CYTOMEGALOVIRUS INFECTION IN INFANCY: REPORT OF 9 CASES AND BRIEF REVIEW OF THE LITERATURE*

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Cytomegalic inclusion disease is the result of infection by the cytomegalovirus and is characterized histologically by the formation of peculiar, large, mononuclear cells with intranuclear and cytoplasmic inclusions. The virus was first isolated by Smith[1] in 1956 from the renal tissue of a dying infant. Considerable literature has since been accumulated describing the nature of the virus and the clinical sequelae of infection.

Infection is by no means uncommon. During the first 6 months of 1968, 9 such cases were encountered at the Transvaal Memorial Hospital for Children. The purpose of this paper is to report these 9 cases and review the pertinent literature.

CASE REPORTS

Case 1

A male infant of 2 months presented with a short history of irritability and fever. The salient features on examination were raised intracranial pressure, optic atrophy and mild splenomegaly. The cerebrospinal fluid contained 445 polymorphonuclear cells, 90 lymphocytes and 340 erythrocytes; the protein content was 121 mg./100 ml.; sugar and chloride content were normal. The provisional diagnosis was aseptic meningitis or partially treated pyogenic meningitis

There was no improvement after 10 days of antibiotic treatment and an air-encephalogram showed symmetrical dilation of the cerebral ventricles. A ventriculo-atrial shunt was then inserted to reduce the intracranial pressure. Chorioretinitis in addition to optic atrophy was now noted. All further investigations, including Wassermann, toxoplasma and antibody tests and liver biopsy were negative. Serum alkaline phosphatase was 28 King-Armstrong units. Cytomegalovirus was isolated from the urine on 4 occasions, but not from the saliva or liver.

Case 2

An 8-week-old male infant was admitted to hospital with deep jaundice, marked hepatosplenomegaly and chorioretinitis. He weighed just under 5 lb. at birth and thrived until 7 weeks when his mother noticed the insidious onset of jaundice. There was no known family history of haemolytic anaemia, but an older sibling had died of congenital heart disease.

Investigations showed a haemoglobin level of 10 G/100 ml.; prothrombin index 66%; alkaline phosphatase 53 KA units; total bilirubin 10·1 mg./100 ml., direct 7·4 mg./100 ml.; the Wassermann reaction was negative; and calcium, phosphorus and serum protein electrophoresis were normal. The jaundice was obstructive in character as evidenced by the absence of bile pigment in urine and faeces. The serum flocculation tests were deranged, with marked elevation of serum transaminases. Liver biopsy demonstrated partial fibrosis with bile-duct proliferation suggestive of commencing cirrhosis. Cytomegalovirus was cultured from the urine on 3 occasions. The hepatitis

gradually resolved with complete disappearance of the jaundice. The child has thrived to date, and mental development appears to be normal.

Case 3

A female infant, one of non-identical twins (sibling of case 4) presented with bronchopneumonia at 3 months of age. Examination revealed hepatosplenomegaly in addition to the pulmonary signs. Investigation demonstrated: a mild haemolytic anaemia with a reticulocyte count of 5%; direct Coombs and Schumms tests negative; haemoglobin 10·6 G/100 ml.; white blood count 13,800/cu.mm. and normal platelet count; alkaline phosphatase 150 KA units; Wassermann reaction negative. The serum hepatic transaminases as well as serum 5-nucleotidase were elevated, but serum protein, calcium and phosphorus levels were normal. Cytomegalovirus was isolated from the urine, but not from a liver biopsy—this showed normal histology. Radiological examination of the wrists demonstrated the absence of ossification centres, and evidence of active rickets.

Case 4

A male infant, twin sibling of case 3, also presented with bronchopneumonia and moderate splenomegaly at 3 months of age. The haemoglobin was 9·7 G/100 ml., with morphological changes in the peripheral blood suggestive of haemolytic anaemia. Investigations showed a reticulocyte count of 5%; lactic dehydrogenase 580 units; hepatic transaminases were elevated; alkaline phosphatase was 81 KA units; and serum protein and electrophoresis were normal. The Coombs, Schumms and toxoplasma tests were all negative. No increase in urinary amino acids was demonstrated, and liver biopsy failed to reveal any pathology. Cytomegalovirus was isolated from the urine.

Case 5

A male infant was admitted to hospital at the age of 4 months, with severe anaemia and hepatosplenomegaly. There was no lymphadenopathy or clinical jaundice. The haemoglobin was 3·2 G/100 ml.; white blood count was 24,000/cu.mm., with a 5% reticulocytosis. Platelets were adequate in number. There was diffuse polychromasia, anisopoikilocytosis and occasional metamyelocytes. The picture was thus suggestive of a leuco-erythroblastic reaction associated with acute haemolysis. The direct Coombs test was positive. The alkaline phosphatase was 24 KA units; and serum bilirubin was 1·7 mg./100 ml. Urine culture was positive for cytomegalovirus. The infant was transfused but the reduction in haemoglobin soon recurred with further haemolysis. Following the administration of prednisone, the haemoglobin levels and blood picture returned to normal. After 3 months the Coombs test was negative, and the haemoglobin level was 17 G/100 ml. with 0·6% reticulocytes.

Case 6

This male infant was born following induction of labour at 36 weeks' gestation, because of maternal Rh

sensitization with a rising titre of anti-Rh antibodies. The jaundiced neonate required 4 exchange transfusions over 72 hours. His jaundice regressed but he was admitted at the age of 6 weeks with a recurrence of the jaundice, as well as hepatosplenomegaly. Bilateral indirect inguinal herniae were also present. The following investigations were done: haemoglobin 8·4 G/100 ml.; white blood cell count 9,400/cu.mm.; reticulocytes 10%; the red cells showed anisopoikilocytosis and polychromasia; direct Coombs test was negative; alkaline phosphatase 43 KA units; serum hepatic transaminase levels were markedly elevated. Liver biopsy showed cholestasis and haemosiderosis. Coliform bacilli were cultured from the blood and cytomegalovirus was cultured from both the urine and hepatic tissue. Parenteral ampicillin successfully eliminated the coliform organisms from the blood, but it was some weeks before the resolution of jaundice occurred. Subsequently the infant has shown a marked predisposition to both viral and bacterial gastro-intestinal infections, requiring 3 further admissions.

Case 7

A male infant was referred from a postnatal clinic at the age of 2 months because of pallor. The birthweight was unknown (both parents are mental defectives). On examination there was marked pallor with hepatosplenomegaly. Laboratory investigations revealed the following: haemoglobin 6·0 G/100 ml.; white blood cell count 52,500/cu.mm.; reticulocytes 9%; peripheral blood smear demonstrated a severe leuco-erythroblastic reaction. There was no hyperbilirubinaemia, but urobilin was present in excess in the urine. The following tests were normal: marrow aspiration, serum calcium and phosphorus, Wassermann reaction, serum iron levels, osmotic fragility, direct Coombs, glucose-6-phosphate dehydrogenase, warm and cold agglutinins, red blood cell sickling and pyruvokinase, Paul-Bunnel and LE cells. The alkaline phosphatase, however, was elevated at 48 KA units and furthermore cytomegalovirus was isolated from the urine. Liver biopsy, apart from mild haemosiderosis, was normal. *Salmonella saint-paul* was cultured from the blood.

The patient received a blood transfusion and the haemoglobin level was maintained initially—during this period the salmonella was eliminated by chloramphenicol. The haemolysis, however, recurred 1 month later in the absence of septicaemia but eventually became quiescent over the next 2 months without any specific therapy. Follow-up over the past 6 months has failed to show any further haemolysis, but it is noteworthy that during this time the infant has suffered 2 attacks of pneumonia while in a convalescent home.

Case 8

A 3-year-old girl presented at the outpatient department with moderate microcephaly (skull circumference measured 18·2 in.), hearing defect and minimal brain dysfunction. She had been investigated at the age of 2 months for failure to thrive, bronchopneumonia and hepatosplenomegaly, and cytomegalovirus had been isolated in the urine at that time.

Case 9

A male infant was admitted at the age of 3 months with gastro-enteritis and septicaemia. He developed bone-marrow maturation arrest following chloramphenicol therapy. This was followed by 3 months' convalescence in the ward, during which time he was in contact with cases 6 and 7. Although initial urine cultures for cytomegalovirus were negative, he was found to have hepatomegaly on outpatient follow-up and cytomegalovirus was then cultured from the urine for the first time.

DISCUSSION AND REVIEW OF THE LITERATURE

Cytomegalovirus infection may be congenital or acquired. Congenital infection results from transplacental transmission of the virus. The severity of the disease so caused varies from mild subclinical features to stillbirth or early neonatal death.

Acquired infection, previously thought to be uncommon, has, as a result of serological studies, been shown to be frequent in both children and adults.[2-5] The infection is usually subclinical, but may be serious and fatal in patients with impaired immune responses. This is exemplified in Hodgkin's disease, leukaemia and immunosuppressive therapy, especially when following renal homotransplantation.[6-9]

As the virus is excreted in the urine and saliva, it is likely that transmission is primarily by contact with these excretions. Neonates who have acquired the infection *in utero* are important sources in the spread of the virus in families and institutions. Infection in early life causes chronic disease characterized by cytomegalic cell formation in the salivary glands and kidneys with prolonged virus excretion in the throat and urine.[10,11] A distinction must be clearly made between infection and overt disease in the older child and adult, since infection in these age-groups is so frequently asymptomatic and subclinical. The infection is thus of brief duration, with or without transient virus excretion. When adults develop the disease, they too excrete the virus for prolonged periods. This virus excretion continues in spite of high serum antibody titres.

It would appear that transmission of the infection requires close physical contact. Patients, particularly infants, with known or suspected active cytomegalovirus disease, like those with rubella infection, should be considered potentially hazardous to pregnant women. Hanshaw[12] has observed groups of virus-positive children in the households of antenatally affected children. Although mothers giving birth to infected infants may excrete the virus for months after delivery, congenital infection in subsequent pregnancies has not been very well documented.[13] The ease of spread is evident from a report by Stern and Elek[3] who found that 80% of children aged 10 - 15 years at boarding school had antibodies to cytomegalovirus, as opposed to 18% incidence in a control group at day schools.

Incidence of Cytomegalovirus Infection

Before 1956 the incidence of infection was based on postmortem findings. This suggested that infection was rare and predominantly affected newborn babies. With the introduction of serological techniques and methods of virus isolation, it is now well established that infection not only occurs *in utero*, but is common in both children and adults.[2-4]

Serological studies. Complement-fixation tests and

neutralizing antibody methods have been widely used to determine the frequency of infection. Approximately 50% of women in the child-bearing age-group have serological evidence of previous infection. Thus, 50% of pregnant women are susceptible to infection by cytomegalovirus. The rate of infection during pregnancy is 6%.[34] In the immune mother the antibody is passively transferred to the foetus. Hanshaw et al.[35] found that in 20 infants under 3 months of age, 7 had antibodies considered to be passively acquired. Between the ages of 5 months and 6 years the incidence of positive serological findings diminished to less than 5%, corresponding with the disappearance of maternal antibodies. Stern and Elek[3] demonstrated antibodies in 4 of 93 normal children in the 5 - 10-year-old group; however, 21% of adolescents tested at the age of 15 years had antibodies. The frequency of positive serology continued to increase, reaching a maximum at the 35-year age-group, where 54% of 85 adult persons tested in London were found to be seropositive.[3]

Studies in Puerto Rico by Mendes-Cashion et al.[36] likewise demonstrated an increasing frequency of positive serology with age, but a higher over-all incidence was found. This greater incidence of infection probably reflects poorer living conditions. Similarly, higher incidences are encountered in institutionalized children; Rowe et al.[10] found complement fixing antibody in 21 of 48 institutionalized preschool children.

Virus culture. Most epidemiological studies have relied on virus isolation from the urine or saliva, although isolated reports exist of virus culture from stool specimens and other visceral biopsy sites.

The most complete epidemiological study to date was recently reported by Stern (1968).[5] Investigating unselected hospital admissions and healthy adults in London, 10% of children between the ages of 2 months and 5 years were found to be excreting cytomegalovirus in the throat and urine, but only 1 excreter aged 6 years was detected among 575 older children and adults (Table I).

TABLE I. EXCRETION OF CYTOMEGALOVIRUS IN UNSELECTED NEONATES AND HOSPITAL ADMISSIONS IN LONDON (STERN, 1968)

Age-groups	No. positive / No. tested	% positive
Neonates	3/118	2·5
2 - 5 months	3/32	9
6 months - 4 years	10/104	10
5 - 9 years	1/101	1
10 - 14 years	0/72	—
15 - 24 years	0/102	—
25 - 34 years	0/100	—
35 - 59 years	0/100	—
60+ years	0/100	—

It is noteworthy that during the period 1963 - 1967 only 10 cases under 1 year of age were encountered by the Polio Research Foundation, in whom cytomegalovirus was isolated, whereas in the present series 9 cases were detected in 6 months. It has not been possible to establish whether this is a genuine outbreak or merely the natural incidence of infection in this area. Certainly, since the first case was encountered in January 1968, there has been an increased awareness of the condition and a high index of suspicion among the medical staff at the hospital.

A seasonal variation in the incidence of infection has not been observed. Likewise, attempts to localize the infection geographically have been without success.

Urine culture in this series was the most important means of isolating the virus. Urine microscopy was found to be of little assistance in demonstrating the inclusion bodies. Isolation of the virus from the saliva was negative in all the described cases. In 1 case of cholestatic jaundice the virus was isolated from the liver.

The ease and rapidity of spread of the infection has been stressed.[3,15] This is exemplified by case 9, admitted with gastro-enteritis due to an enteropathogenic *E. coli.* serotype 0111/B4 infection. This infant, while in a debilitated state, came into contact with 2 cases of proved cytomegalovirus infection. Initial attempts at virus isolation in the urine were negative. Shortly after discharge he was noted to have developed hepatomegaly and, for the first time, the cytomegalovirus was cultured in the urine. It has been demonstrated serologically that the incidence of infection rises rapidly with age, especially after the first decade, whereas virologically it is uncommon to isolate the virus after the first few years of life. Stern[5] explains this paradox on the basis that primary infection in the older group is mostly subclinical, of brief duration and occurring without or with only transient virus excretion, whereas, in early life, infection causes a chronic disease with prolonged excretion in the pharynx and urine. It is noteworthy, however, that when adults actually develop the disease, they also excrete the virus for prolonged periods and thus simulate the childhood state.

Significance of Cytomegalovirus Isolation

Stern[5] also concluded from the above-mentioned data that isolation of the cytomegalovirus after childhood was of diagnostic significance. By contrast, the high incidence and prolonged duration of the virus excretion in children under 5 or 6 years of age make it difficult to assess the significance of viuria in relation to any particular presenting illness. The interpretation of viuria should be made in the light of known, well-described, clinical sequelae of infection in childhood, particularly hepatic and respiratory. Positive virus isolation should not be ignored, but, on this basis alone, the clinician would be wrong to attribute too many clinical features to the isolated suspected virus.

The isolation of cytomegalovirus from apparently healthy newborn babies emphasizes the fact that not all congenital infections are fatal or result in severe sequelae. Since 2 out of 3 such cases described by Stern[5] subsequently showed some central nervous system abnormality, an extremely guarded prognosis should nevertheless be given for the newborn from whom the virus is isolated.

CLINICAL FEATURES OF CYTOMEGALIC INCLUSION DISEASE
Intra-uterine infection results in a wide clinical spectrum ranging from stillbirth, early neonatal death and serious neurological involvement to apparently healthy babies with no manifest disease.

This latter group of infants with no obvious disease has been the subject of much study of late. Disturbances of hepatic function have been demonstrated in many such infants.[5,12] Furthermore, although congenital infec-

tion has been reported with initial normal mental and physical development,[17] these infants may present much later with evidence of minimal brain dysfunction and, in addition, microcephaly may only be detected in the second year of life.[11]

The central nervous system usually bears the brunt of both congenital and infantile infection. Thus, of 17 infants studied by Weller, 14 were microcephalic, 5 of whom were evident at birth. Other CNS defects noted included paraparesis or diplegia with spasticity, chorioretinitis, cerebral calcification, seizures, blindness and optic atrophy. These clinical findings have been confirmed by Medearis[13] and Hanshaw.[12] Even in the absence of any neurological abnormality, almost all the affected infants were shown on follow-up examination to have low IQ levels.

The preschool child with perceptual disorders, behavioural problems, deafness and learning difficulties could well be the result of minimal intra-uterine or early infantile encephalitis following cytomegalovirus infection.

In this series there was a notable paucity of overt neurological disease, except for 1 case which presented with obstructive hydrocephalus. Evidence of chorioretinitis was found in 3 cases. Not one of the described patients had radiological evidence of intracranial calcification (Fig. 1). The prognosis for normal mental development is, however, guarded. Case 8 illustrates this in that she presented some 3 years after the original infection with features of minimal brain dysfunction.

The most frequently observed extraneural manifestations in congenital disease are hepatosplenomegaly, hepatitis, pneumonitis, papular or purpuric rashes, haemolytic anaemia, thrombocytopenia and chronic diarrhoea. One or more of these symptoms usually become manifest during the neonatal period. The onset of symptoms after this period raises the possibility of acquired infection. Jaundice, which is the most common finding, is early in onset, prolonged, and results from a combination of cholestatic hepatitis and haemolytic anaemia. The association of hepatosplenomegaly, jaundice and thrombocytopenia is to be differentiated from the rubella syndrome, toxoplasmosis, congenital syphilis and septicaemia.

Zuelzer et al.[18] reported 22 cases of acquired haemolytic anaemia in children with CID. These children ranged in age from birth to 12 years and the direct antiglobulin tests were positive in 18 cases.

Chronic diarrhoea with failure to thrive may also be the presenting symptoms in the infected infant. Benyesh-Melnick et al.[19] isolated the cytomegalovirus from renal cultures derived from 2 infants with congenital heart disease, dying under 6 weeks of age. This observation suggests the possibility that this virus, as well as rubella, may infect the infant during the first trimester and produce comparable, early teratogenic effects. It is of interest that case 2 had an older sibling who had died of congenital heart disease soon after birth.

The most common clinical features in this series were hepatosplenomegaly, jaundice and anaemia (Table II).

Haemolytic anaemia was present in 5 cases out of 9, including 1 infant with a positive direct Coombs antibody reaction. Five of the 9 cases were admitted with superimposed infection, 2 with bronchopneumonia, 2 with Gram-negative septicaemia and 1 with meningo-encephalitis. Subsequent follow-up of the cases revealed a predisposition to intercurrent infection, usually bacterial in type. A disordered immune mechanism was not demonstrated.

Acquired infection may occur at any age. Early infection in the neonatal period and first few months produces any of the neurological and systemic features described above. As a rule, however, the prognosis for early postnatal infections is far better and consistent with normal mental and physical development. Until recently, the prevailing concept of acquired cytomegalovirus infection was based on the premise that infection is primarily asymptomatic after early life, except in persons who have some defect in antibody synthesis or other host defence mechanism. Patients with generalized neoplastic disease such as leukaemia, lymphoma and Hodgkin's disease have a greater incidence of subclinical and clinical disease.[8,20] Pathologists have observed the typical inclusions in patients dying of many debilitating illnesses.[20] Patients undergoing immunosuppressive therapy for renal transplantation have recently been found to carry a high risk of infection and disease.[6,21]
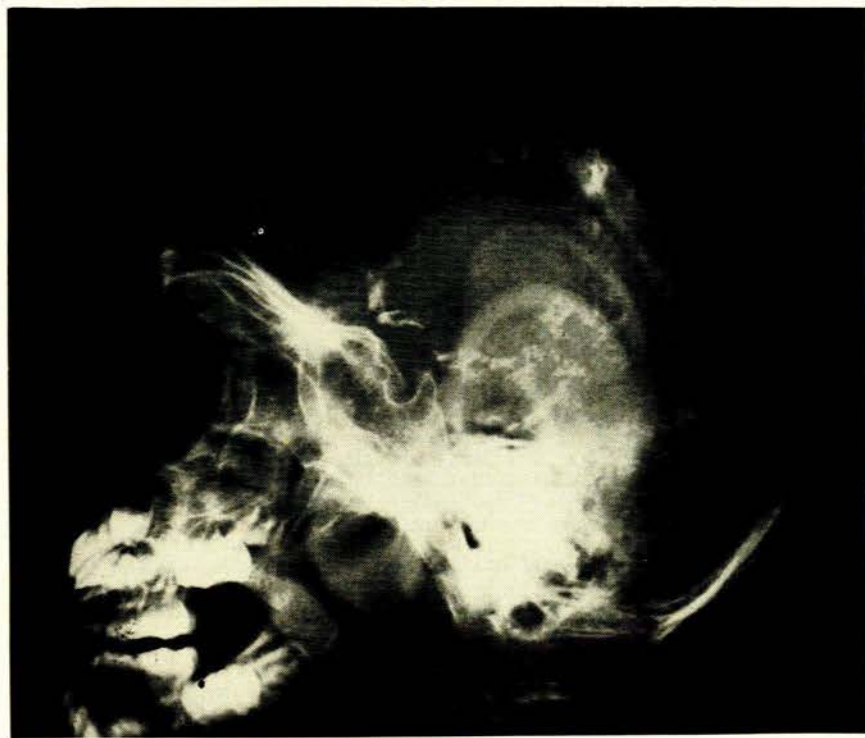

Fig. 1. Lateral X-ray of the skull showing microcephaly and paraventricular calcification in a case of congenital cytomegalovirus infection.

TABLE II. SUMMARY OF CLINICAL FEATURES ENCOUNTERED IN 9 CASES REPORTED

Case No.	Sex	Age (months)	Hepato-megaly	Alk. phos. (KA units)	Spleno-megaly	Haemolytic anaemia	CNS abnormalities	Chorio-retinitis
1	M	2	—	28	—	—	Hydro-cephalus	+
2	M	2	++	53	++	—	—	+
3	F	3	++	120	++	+	—	—
4	M	3	+	81	++	+	—	—
5	M	4	++	24	++	+++	—	—
6	M	2	++	43	++	+	—	—
7	M	3	++	53	++	++	—	+
8	F	2	+	—	+	—	Micro-cephaly	—
9	M	8	+	—	—	—	—	—

Whether this constitutes a new infection or reactivation of a dormant virus is uncertain. The common clinical manifestations include ulcerative gastro-enteritis, pneumonitis, polyneuritis, myocarditis, pericarditis and, most important, hepatitis.

Recent reports, however, have indicated that acquired infection may produce symptoms in previously healthy subjects. Thus, investigating apparently normal children with viuria, the majority of these had hepatosplenomegaly, and almost all had disturbed liver-function tests.[3,10,12] The abnormal function tests most frequently encountered are the serum alkaline phosphatase and serum transaminases. These observations suggest that chronic infection may be clinically significant and that localization tends to occur in the liver with resultant subclinical or overt hepatitis. Toghill et al.[22] also reported 2 cases of acute clinical hepatitis with prominent cholestasis and haemolytic anaemia in adults.

The present series has demonstrated the importance of elevated levels of alkaline phosphatase, in that raised levels were present in 7 out of 9 cases. A mean value of 56 King-Armstrong units was found. Isoenzyme studies confirmed that the alkaline phosphatase was of hepatic origin, except for a pair of twins in whom rickets was an additional source of alkaline phosphatase. The highest levels were encountered in the cases with cholestatic jaundice, as confirmed by biopsy.

It is well known that the hepatic alkaline phosphatase estimation is not a good index of hepatocellular damage: there is nevertheless a close correlation between hepatic infection by cytomegalovirus and serum levels of alkaline phosphatase. This suggests that elevated levels of alkaline phosphatase reflect subclinical as well as clinical hepatitis and may therefore be a valuable aid in confirming cytomegalovirus infection in infancy.

Other clinical manifestations of cytomegalovirus disease in childhood are mild respiratory symptoms, including bronchitis or pneumonia, with a persistent cough suggestive of viral pneumonitis, and often resembling pertussis. Whether respiratory symptoms accompany primary infection in older age-groups is unknown. In view of the number of mothers of infants with CID who have a history of chronic cough or influenza-like illness during pregnancy, it seems likely that cytomegalovirus induces a primary respiratory infection of variable severity as one manifestation of acquired disease.[11,13]

Adults may develop an atypical Paul Bunnel-negative syndrome which also occurs as a complication of operations requiring massive transfusions of fresh blood.[23,24] The condition is characterized by hepatitis and jaundice, or polyneuritis and pericarditis. Atypical mononucleosis tends to be more common over 30 years of age, and results in a syndrome of pyrexia of 3 - 6 weeks associated with a glandular-fever-like blood picture and abnormal liver-function tests, but without exudative tonsillitis or significant lymphadenopathy. Recently Carlström et al.[25] described additional symptoms of lymphadenopathy, anaemia, conjunctivitis, exanthema, gastro-enteritis and headache to a growing list of clinical features associated with acquired cytomegalovirus infection of adults.

SUMMARY

Nine cases of cytomegalovirus infection in infants less than 1 year of age, encountered at the Transvaal Memorial Hospital for Children during the first 6 months of 1968, are described. The common incidence of this infection is stressed.

The most common clinical features were hepatosplenomegaly, jaundice and anaemia. Haemolytic anaemia, including 1 case with a positive direct Coombs auto-antibody reaction, was a frequent finding. Chorioretinitis was present in 3 cases and 1 case presented with obstructive hydrocephalus. The diagnosis of cytomegalovirus infection was confirmed by isolation of the virus in the urine. These cases demonstrated a striking elevation of serum alkaline phosphatase levels in 7 out of 9 cases, with a mean value of 56 King-Armstrong units. Isoenzyme studies confirmed that this alkaline phosphatase was of hepatic origin. The raised levels of alkaline phosphatase reflect subclinical as well as overt hepatitis and it is suggested that this may be a valuable aid in the confirmation of cytomegalovirus disease of infancy.

A review of the literature regarding cytomegalovirus infection and disease is presented.

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