor difficulties were predominant and cleared gradually in all the cases.

Pericarditis (myocarditis). Transient electrocardiographic alterations can be seen in young persons with acquired CMV mononucleosis (64). In one patient, the presenting clinical manifestation was chest pain and electrocardiographic abnormalities typical of pericarditis. In this patient, hepatic function tests were mildly deranged and there was an absolute and relative lymphocytosis. Atypical lymphocytes were reported as being absent. Electrocardiographic changes reverted. Cardiac dilatation and pericardial effusion were not present.

Comment:

It appears inevitable that more clinical syndromes will be associated with CMV infection. It is important to keep several facts in mind when considering the etiologic relationship of these syndromes to CMV: 1) CMV excretion in children,

in particular, is common and may be prolonged. The particular syndrome in question may be due to another etiology but may be engrafted on a state where the host coincidentally is excreting CMV. 2) As in herpes simplex viral infections, another agent may reactivate a latent CMV infection. A diagnostic rise in CF titer may occur with reactivation of infection. A healthy skepticism with regard to new syndromes associated with CMV infection appears warranted.

DIAGNOSIS

A summary of diagnostic tests for CMV infection is presented (Table 12).

TABLE 12

LABORATORY TESTS FOR CMV INFECTION

TEST	SPECIMENS	REMARKS
Virus isolation	Urine, saliva, sputum, blood (buffy coat), tissue specimens	Should be inoculated into tissue culture immediately after collection. Freezing reduces titer
Cytology	Urine, sputum, saliva	Relatively insensitive. Helpful when positive
Complement fixation test (CF)	Serum	Sensitive and specific. Widely available. Ineffective in detecting IgM antibody
Indirect immunofluorescent (anti-IgM) test	Cord blood Serum	Detects specific IgM antibody and is diagnostic of congenital infection. Restricted availability
Indirect hemagglutination test (IHA)	Cord blood Serum	Detects both IgG & IgM antibody. Ease of performance. When widely available, may circumvent need for indirect immunofluorescent test
Neutralization test	Serum	Not practical at present. Research uses.

PREVENTION

A vaccine is not available and may not be feasible due to the existence of multiple serotypes. Prevention of severe disease in the neonate represents an area of particular concern. High risk factors need to be better defined. Young maternal age, primiparity, primary infection during pregnancy appear to be significant. The stage of pregnancy when infection occurs may also be important. At present, it is thought that mothers do not give birth to second infants with manifest disease due to CMV.

Efforts to prevent CMV post-transfusion mononucleosis are clearly indicated. Since most adults have CF antibody, it does not appear practical to exclude persons with detectable antibody from donating blood. Techniques to detect CMV antigen in blood are needed. Virus isolation attempts probably require too much time to be useful.

THERAPY

Congenital disease due to CMV and generalized infection in the compromised host may be therapeutically accessible by presently developed chemotherapeutic agents [cytosine arabinoside (CA) and 5-iodo-2-deoxyuridine (IDU)]. Of these two drugs, CA may be the more useful practically and hence will be reviewed in detail.

CYTOSINE ARABINOSIDE

Mechanism of action. Cytosine arabinoside [1-β-D-arabinofuranosyl-cytosine (ara-C) (CA)] exerts its inhibitory action on viral growth by preventing DNA synthesis and formation of intact virions. In mammalian cell cultures, CA inhibits host cell DNA synthesis while RNA and protein synthesis continue. It has been postulated from experiments with clones of cells resistant to the action of CA that the effective inhibitor is actually a phosphorylated derivative (89).

Metabolism. Systemic administration of CA in man and animals results in the rapid formation of a deaminated product [1-β-D-arabinofuranosyl-uracil (ara-U)], which is inactive and either an antitumor or antiviral agent. In man, metabolic conversion of CA occurs primarily in liver and kidney.

Distribution and half-life. The drug does penetrate the blood-brain barrier and enters the cerebrospinal fluid. Brain levels in man are not known. Intrathecal administration of CA has been attempted and is a promising line of investigation. The serum $T\frac{1}{2}$ in man is extremely rapid. 90-95% of CA is excreted in the urine as the deaminated derivative (ara-U). 5% of administered CA is excreted unchanged in the urine.

Toxicity. Hematological changes are of primary importance. Anemia, reticulocytopenia and megaloblastoid bone marrow changes occur. Leucopenia, related to a decrease in the number of circulating granulocytes, and thrombocytopenia may supervene. Anorexia, nausea and vomiting are the chief manifestations of gastrointestinal toxicity. Mucosal ulceration is rare. Chromosomal changes have been reported acutely and CA is teratogenic in experimental animals (rats, chicks). The question of hepatic toxicity is unresolved but hepatic function should be monitored routinely during administration. SGOT, SGPT, LDH and alkaline phosphatase levels have been reported on occasion to be increased. Phlebitis at the

infusion site can occur. Corneal "speckling" may occur in therapy of herpetic keratitis.

Viral spectrum. CA has been used in therapy of DNA viral infections of man, e.g., herpes simplex (keratitis, generalized infections, eczema herpeticum, encephalitis), varicella-zoster (pneumonia, other evidence of generalized infection) and CMV. The number of patients treated is limited and generally without controls but individual case reports indicate promise. Only a few patients with congenital disease due to CMV have been treated with CA. At present, no patient with acquired generalized disease due to CMV has been reported as having been treated either with CA or with IDU.

Dose and administration. In contrast to IDU, CA is readily soluble in water. CA is also generally readily available because of its use in chemotherapy of leukemias and lymphomas. CA is administered intravenously as a solution in 5% dextrose in water or saline. Dosage regimens are presently being worked out.

REFERENCES

1. Anderson, J. P.: Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. Brit. Med. J. 1:672, 1966 (March 12).

2. Armstrong, D., Ely, M., and Steger, L.: Post-transfusion cytomegaloviremia and persistence of cytomegalovirus in blood. Inf. & Immun. 3:159, 1971 (#1).

3. Becker, F. P., Eddy, J. R.: Autoimmune hemolytic anemia with terminal lymphoma and cytomegalic inclusion disease. New York J. Med. 64:1211, 1964.

4. Benyesh-Melnick, M., Dessy, S. I., and Fernbach, D. J.: Cytomegalovirus in children with acute leukemia and other children. Proc. Soc. Exp. Biol. & Med. 117:624, 1964 (#2).

5. Benyesh-Melnick, M., Probstmeyer, F., McCombs, R., Brunschwig, J. P., and Vonka, V.: Correlation between infectivity and physical virus particles in human cytomegalovirus. J. Bact. 92:1555, 1966 (#5).

6. Benyesh-Melnick, M., Rosenberg, H. S., and Watson, B.: Viruses in cell cultures of kidneys of children with congenital heart malformations and other diseases. Proc. Soc. Exp. Biol. & Med. 117:452, 1964 (#2).

7. Benyesh-Melnick, M., Vonka, V., Probstmeyer, F., and Wimberly, I.: Human cytomegalovirus: properties of the complement-fixing antigen. J. Immunol. 96:261, 1966 (#2).

8. Bernstein, M. T., and Stewart, J. A.: Indirect hemagglutination test for detection of antibodies to cytomegalovirus. Appl. Microbiol. 21:84, 1971 (#1).

9. Blanc, W. A.: Cytologic diagnosis of cytomegalic inclusion disease in gastric washings. Am. J. Clin. Path. 28:46, 1957 (#1).

10. Blanc, W. A., and Gaetz, R.: Simplified millipore filter technique for cytologic diagnosis of cytomegalic inclusion disease in examination of urine. Pediatrics 29:61, 1962 (Jan.).

11. Bodey, G. P., Wertlake, P. T., Douglas, G., and Levine, R. H.: Ann. Int. Med. 62:899, 1965 (#5).

12. Brandes, J., and Wetter, C.: Serologic differentiation of viruses responsible for cytomegalic inclusion disease. Virology 12:130, 1960.

13. Cangir, A., and Sullivan, M. P.: The occurrence of cytomegalovirus infections in childhood leukemia. JAMA 195:616, 1966 (#8).

14. Carlstrom, G., Alden, J., Belfrage, S., Hedenstrom, G., Holmberg, L., Nordbring, F., and Sterner, G.: Acquired cytomegalovirus infection. Brit. Med. J. 2:521, 1968 (June) (#5604).

15. Coombs, R.R.H.: Cytomegalic inclusion-body disease associated with acquired auto-immune haemolytic anaemia. Brit. Med. J. 2:743, 1968.

16. Craighead, J. E., Hanshaw, J. B., and Carpenter, C. B.: Cytomegalovirus infection after renal allotransplantation. JAMA 201:725, 1967 (#10).

17. Craighead, J. E.: Immunologic response to cytomegalovirus infection in renal allograft recipients. Am. J. Epidemiol. 90:506, 1969 (#6).

18. Crawford, L. V., Lee, A. J.: The nucleic acid of human cytomegalovirus. Virology 23:105, 1964.

19. Coombs, R.R.H.: Cytomegalic inclusion-body disease associated with acquired autoimmune haemolytic anemia. Brit. Med. J. 2:743, 1968 (June) (#5607).

20. Darlington, R. W., and Moss, L. H., III: The envelope of herpesvirus, in Progress in Medical Virology. Vol. 11. S. Karger, Basel and New York, 1969.

21. Delvaux, T. C.: Viral lesions complicating lymphoma in an adult. Am. J. Clin. Path. 28:286, 1957.

22. Diosi, P., Babusceac, L., and David, C.: Cytomegalovirus type cytopathic changes in spontaneously degenerating human embryonic cell cultures. Nature 214:419, 1967 (Apr. 22).

23. Diosi, P., et al.: Cytomegalovirus infection associated with pregnancy. Lancet 2:1063, 1967 (Nov. 18).

24. Diosi, P., Babusceac, L., Nevinglovschi, O., and Stoicanescu, A.: Duration of cytomegaloviruria in nursery children. Path. Microbiol. 29:513, 1966.

25. Donnellan, W. L., Chantra-Umporn, S., and Kidd, J. M.: The cytomegalic inclusion cell. Arch. Path. 82:336, 1966 (Oct.).

26. Dudgeon, J. A., Marshall, W. C., Soothill, J. F.: Immunological responses to early and late intrauterine virus infections. J. Pediatrics 75:1149, 1969 (Dec.).

27. Duvall, C. P., Casazza, A. R., Grimley, P. M., Carbone, P. P., and Rowe, W. P.: Recovery of cytomegalovirus from adults with neoplastic disease. Ann. Int. Med. 64:531, 1966 (#3).

28. Eichenwald, H. F., McCracken, G. H., Jr., and Kindberg, S. H.: Virus infections of the newborn, in Progress in Medical Virology. Vol. 9. S. Karger, Basel and New York, 1967.

29. Editorial: Cytomegalic inclusion disease in leukemia. JAMA 195:168, 1966 (#8).

30. Emanuel, I., and Kenny, G. E.: Cytomegalic inclusion disease of infancy. Pediatrics 38:957, 1966 (Dec.).

31. Embil, J. A., Haldane, E. V., et al.: Cytomegalovirus infection following extracorporeal circulation in children. Lancet, Nov. 30, 1968.

32. Fetterman, G. H.: A new laboratory aid in the clinical diagnosis of inclusion disease of infancy. Am. J. Clin. Path. 22:424, 1952 (#5).

33. Foster, K. M., and Jack, I.: A prospective study of the role of cytomegalovirus in post-transfusion mononucleosis. New Eng. J. Med. 280:1311, 1969 (June) (#24).

34. Fuccillo, D. A., Moder, F. L., et al.: Micro indirect hemagglutination test for cytomegalovirus. Appl. Microbiol. 21:104, 1971 (#1).

35. Glasgow, Hanshaw, J. B., Merigan, T. C., and Petralli, J. B.: Interferon and cytomegalovirus in vivo and in vitro. Proc. Soc. Exp. Biol. & Med. 125:843, 1967 (July).

36. Goodheart, C. R., and Jaross, L. B.: Human cytomegalovirus. Assay by counting infected cells. Virology 19:532, 1963.

37. Hanshaw, J. B., and Weller, T. H.: Urinary excretion of cytomegaloviruses by children with generalized neoplastic disease. J. Pediatrics 58:305, 1961 (#3).

38. Hanshaw, J. B.: Clinical significance of cytomegalovirus infection. Postgrad. Med. 35:472, 1964 (May).

39. Hanshaw, J. B., Betts, R. F., Simon, G., and Boynton, R. C.: Acquired cytomegalovirus infection: association with hepatomegaly and abnormal liver-function tests. New Eng. J. Med. 272:602, 1965 (March).

40. Hanshaw, J. B., Betts, R. F., Simon, G., et al.: Acquired cytomegalovirus infection. New Eng. J. Med. 272:602, 1965.

41. Hanshaw, J. B.: Cytomegalovirus complement-fixing antibody in microcephaly. New Eng. J. Med. 275:476, 1966.

42. Hanshaw, J. B.: Congenital and acquired cytomegalovirus infection. Pediatric Clin. No. Amer. 13:279, 1966 (May).

43. Hanshaw, J. B., Steinfield, H. J., and White, C. J.: Fluorescent-antibody test for cytomegalovirus macroglobulin. New Eng. J. Med. 279:566, 1968 (#11).

44. Hanshaw, J. B. (Editorial): Viruses in circulating lymphocytes. New Eng. J. Med. 280:1353, 1969 (#24).

45. Hanshaw, J. B.: Congenital cytomegalovirus infection: Laboratory methods of detection. J. Pediatrics 75:1179, 1969 (Dec.).

46. Hanshaw, J. B.: Developmental abnormalities associated with congenital cytomegalovirus infection. Adv. in Teratology, Vol. 4, 1970.

47. Hanshaw, J. B.: Review: Congenital cytomegalovirus infection: A fifteen year perspective. J. Inf. Dis. 123:555, 1971 (May).

48. Harnden, D. G., Elsdale, T. R., Young, D. E., and Ross, A.: The isolation of cytomegalovirus from peripheral blood. Blood 30:120, 1967 (#1).

49. Hedley-Whyte, M. B., and Craighead, J. E.: Generalized cytomegalic inclusion disease after renal homotransplantation: report of a case with isolation of virus. New Eng. J. Med. 272:473, 1965 (#9).

50. Henle, W., Henle, G., Scriba, M., Joyner, C., Harrison, F., von Essen, R., Palocheimo, J., and Klemola, E.: Antibody responses to the Epstein-Barr virus and cytomegaloviruses after open-heart and other surgery. New Eng. J. Med. 282:1068, 1970 (May 7).

51. Hildebrant, R. J., Sever, J. L., and Anderson, B.: Preservation of infectious cytomegalovirus. Proc. Soc. Exp. Biol. & Med. 129:504, 1968 (#2).

52. Hildebrandt, R. J., Sever, J. L., Margileth, A. M., and Callagan, D. W.: Cytomegalovirus in the normal pregnant woman. Am. J. Obst. & Gynec. 98: 1125, 1967 (#8).

53. Hill, R. B., Dahrling, B. E., Starzl, T. E., and Rifkind, D.: Editorial: Death after transplantation: an analysis of sixty cases. Am. J. Med. 42: 327, 1967 (#3).

54. Hill, R. B., Rowlands, D. T., et al.: Infectious pulmonary disease in patients receiving immunosuppressive therapy for organ transplantation. New Eng. J. Med. 271:1021, 1964 (#20).

55. Holswade, G. R., Engle, M. A., Redo, S. F., et al.: Development of viral diseases and a viral disease-like syndrome after extracorporeal circulation. Circulation 27:812, 1963.

56. Ironside, A. G., and Tobin, J. O'H.: Cytomegalovirus infection in the adult. Lancet 2:615, 1967 (Sept.) (#7516) (Letters to the Editor).

57. Jacox, R. F., Mongan, E. S., Hanshaw, J. B., and Leddy, J. P.: Hypogammaglobulinemia with thymoma and probable pulmonary infection with cytomegalovirus. New Eng. J. Med. 271:1091, 1964 (#21).

58. Kääriäinen, L., Klemola, E., and Paloheimo, J.: Rise of cytomegalovirus antibodies in an infectious-mononucleosis-like syndrome after transfusion. Brit. Med. J. 1:1270, 1966 (May) (#5498).

59. Kääriäinen, E. K., von Essen, R., Haltia, K., Koivuneimi, and von Bonsdorff, C. H.: Further studies on cytomegalovirus mononucleosis in previously healthy individuals. Acta Med. Scand. 182:311, 1967 (fasc. 3).

60. Kanich, R. E., and Craighead, J. E.: Cytomegalovirus infection and cytomegalic inclusions disease in renal homotransplant recipients. Am. J. Med. 40:874, 1966 (#6).

61. Kantor, G. L., Johnson, B. L., Jr.: Cytomegalovirus infections associated with cardiopulmonary bypass. Arch. Int. Med. 125:488, 1970.

62. Kantor, G. L., Goldberg, L. S., Johnson, L., Jr., Derechin, M. M., and Barnett, E. V.: Immunologic abnormalities induced by postperfusion cytomegalovirus infection. Ann. Int. Med. 73:553, 1970.

63. Klemola, E., Weckman, N., Haltia, K., and Kaariainen, L.: The Guillain-Barre syndrome associated with acquired cytomegalovirus infection. Acta Med. Scand. 181:603, 1967 (fasc. 5).

64. Klemola, E., von Essen, R., Wager, O., Haltia, K., Koivuniemi, A., and Salmi, I.: Cytomegalovirus mononucleosis in previously healthy individuals. Ann. Int. Med. 71:11, 1969 (#1).

65. Lamb, S. G., and Stern, H.: Cytomegalovirus mononucleosis with jaundice as presenting sign. Lancet 2:1103, 1966 (Nov.) (#7471).

66. Lang, D. J.: The association of indirect inguinal hernia with congenital cytomegalic inclusion disease. Pediatrics 38:913, 1966 (#5).

67. Lang, D. J., and Hanshaw, J. B.: Cytomegalovirus infection and the post-perfusion syndrome. New Eng. J. Med. 280:1145, 1969.

68. Lang, D. J., and Noren, B.: Cytomegaloviremia following congenital infection. J. Pediatrics 73:812, 1968 (Dec.).

69. Lang, D. J., Scolnick, E. M., Willerson, J. T.: Association of cytomegalovirus infection with the postperfusion syndrome. New Eng. J. Med. 278:1147, 1968.

70. Levinsohn, E. M., Foy, H. M., Kenny, G. E., Wentworth, B. B., and Grayston, J. T.: Isolation of cytomegalovirus from a cohort of 100 infants throughout the first year of life. Proc. Soc. Exp. Biol. & Med. 132:957, 1969.

71. Li, F., and Hanshaw, J. B.: Cytomegalovirus infection among migrant children. Am. J. Epidemiol. 86:137, 1967 (#1).

72. Luse, S. A., and Smith, M. G.: Electron microscopy of salivary gland viruses. J. Exp. Med. 107:623, 1958 (#5).

73. Luse, S. A., and Smith, M. G.: Electron microscope studies of cells infected with the salivary gland viruses. Ann. N. Y. Acad. Sci. 81:133, 1959.

74. McAllister, R. M., Wright, H. T., and Tasem, W. M.: Cytomegalic inclusion disease in newborn twins. J. Pediatrics 64:278, 1964 (#2).

75. Martin, A. M., and Kurtz, S. M.: Cytomegalic inclusion disease. Arch. Path. 82:27, 1966 (#1).

76. Medearis, D. N.: Cytomegalic inclusion disease: An analysis of the clinical features based on the literature and six additional cases. Pediatrics 19: 467,1957.

77. Medearis, D. N.: Observations concerning human cytomegalovirus infection and disease. Bull. Johns Hopkins Hosp. 114:181, 1964.

78. Mirkovic, R., Werch, J., South, M. A., and Benyesh-Melnick, M.: Incidence of cytomegaloviremia in blood-bank donors and in infants with congenital cytomegalic inclusion disease. Inf. & Immun. 3:45, 1971 (#1).

79. Mongan, E. S., Kern, W. A., Jr., and Terry, R.: Hypogammaglobulinemia with thymoma, hemolytic anemia, and disseminated infection with cytomegalovirus. Ann. Int. Med. 65:548, 1966 (#3).

80. Mott, M.G.: Cytomegalovirus infection extracorporeal circulation. Lancet, Dec. 14, 1968.

81. Nelson, J. S., and Wyatt, J. P.: Salivary gland virus disease. Medicine 38: 223, 1959.

82. Numazaki, Y., Yano, N., Morizuka, T., Takai, S., and Ishida, N.: Primary infection with human cytomegalovirus: virus isolation from healthy infants and pregnant women. Am. J. Epidemiol. 91:410, 1970 (#4).

83. Osborn, J. E., and Medearis, D. N.: Studies of relationship between mouse cytomegalovirus and interferon. Proc. Soc. Exp. Biol. & Med. 121:819, 1966.

84. Osborn, J. E., Blazkovec, A. A., and Walker, D. L.: Immunosuppression during acute murine cytomegalovirus infection. J. Immunol. 100:835, 1968.

85. Pirofsky, B., Sutherland, D. W., Starr, A., et al.: Hemolytic anemia complicating aortic-valve surgery. New Eng. J. Med. 272:235, 1965.

86. Plotkin, S. A., Stetler, H.: Treatment of congenital cytomegalic inclusion disease with antiviral agents. Antimicrobial Agents & Chemotherapy, 1969, 372-379.

87. Plummer, G., and Benyesh-Melnick, M.: A plaque reduction neutralization test for human cytomegalovirus. Proc. Soc. Exp. Biol. & Med. 117:145, 1964 (#1).

88. Potter, E. L.: Placental transmission of viruses. Am. J. Ob. & Gyn. 74:505, 1957 (#3).

89. Renis, H. E.: Nucleoside antiviral agents and resistance development. Presented to the APHA Academy of Pharmaceutical Sciences, Washington, D.C., Nov. 1968, "Symposium on Antiviral Chemotherapy."

90. Rifkind, D.: Cytomegalovirus infection after renal transplantation. Arch. Int. Med. 116:554, 1965 (#4).

91. Rifkind, D., Goodman, N., and Hill, R. B.: The clinical significance of cytomegalovirus infection in renal transplant recipients. Ann. Int. Med. 66:1116, 1967 (#6).

92. Rifkind, D., Starzl, T. E., Marchioro, T. L., Waddell, W. R., Rowlands, D. T., and Hill, R. B.: Transplantation pneumonia. JAMA 189:114, 1964 (Sept.).

93. Rosenstein, D. L., and Navarrete-Reyna, A.: Cytomegalic inclusion disease. Am. J. Obst. & Gyn. 89:220, 1964 (#2).

94. Rowe, W. P., Hartley, J. W., Waterman, S., Turner, H. C., and Huebner, R. J.: Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids (22497). Proc. Soc. Exp. Biol. & Med. 92: 418, 1956.

95. Rowe, W. P., Hartley, J. W., et al.: Detection of human salivary gland virus in the mouth and urine of children. Am. J. Hyg. 67:57, 1958.

96. Saikku, P., and Räsänen, V.: Cytomegalovirus hepatitis. Lancet 2:772, 1967 (Oct.).

97. Seaman, A. J., and Starr, A.: Febrile postcardiotomy lymphocytic splenomegaly. A new entity. Ann. Surg. 156:956, 1962.

98. Smith, M. G.: Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease (22498). Proc. Soc. Exp. Biol. & Med. 92:424, 1956.

99. Smith, M. G.: The salivary gland viruses of man and animals (cytomegalic inclusion disease), in Progress in Medical Virology. Vol. 2. Hafner Publishing Co., Inc., N. Y., 1959.

100. Starr, J. G., Bart, R. D., and Gold, E.: Inapparent congenital cytomegalovirus infection. Clinical and epidemiologic characteristics in early infancy. New Eng. J. Med. 282:1075, 1970.

101. Starr, J. G., Calafiore, D., and Casey, H. L.: Experience with a human cytomegalovirus complement fixing antigen. Am. J. Epidemiol. 86:507, 1967 (#3).

102. Starr, J. G., and Gold, E.: Screening of newborn infants for cytomegalovirus infection. J. Pediatrics 73:820, 1968 (Dec.).

103. Stern, H.: Isolation of cytomegalovirus and clinical manifestations of infection at different ages. Brit. Med. J. 1:665, 1968 (March) (#5593).

104. Stern, H., and Elek, S. D.: The incidence of infection with cytomegalovirus in a normal population. J. Hygiene 63:79, 1965.

105. Stoker, M.G.P.: Latent infections with viruses and rickettsiae. Brit. Med. J. 1:5025, 1957 (April) (Part 2).

106. Stulberg, C. S., Zuelzer, W. W., Page, R. H., Taylor, P. E., and Brough: Cytomegalovirus infections with reference to isolations from lymph nodes and blood. Proc. Soc. Exp. Biol. & Med. 123:976, 1966 (#3).

107. Toghill, P. J., Bailey, M. E., William, R.: Cytomegalovirus hepatitis in the adult. Lancet 1:1351, 1967.

108. Tsukahara, S., Ueno, I., and Kawanishi, H.: Retinal changes in human cytomegalovirus infection. Am. J. Ophthalmol. 62:1153, 1966 (#6).

109. Vaczi, L., Gonczol, E., Lehel, F., and Geder, L.: Isolation of cytomegalovirus and incidence of complement-fixing antibodies against cytomegalovirus in different age groups. Acta Microbiol. 12:117, 1965/66.

110. Wager, O., Rasanen, J. A., Hagman, A., et al.: Mixed cryoimmunoglobulinaemia in infectious mononucleosis and cytomegalovirus mononucleosis. Int. Arch. Allerg. 34:345, 1968.

111. Weller, T. H.: Observations on the behavior of certain viruses that produce intranuclear inclusion bodies in man. The Harvey Lectures 1956-57.

112. Weller, T. H., Macauley, J. C., Craig, J. M., and Wirth, P.: Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc. Soc. Exp. Biol. & Med. 94:4, 1957 (Jan.-April).

113. Weller, T. H., Hanshaw, J. B., and Scott, D. E.: Serologic differentiation of viruses responsible for cytomegalic inclusion disease. Virology 12:130, 1960.

114. Weller, T. H. and Hanshaw, J. B.: Virologic and clinical observarions on cytomegalic inclusion disease. New Eng. J. Med. 266:1233, 1962 (June) (#24).

115. Weller, T. H.: Cytomegaloviruses, in Viral and Rickettsial Diseases of Man. 4th Edition. J. B. Lippincott Co., Philadelphia & Montreal, 1965.

116. Weller, T. H.: Cytomegalovirus: The difficult years. J. Inf. Dis. 122:532, 1970 (Dec.).

117. Zulzer, W. W., Stulberg, C. S., Page, R. H., Teruya, J., and Brough, A. J.: Etiology and pathogenesis of acquired hemolytic anemia. Transfusion 6:438, 1966.

118. Prince, A. M., Szmuness, W., Millian, S. J., and David, D. S.: A serologic study of cytomegalovirus infections associated with blood transfusions. New Eng. J. Med. 284:1125, 1971.

119. McCracken, G. H., Shinefield, H. R., Cobb, K., Rausen, A. R., Dische, R., and Eichenwald, H.: Congenital cytomegalic inclusion disease. Am. J. Dis. Child. 117:522, 1969.