Cytomegalic Inclusion Disease: A Case Report with Isolation of Virus

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The occurrence of large inclusion bodies in human tissues, both in health and in disease, has been known for many years. In 1904, Jesionek and Kiolemenoglou noted the presence of such inclusions in children, and vonGlahn and Pappenheimer later reported their occurrence in adults. The presence of such inclusions in the salivary glands has led to the designation “salivary gland virus (SGV) disease”. In reviews of the literature on this subject Nelson and Wyatt and Wyatt et al. noted that SGV was pantropic in character, was latent in nature, and could affect the otherwise healthy individual. At postmortem examination 10% of infants are said to harbour the virus in the salivary glands, regardless of the cause of death, according to Farber and Wobach. Medearis estimated that 10% to 30% of pediatric patients coming to necropsy may harbour the virus in the salivary gland, from which site it is seldom disseminated. Some indication of the distribution of the virus in healthy, hospitalized and institutionalized children may be derived from the research of Hanshaw et al. who found cytomegalovirus (CMV) infection in 1% of a group of 200 well children and 100 unselected patients from pediatric hospital admissions. Institutionalized children and close contacts of virus-positive children had infection rates of 23% and 28%, respectively. Of 23 children with unexplained hepatomegaly or chronic liver disease, a CMV was isolated from nine (39%). Newborn and premature infants are particularly susceptible to infection and develop the disseminated form of the disease. The acquisition of in utero infection passing from mother to child is, accordingly, a strong possibility. Primary infection of the pregnant mother with CMV has also been postulated by Medearis.

Knowledge of cytomegalic inclusion disease (CMI) has been increased by the isolation of salivary gland virus from human adenoid tissue by Rowe et al. The resemblance of the agent recovered by Rowe et al. to a virus isolated from human salivary gland, as well as to viruses from cytomegalic inclusion disease, has suggested the identity of these agents. Further evidence has been provided by Smith, who recovered a cytopathic virus in human fibroblast cells causing large intranuclear inclusions resembling those found in SGV disease from the tissue of two infants, one of whom died from generalized CMD.

According to Weller, viruses that produce intranuclear inclusion bodies appear to have an unusual opportunity for self-perpetuation, because the immune host, on occasion, becomes an agent of viral dissemination. Thus, this mode of dissemination may prove to be more commonplace than has been suspected.

Serological studies on human CMV and SGV agents by Weller, Hanshaw and Scott have further indicated that these agents do not constitute single entities but should be referred to as the “cytomegalovirus” group.

Human Cytomegalovirus Infection

The clinical significance of cytomegalovirus infection in man has recently been assessed by Hanshaw, who has described effects following infection in the congenital, postnatal, childhood and adolescent periods of life.

Data adduced relative to the congenital stages of development revealed that the mother was usually primiparous and most often had an uneventful pregnancy. The average birth weight of the child was 2279 g. Illness commenced in the first 48 hours of life, or occasionally in the second or third week, and was characterized by petechial rash, thrombocytopenia, jaundice and hepatosplenomegaly. The latter may persist into the second or third year of life.

Several clinical patterns of illness may occur. Microcephaly occurs in most cases and may be present either at birth or at the end of the first year of life. This may be accompanied by chorioretinitis, optic atrophy, cerebral calcifications and spastic paralysis. Sometimes neurological sequelae may be delayed until the second year of life. It is also of special interest to note that CMV may be excreted in the urine from two to three years after birth. Hanshaw observed 17 patients; of this number, one died and two escaped mental damage, but among the remaining 14, mental and motor dysfunction, blindness, seizures, spastic paralysis, microcephaly and mental retardation were observed. In the neonatal period the differential diagnosis includes congenital toxoplasmosis, erythroblastosis fetalis, neonatal sepsis, biliary atresia, and

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other virus diseases which may cause hepatosplenomegaly in this period, such as Coxsackie B and herpes simplex viruses. In a case studied by Kluge, Wicksman and Weller,14 jaundice, purpura and hepatosplenomegaly were reported shortly after birth. At the end of a two-year period of observation, signs of developmental difficulty were noted, as indicated by impairment of speech and a head circumference below the 3rd percentile for age. CMV was isolated from the urine at two and six weeks and at three, 16, 21 and 26 months of age. Neutralizing antibody for CMV was present in the patient's serum during the period of viruria. Hanshaw and Weller16 have described the details of the excretion of cytomegaloviruses by children with generalized neoplastic disease, and have presented a correlation of clinical and histopathological changes in this disorder.

Hanshaw23 has observed that during the early postnatal period neurological impairment may not be evident in the affected infant. The association, in a fatal case of Pneumocystis carinii pneumonia, of generalized cytomegalic inclusion disease (CMI) and hypogammaglobulinemia has been reported by Kramer, Citrone and Moore.18 CMV was also isolated by Stern, Lambert and Shakespeare17 from the throat and urine of a 6-month-old patient with a large retroperitoneal angiosarcoma. Infants with congenital heart malformations18 and children suffering from leukemia19 have also been shown to harbour CMV. Retinal involvement and cataract in a stillborn infant with CMD has been reported by Dvorak-Theobald.20 An unsuccessful search for cytomegalic inclusions in the placenta of a fetus with evidence of CMD has been described by Quan and Straus.21 In the first case of congenital CMD described from Norway by Gardborg and Hanssen22 inclusions were demonstrated in the ducts of the submandibular glands, the bronchi, lungs, pancreas and renal tubules. The first isolation of cytomegalovirus in Sweden was recently accomplished by Carlström.23

In adults, CMV infection has been linked with certain abnormalities, pathological conditions and debilitated states. These include diabetes;24 hypogammaglobulinemia with thymoma;25 and Wegener's granulomatosis, thrombotic purpura and Hodgkin's disease.26 Of great interest is the report of the development of generalized CMI pneumonia following a course of immunosuppressive therapy, undertaken prior to the performance of a renal homograft operation, as described by Hedley-Whyte and Craighead.27 The latter contend that CMI disease may be a potential hazard of renal homotransplantation and may represent a hitherto unrecognized complication of immunosuppressive therapy. Retinal involvement in patients with CMI infection has been described by Smith,28 and a general consideration of the relative resistance of adults to infection by this agent is contained in a report by Wong and Warner.29 Pulmonary CMI disease in adults has been discussed by Capers and Lee.30

Canadian medical literature contains few references to CMI disease. McMillan24 described a case of inclusion pneumonitis affecting a 60-year-old Japanese woman in Montreal, the second case to be reported in an adult in the then current literature. Lauzé32 reported a case from Montreal affecting a 35-year-old diabetic man who also suffered from pulmonary tuberculosis, and two other cases were reported from Montreal by Chagnon.33 Three cases from Calgary, Alberta, in which periventricular cerebral necrosis was observed in two premature infants were reported by Elliott and Elliott.34 The case described in the following report, to our knowledge, represents the first case in Canada from which the virus has been propagated in tissue culture. The presenting signs and symptoms in this patient were initially those of a mild respiratory illness followed later by progressive mental deterioration.

**First Admission—Age two months**

This infant was first seen at the Children's Hospital, Halifax, at the age of two months with a three-day history of upper respiratory infection associated with occasional projectile vomiting. During this time the infant was markedly irritable but took her feeding well.

The past history was unremarkable. The baby was full-term and weighed 8 lb. 8 oz. at birth. There were no complications during the pregnancy, and the delivery and post-delivery course were normal. There was no history of jaundice or purpura in the neonatal period. The infant did well at home following discharge from hospital, and as far as could be ascertained, seemed to develop normally during the first two months of life. She was fed on an evaporated milk formula and no problems were noted except for occasional vomiting after feeding, beginning around two weeks of age. The mother did notice that the infant exhibited occasional episodes of unexplained irritability.

The patient had one sister, aged 20 months, who was well. The mother and father were both 29 years of age and were in good health.

At physical examination on her first admission to hospital, the baby weighed 12 lb. 13 oz. Her nutritional status was good. She seemed bright and alert and able to hold her head up without difficulty when lying in the prone position. No neurological abnormalities were detected. There was no hepatosplenomegaly. The remainder of the physical examination revealed only evidence of a mild upper respiratory infection with a left otitis media. Funduscopic examination was not attempted. Within 12 hours of admission the infant developed swelling of the lips and mucous membranes of the mouth associated with vesicular stomatitis which extended to involve the nares on the following day. In addition, a diffuse erythema of the skin developed, which very shortly began to exfoliate. During the succeeding week the baby showed a marked exfoliative dermatitis associated with an ulcerative stomatitis without evidence of conjunctivitis. Cultures of the oropharynx failed to grow herpes simplex virus. The infant was treated with ampicillin 70 mg. every six hours, orally, and prednisone 15 mg. daily in divided doses.
During the next two weeks she made an uneventful recovery. The exfoliative dermatitis and stomatitis at that time remained unexplained, but the illness bore a resemblance to the Stevens-Johnson syndrome. The infant was discharged apparently well.

Laboratory data on this admission showed a hemoglobin of 13.2 g./100 ml. The white blood cell count was 26,600/c.mm. with normal platelets, and the differential count revealed 40% neutrophils, 8% eosinophils and 52% lymphocytes. Nasopharyngeal and stool cultures showed no evidence of pathogenic bacteria. A lumbar puncture revealed a normal cerebrospinal fluid pressure, with normal values for protein, sugar and chlorides. The blood urea nitrogen was 19 mg./100 ml.; serum oxalacetic transaminase (SGOT) was 60 units, and the serum glutamic pyruvic transaminase (SGPT) was 40 units. Repeated urinalyses were within normal limits. The serum alkaline phosphatase was 23.5 King-Armstrong units.

SECOND ADMISSION—Age seven months

At this time the infant was admitted because of a "convulsion". The seizure, which was described as being tonic in type and associated with cyanosis, was of short duration and occurred following a short episode of upper respiratory infection associated with otitis media. The infant was treated at home with ampicillin, and was referred to the hospital for admission following the seizure.

On this admission the physical examination and history revealed a marked degree of developmental retardation. The infant could not roll over from the prone position, could not hold her head up without support, and would not reach for objects. A strong grasp reflex was also evident. In addition she showed peculiar aimless movements of both upper extremities. At that time she weighed 17 lb. 1 oz. and her nutritional status was good. Her head circumference was 17" in. and her body length was 27" in. General neurological examination showed no localized abnormality. Muscle tone was average. The infant did not appear to be deaf. Funduscropy showed no evidence of chorioretinitis. The liver and spleen were not palpably enlarged.

The laboratory findings were as follows. Lumbar puncture showed a clear spinal fluid with a normal colloidal gold curve, normal values for protein, chloride and sugar, and there was no growth in bacteriological culture. The tuberculin test (PDD) was negative. Radiographs of the skull were within normal limits. A radiograph of the chest and an intravenous pyelogram showed no evidence of abnormality. An electroencephalogram was within normal limits. The hemoglobin was 12.8 g./100 ml. The white blood cell count was 13,650/c.mm. with 19% neutrophils, 80% lymphocytes and 1% eosinophils. Urine analyses were within normal limits. Fasting and post-prandial blood sugar estimations were within normal limits. The serum phosphorus was 4.8 mg./100 ml. The total serum calcium was 10 mg. % and the serum alkaline phosphatase was 19 King-Armstrong units. The total platelet count was 369,000 and the blood urea nitrogen was 12 mg./100 ml. The blood phenylalanine level was reported at 1.3 mg./100 ml. and the blood tyrosine was 1.3 mg. %.

No further seizures were noted in hospital and the infant was discharged, with no explanation for the developmental retardation. On the basis of the physical examination she was assigned an approximate developmental age of 3 months.

Following discharge from the hospital the infant again developed seizures of the type previously described. The seizures were generalized, associated with clonic movements, and lasted approximately one minute. These seizures occurred several times daily. The infant was readmitted to hospital after failing to respond to anticonvulsant medication at home.

THIRD ADMISSION—Age eight months

On this readmission the physical findings were as before and further investigations were performed. A six-hour glucose tolerance test failed to reveal evidence of hypoglycemia. Chromatographic screening of the urine for sugar and amino acids showed no abnormality. The electroencephalogram was not repeated.

The infant was treated with phenobarbital 8 mg. every six hours. During the final week prior to her discharge no further seizures were noted and she was sent home, with a diagnosis of mental retardation and convulsive disorder of unknown etiology.

FOURTH ADMISSION—Age nine months

The infant was readmitted to hospital with evidence of progressive developmental retardation, failure to gain weight, and recurrent tonic convulsions which were not controlled by anticonvulsant therapy, consisting of phenobarbital and diphenylhydantoin (Dilantin).

Physical examination showed some evidence of failure to thrive and marked developmental delay, her developmental age being assessed at the three- to four-month level. The neurological examination otherwise was unremarkable except for evidence of generalized hypotonia and athetoid movements of the upper extremities. It was also apparent at that time that the infant had a marked hearing deficit.

The following investigations were performed. The blood hemoglobin was 13.2 g./100 ml., and the white blood cell count was 16,950/c.mm. The differential white blood cell count showed 27% neutrophils, 15% eosinophils and 58% lymphocytes. Repeat examinations of urinary sediment showed no metachromatic granules. A ventriculogram showed no abnormality. The cerebrospinal fluid examination and culture were normal. Repeated white blood cell counts showed a persistent eosinophilia, varying from 14% to 20%. The total eosinophil count on one occasion was 2693/c.mm. Serum chloride, potassium and sodium were normal. The blood pH was 7.438. The blood group was B, Rh-positive. Titration of blood group agglutinins showed an anti-A titre of 1/164 and an anti-B titre of less than 1 in 4. An electrocardiogram showed no abnormality. A serum complement fixation test for toxoplasmosis was negative at a dilution of 1 in 4. Chromatographic examination of the urine for reducing substances and amino acids was normal. Microscopic examination of the urinary sediment for cytomegalic inclusion bodies was negative. Simultaneously the urine was cultured for cytomegalovirus.

Because of the subsequent isolation of a characteristic cytopathic virus, a tentative diagnosis of cytomegalic inclusion virus encephalitis was made. The infant was discharged, unimproved, following a trial
period of therapy with pyridoxine 10 mg. daily for two weeks. Anticonvulsant medication, consisting of phenobarbital 15 mg. four times daily and diphenylhydantoin 60 mg. daily, was continued. Her subsequent course at home remained unchanged except that her seizures were better controlled and occurred much less frequently.

**Fifth Admission—Age 14 months**

At this stage the infant's physical and mental status was essentially unchanged except for evidence of poor weight gain.

Funduscopia showed no evidence of chorioretinitis. An electroencephalogram showed periodic, isolated low voltage, sharp discharge occurring in the right hemisphere, mainly in the frontotemporal region, followed by low-voltage slow waves. There was a light background asymmetry with lower voltages on the left side. The electroencephalogram was interpreted as an abnormal tracing with epileptogenic activity over the right hemisphere, mainly in the right frontal region. An examination of the blood for immunoglobulins at this time revealed that all immunoglobulins were present; immunoglobulin A was elevated and the levels of immunoglobulin M were low. Examinations of the urine sediment for cytomegalic inclusion bodies again were negative.

A repeat urine culture taken at this age, five months after the original isolation, again revealed evidence of excretion of a cytomegalovirus. The patient's weight on discharge was 16 lb. 14 oz.

**Materials and Methods**

**Tissue Cultures**

A modification of the “sandwich” technique advised by Therkelsen was used in the preparation of tissue cultures. Less frequently, a method similar to that described by Morann and Melnick was employed. Human foreskin, as well as human embryonic skin and muscle, was used as a source of tissue. A 16 x 125 mm. Leighton tube containing a 9 x 35 mm. glass cover slip was used for cultivation.

Tissues were cut into small fragments measuring approximately 0.5 mm. in size. With the aid of the tip of a Pasteur pipette, single fragments were picked up and placed on the flat surface of the Leighton tube, which was held uppermost toward the operator. Thereafter the tube was slowly rotated so that the cover slip was allowed to fall upon the fragments of tissue, so as to form a sandwich. Firm pressure was applied to the coverslip with the tip of a bent Pasteur pipette. The following serum dilutions and media were observed to give optimum results.

The best growth medium for human foreskin was found to be 80% Hank's lactalbumin containing 20% calf serum. For growth of human embryo skin and muscle, 90% Eagle's MEM medium with 10% fetal calf serum was used. Optimum maintenance medium for human foreskin was 93% Hank's lactalbumin with 7% calf serum, and for human embryonic skin and muscle, 93% Eagle's MEM medium plus 7% fetal calf serum proved effective.

Cultures were incubated at 37° C. and left undisturbed for four to five days; thereafter, medium changes were made at intervals of three days. After dense growth had developed, the cultures were used for inoculation.

The sandwich tissue cultivation technique has some advantages over the plasma clot method. The growth of cells was rapid, and in the logarithmic phase of growth the generation time was 16-24 hours according to Therkelsen. The only apparent drawback was that growth could not be maintained for longer than 75 days.

**Urine**

Twelve to 15 ml. of the patient's urine was centrifuged at 2700 r.p.m. for 30 minutes; 1.5 to 2 ml. of supernatant fluid was removed; one drop of fungizone was added and 0.5-ml. amounts were distributed to each of two tubes containing 1 ml. of maintenance medium. The centrifuged deposit was resuspended in 1.5 ml. of maintenance medium; one drop of fungizone was added and thereafter inoculated in 0.5-ml. amounts into a similar number of tissue culture tubes containing 1 ml. of maintenance medium. Culture medium was examined for evidence of toxicity after 17 to 72 hours of incubation; if toxicity was present, the medium was replaced with fresh maintenance medium. Routine fluid changes were made at intervals of three to five days throughout the period of observation.

**Isolation of Virus**

After 14 days' incubation at 37° C., initial focal cytopathic changes were observed (Figs. 1 and 2). These consisted of giant cell formation which gradually extended to affect the whole cell sheet so that at the end of 28 days some 80% of the tissue showed cytopathogenic effect (CPE). Virus was isolated from both the urinary sediment and the supernatant fluid. The
agent was transmitted by trypsinization of cultures with 2 ml of 0.25% concentration of National Biochemical Co. (1:300) trypsin in balanced Hanks' salt solution without phenol red, centrifugation, resuspension of the pellet with 2 ml of maintenance medium, and reinoculation of new cultures. Human amnion cells were also found to be susceptible to the virus isolated. Antibiotics added to media consisted of 500 I.U./ml of penicillin-G sodium and 500 μg/ml streptomycin sulfate.

Altogether the virus had been propagated for serial subcultures for a period of five months. On subculture CPE was noted to occur in seven days accompanied by the formation of focal necrosis with inclusion bodies.

Histological examination of tissue culture showing CPE revealed well-marked intranuclear "owl-eye" type A inclusions which stained by the Geimsa and H and E methods and were characteristic of CMV infection as described by Rowe et al.37 and by Weller and Rowe38 (Fig. 3). The use of "touch" preparations for rapid diagnosis has been reported by Lysaught,39 Hanshaw13 and others. Rapp, Rasmussen and Benyesh-Melnick40 described an immunofluorescent technique for quantitative assay of cytomegalovirus foci of antigen-containing cells. The electron microscopic appearance of CMV inclusions has been described by Stern and Friedmann.41

Details of other diagnostic methods can be found in papers by Blanc and Gaetz,42 Plummer and Benyesh-Melnick43 and Carlström.29

DISCUSSION

The clinical symptomatology of the case presented, at the neonatal stage, did not conform to the classical description of CMI disease. However, the relatively higher incidence of CMV infection in retarded children has raised the question of its possible association with arrested mental development.

In most instances the disease process linked with CMV is acquired in utero. Infants thus affected present with microcephaly, chorioretinitis, cerebral calcification, jaundice and hepatosplenomegaly. In other cases the classical features of the disease may be absent at birth, but over the following two years the infant may develop progressive signs of mental retardation, deterioration and allied sequelae. It is possible that the examination of urine and throat swabs by virological tissue cultivation procedures affords a better chance of establishing a diagnosis than following the earlier practice of searching urinary deposit for intranuclear inclusions. In our case the latter examination was conducted several times but with negative results, whereas two specimens of urine taken five months apart yielded virus in tissue culture. At the present time little information is available concerning the role of CMV infection in retarded children.

There is growing evidence that CMI virus is linked with a train of events in the life of the newborn child which may ultimately lead to progressive mental retardation and cognate neurological phenomena.

More recently, the work of Hedley-Whyte and Craighead27 has shed light on a different aspect
of CMV infection. Their findings not only support the observation of Hanshaw and Wellersh 15 that the administration of corticosteroids and antimetabolites predisposes to CMV infection, but also indicate that immunosuppressive drugs, such as azathioprine, methylprednisolone, azaserine and actinomycin C, can provoke the same effect.

Despite the available knowledge, there is still no conclusive proof that CMV is directly responsible for the sequence of clinical events described.

There is some hesitancy in attributing a pathogenic role to CMV in this disease, because CMV has been recovered from children with a number of different maladies in which CMV cannot be incriminated. Furthermore, it has been demonstrated that normal persons may harbour CMV in their salivary glands and other organs.

The nub of the problem would seem to be the nature of the as yet unknown factors which may precipitate the onset of CMV disease in certain children, yet allow others to escape. Precisely the same question may be asked in connection with the pathogenesis of herpes simplex labialis. Here again, the basic mechanism whereby virus undergoes transition from a latent to a pathogenic role remains unsolved.

The epidemiology of CMV in relation to human cases is obscure. Nothing is known of the distribution, frequency and mode of transmission of CMV virus in the population of Canada.

**Summary**

A case of cytomegalic inclusion disease affecting a 14-month-old retarded female child is reported. Virus was isolated from urine by tissue cultivation methods, was demonstrated to be CMV, and observation and also the experience he has obtained from study and observation and also the experience he has obtained from study and observation. The nub of the problem would seem to be the nature of the as yet unknown factors which may precipitate the onset of CMV disease in certain children, yet allow others to escape. Precisely the same question may be asked in connection with the pathogenesis of herpes simplex labialis. Here again, the basic mechanism whereby virus undergoes transition from a latent to a pathogenic role remains unsolved.

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**References**