# Cytomegalovirus Infection in Black Children in Durban

## A MORBID ANATOMICAL STUDY

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## SUMMARY

**Histological examination of salivary glands from Black children revealed that cytomegalovirus (CMV) infection is most common between 2 and 12 months of age, 25% of salivary glands showing cytomegalic cells and 4% chronic sialitis. A high incidence of infection in association with congenital biliary cirrhosis is noted. Six cases of congenital CMV infection were found, only 2 of which were disseminated. A review of records of postmortem findings revealed 15 cases of postnatal disseminated CMV infection, all in infants with kwashiorkor.**


*S. Afr. Med. J.*, **48**, 1408 (1974).

Dissemination of the herpes simplex virus is quite common in kwashiorkor, where there is believed to be a depression of cellular immunity. The sporadic observation of cytomegalic virus cells in lungs made us question whether disseminated CMV infection was not also prevalent in kwashiorkor, and prompted this investigation of the incidence of CMV infection in our hospital's necropsy cases.

## PATIENTS AND METHODS

One submaxillary gland was collected from routine hospital necropsies on children under 5 years of age over a 3-year period at King Edward VIII Hospital, Durban. Cases were not entirely consecutive but there was no element of selection. The tissue was fixed in formol saline and sections stained by haematoxylin and eosin. Some sections were stained by PAS to depict intracytoplasmic granules more clearly. In all, 1 010 glands were collected.

Sections were examined for the presence of cytomegalic cells and plasma cell and lymphocytic infiltration. An assessment of the number of cells infected by the virus was made on a + basis and the extent and severity of the cellular infiltrate were likewise graded.

In a retrospective study records were searched for cases of CMV infection over the past 5 years. Sections of all available tissues were re-examined for indications of dissemination of the virus.

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## RESULTS

### Histological Features

The mature CMV cell is highly characteristic, with a large basophilic nuclear inclusion body separated by a clear zone from the peripherally condensed chromatin. The cytoplasm develops granules, their size and staining reactions varying with the stage of development, becoming PAS-positive on maturity.

In salivary glands CMV cells are seen mainly in intercalated ducts (Fig. 1), but occasionally in acinar cells. They appear to rupture into the ducts, the virus thus being excreted in the saliva. In all cases where CMV cells were found there was an associated chronic sialitis with plasma cell and lymphocyte infiltration. This might be either focal, lobular or diffuse. In mild or early cases inflammatory cells were mainly seen round intercalated ducts, but in more active cases the cells penetrated between the acini (Fig. 2). Some infections were severe with extensive necrosis of acini, the necrotic cells being lysed so that only ducts remained in an oedematous stroma (Fig. 3). Later there was epithelial regeneration (Fig. 4), the gland presumably returning to normal. At a late stage lymph follicles formed, and the plasma cells decreased in number. It is evident that these lymph follicles eventually disappear,

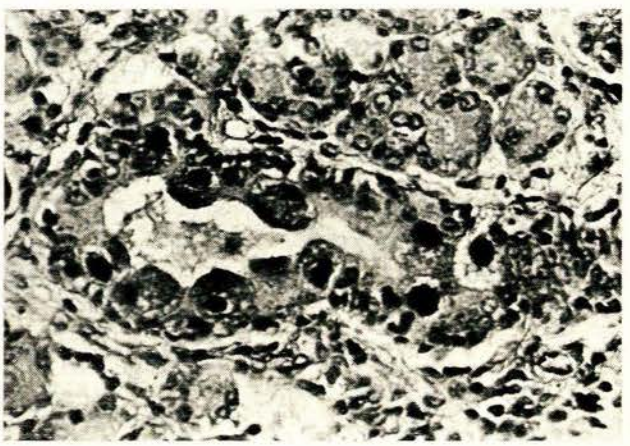

**Fig. 1. Submaxillary salivary gland, CMV cells in ductular epithelium, minimal inflammatory infiltrate (H. and E. × 390).**

and are rarely seen in older children. Polymorphs are not commonly seen and, if present, they are intraductal, suggesting secondary bacterial infection.

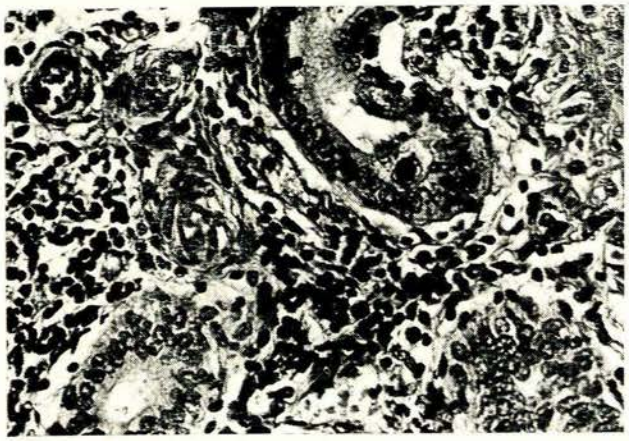

**Fig. 2. Submaxillary salivary gland, CMV cells in ductular epithelium, severe inflammatory infiltrate (H. and E. × 390).**

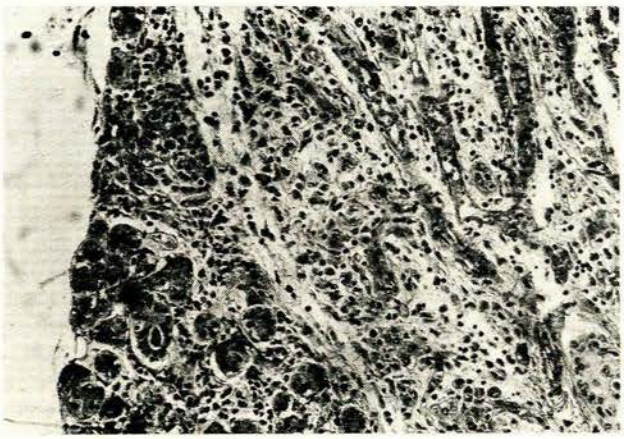

**Fig. 3. Submaxillary salivary gland, necrosis of acini, ducts in oedematous stroma with inflammatory cells (H. and E. × 150).**

## Incidence of Infection

The number of CMV cells in positive cases in different age groups is assessed in Table I. In some cases only one or two infected cells were seen in a cross-section of the gland, while in other cases they were numerous. The severity of the cellular infiltrate was also variable, not necessarily related to the number of CMV cells. A severe infiltrate was sometimes found with only one or two CMV cells, and at times no cytomegalic cells were detected. While it is possible that in these cases the infection may be due to some other virus, the occurrence among other positive cases leads one to think that the virus-containing cells may in fact have been destroyed by the immunological

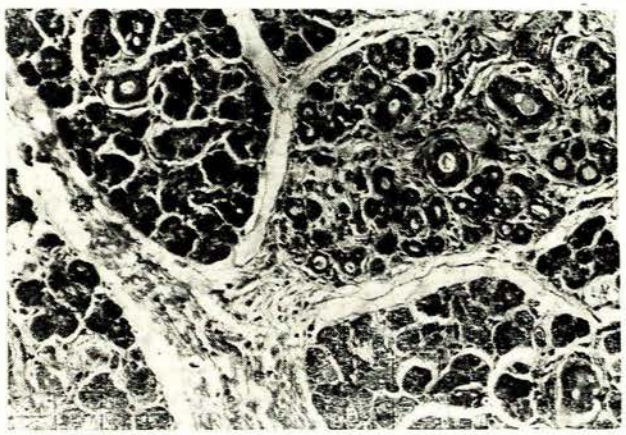

**Fig. 4. Submaxillary salivary gland, regenerative lobule with small acini (H. and E. × 150).**

response. If this is so, chronic sialitis may be a better indication of the presence of infection than the diagnostic cytomegalic cells themselves.

Table II shows that the incidence of chronic sialitis parallels at a higher level the incidence of CMV cells.

TABLE I. NUMBER OF CYTOMEGALIC CELLS

| | 1st mo. | 2nd - 6th mo. | 7th - 12th mo. | Over 1 year |
|---|---|---|---|---|
| + | 6 | 20 | 32 | 9 |
| ++ | 1 | 9 | 7 | 2 |
| +++ | 3 | 4 | 1 | 0 |
| ++++ | 0 | 6 | 0 | 0 |

TABLE II. INCIDENCE OF CYTOMEGALIC CELLS AND CHRONIC SIALITIS

| Age group | Cases | CMV + | % Incidence | Chronic sialitis | % Incidence |
|---|---|---|---|---|---|
| Under 2 weeks | 297 | 3 | 1,0 | 31* | 9,7 |
| 2 - 4 weeks | 85 | 3 | 3,5 | 20 | 23,5 |
| 2 - 3 months | 92 | 16 | 17,3 | 31 | 33,7 |
| 4 - 5 ,, | 58 | 18 | 31,0 | 25 | 43,1 |
| 6 - 7 ,, | 62 | 22 | 35,5 | 27 | 43,4 |
| 8 - 9 ,, | 72 | 20 | 28,0 | 39 | 54,1 |
| 10 - 11 ,, | 54 | 5 | 9,3 | 21 | 39,9 |
| 1st year | 136 | 10 | 7,4 | 43 | 31,6 |
| 2nd ,, | 66 | 1 | 1,5 | 22 | 33,0 |
| 3rd ,, | 88 | 0 | 0 | 17 | 19,3 |

* Five cases with congenital syphilis excluded.

Most infections occur between the ages of 2 and 12 months. Of 321 cases in this age group 81 showed CMV cells, an incidence of 25%, but, as indicated above, the true incidence of infection may be 10 - 15% higher. This is

clearly postnatal infection, and indeed crops of cases occur, suggesting some outbreaks of infection. Over a 3-year period it seems that infection is least common in the summer months, especially January and February (Fig. 5).

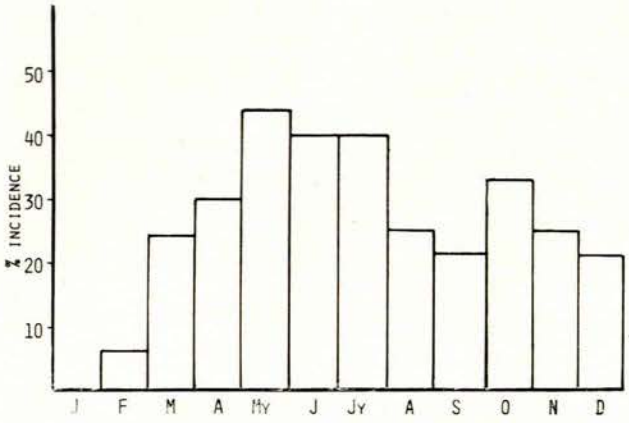


**Fig. 5. Monthly incidence CMV cells.**

It has been suggested that infection detected in the first month of life can probably be regarded as congenital,[1] either the result of infection *in utero* via the maternal blood stream, or infection acquired from the genital tract during birth. On this basis, of the 382 cases in this age group there were only 6 with congenital CMV infection.

## RETROSPECTIVE STUDY

Review of our records revealed some 15 cases of pulmonary CMV infection over the past 5 years (Table III). Salivary glands had not been examined as a routine, so that we are unable to say whether there was a concomitant sialitis in every case. Where salivary gland sections were available, in 2 cases CMV cells were not found, while in 2 further cases CMV cells were present. It is clear that these 15 cases do not reflect the true incidence of pulmonary CMV infection, since virus-containing cells may be comparatively few and confined to part of a section. A random block of lung tissue may well miss them. In examining these lungs care has to be taken to differentiate CMV cells from infection by adenovirus. Here the intranuclear inclusions are smaller and more densely basophilic, lacking a halo, and cytoplasmic inclusions are absent.

In all the above cases CMV cells were present in the lung, the cytomegalic cells either lining alveolar walls or lying free within alveoli (Fig. 6). In most cases CMV cells were not numerous. A chronic interstitial pneumonitis with plasma cells and lymphocytes was the usual reaction, but in 5 cases where CMV cells were abundant, the lung showed a haemorrhagic pneumonia with extensive necrosis.

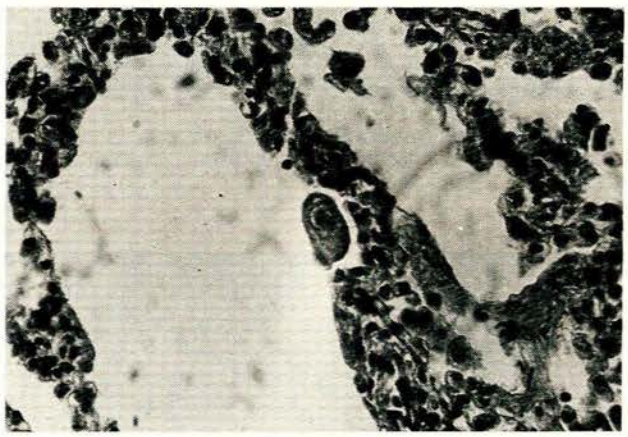

**Fig. 6. Lung, CMV cells in alveolar sac (H. and E. × 390).**

In 8 cases CMV cells were also found in the liver, mostly associated with tiny focal necroses, often with neutrophil polymorphs (Fig. 7). It is of interest that the cellular response to CMV cells in the liver was mainly polymorphs, whereas in lung and salivary gland it was lymphocytes and

**TABLE III. POSTNATAL DISSEMINATED CMV INFECTION IN KWASHIORKOR**

| Case | Age (mo.) | CMV cells | Other pathology | Liver |
|---|---|---|---|---|
| 1 | 9 | L. (Lung) | Br. pn. | Giant cell hepatitis |
| 2 | 12 | L. Liv. | Br. pn. | Cholestasis focal necrosis |
| 3 | 17 | L. Liv. | GE | Focal necrosis |
| 4 | 7 | L. Liv. | Supp. pn. | Focal necrosis |
| 5 | 36 | L. | Haem. pn. | Focal necrosis |
| 6 | 12 | L. Liv. | Br. pn. | Focal necrosis |
| 7 | 36 | L. Liv. | Br. pn. | Focal necrosis |
| 8 | 24 | L. Liv. | Haem. pn. | Giant cell + focal necrosis |
| 9 | 42 | L. | Br. pn. | Giant cell hepatitis |
| 10 | 18 | L. | Myocarditis | Fatty |
| 11 | 20 | L. Liv. Adr. | Br. pn. | Focal necrosis |
| 12 | 7 | L. Sal. | Mucormycosis | Fatty |
| 13 | 3 | L. K. | Tuberculosis | Fatty |
| 14 | 18 | L. Liv. | Br. pn. | Giant cell hepatitis |
| 15 | 10 | L. | Haem. pn. | Fatty |

plasma cells. Case 7, in addition to CMV cells and focal necrosis, also showed multinucleated parenchymal giant

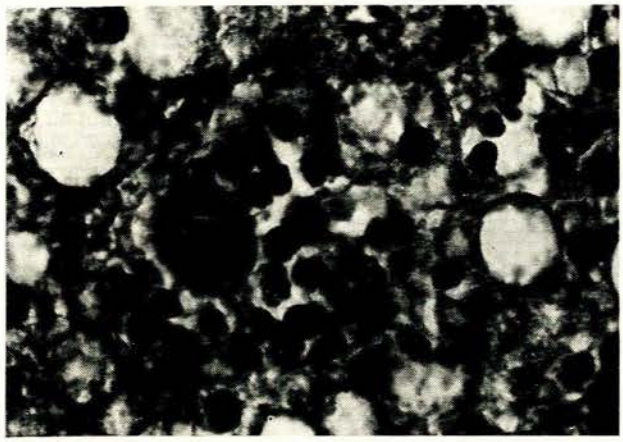

**Fig. 7. Fatty liver with focal necrosis containing CMV cells (H. and E. × 960).**

cells, a reparative process. Of livers without CMV cells 2 showed giant cell hepatitis and 1 focal necrosis alone. Thus in 11 of 15 cases there was evidence of liver involvement. It is perhaps surprising that renal and adrenal involvement was found in only 1 case.

## DISCUSSION

Much has been added to the knowledge of cytomegalic inclusion disease since Farber and Wolbach[2] observed CMV cells in the salivary glands in 12% of 183 necropsies between the ages of 2 days and 18 months. Fetterman[3] added new impetus by finding CMV inclusion cells in urinary sediment during life. The virus was isolated from the human salivary gland by Smith[4] and from adenoids by Rowe *et al.*[5] Weller *et al.*[6] unexpectedly isolated the virus in tissue culture from a liver biopsy in a case of microcephaly, thought to be caused by *Toxoplasma gondii*. This finding lent emphasis to the neurological complications of CMV infection. Further virological studies revealed that CMV could be found in urine from apparently normal newborn infants as well as older children.

A group investigation[1] in England in 1970 found 45 of 1 395 children excreting virus in the urine, the incidence of virus-excretors being 1,8% in the first 2 months, 6% 6 - 11 months, and 1,5% over 4 years of age. In schoolchildren virus excretions ranged from 3% to 24,6% in different schools, the higher levels being in the lower socio-economic groups. Serological studies after the age of 3 months, by which time transferred maternal antibody is lost, indicated that up to 12% were infected in the first year, and 18% by the time of leaving primary school. In London 7% of children were found to have acquired antibodies by 7 years of age, 21% by 15 years, and 54% of adults by 35 years.[7] We have not been able to repeat these virological studies, but our postmortem examination incidence of 25% between 2 - 11 months of age indicates a much higher postnatal infection rate in Black children than is found in England.

In the Collaborative Study[1] in England 36 cases of congenital cytomegalic infection were diagnosed, all except 2 excreting virus at the first 4 weeks of life. These cases were grouped as follows:

| | |
|---|---|
| CNS involvement ± other systems ... ... ... | 6 |
| Hepatomegaly ± jaundice and purpura ... ... | 11 |
| Marasmus and diarrhoea ... ... ... ... ... | 5 |
| Apparently normal ... ... ... ... ... ... ... | 14 |

Of the 6 cases in our survey which occurred within the first month and which may be regarded as congenital CMV infection, only 2 showed dissemination, the CMV cells being confined to the salivary gland in the other 4 cases. One disseminated case was an infant with microcephaly, microphthalmia, cleft palate and hare-lip, extra digits and atrial and ventricular septal defects (CMV cells detected in lung, liver and kidney). The other infant had diarrhoea and marasmus (CMV cells in lung and kidney). However, failure to find CMV does not entirely exclude dissemination, since virus-containing cells may be very scanty. In the above case of microcephaly the disease was missed on routine postmortem histology and only after very thorough microscopy were the characteristic cells found.

Of particular interest in the Collaborative Study is that 7 of 11 cases with hepatosplenomegaly had neonatal jaundice. This faded after weeks, or, in some cases, months, except for one case with biliary cirrhosis. Stern and Tucker[8] also described an infant with persistent jaundice from the second day in which CMV was repeatedly isolated from the urine. Biopsy and necropsy revealed biliary cirrhosis with multinucleated parenchymal cells but no CMV cells. Nor were cytomegalic cells found in any tissue, although the virus was isolated from liver, kidney and lungs. Stern and Tucker thought that it must remain problematic whether the CMV infection caused biliary cirrhosis or was superimposed fortuitously. They quote Seifert and Oehme[9] as also describing biliary cirrhosis in association with CMV infection. McCracken[10] likewise comments on reports describing 'neonatal hepatitis, hepatic fibrosis and biliary cirrhosis' with CMV infection. He himself found only mild cholangitis, giant cells, portal and interstitial fibrosis in a series of 12 infants with hepatosplenomegaly whom he followed for periods of 1 to 8 years.

In the present study 5 of 7 cases with congenital biliary cirrhosis between 2 and 6 months had CMV cells in the salivary gland, an incidence of 70% compared with the average of 23% for that age group. Another 6-day-old infant with biliary cirrhosis showed severe chronic sialitis without CMV cells. But in no instance were CMV cells found in the liver. We have found focal necrosis and giant cell hepatitis to be the usual response to postnatal CMV infection, but it is possible that infection at an early stage *in utero* might result in biliary atresia and cirrhosis. The failure to find CMV cells in the liver clearly does not exclude this possibility, but on the other hand debilitation in association with biliary atresia might increase susceptibility to postnatal infection. By comparison 2 of 6 infants with congenital fibro-elastosis and 7 of 20 cases with other forms of congenital heart disease showed CMV cells in the salivary gland, an incidence of 33%. A survey of all

neonates with other types of congenital abnormalities over a 2-year period failed to reveal any evidence of disseminated CMV disease.

In describing a patient with haemolytic anaemia who died 2 hours after birth, McEnery and Stern[11] comment that the 'remarkable feature was the localisation of cytomegalic cells to the salivary gland', and that 'such localisation is characteristic of acquired infection of early childhood, but is rarely observed under 2 months of age and does not appear to have been reported previously in the newborn'. In our series 4 of 6 cases occurring in the first month were localised to the salivary gland, 2 being 1-day-old infants. However, even in disseminated cases with pathological changes, CMV cells may be very scanty and easily missed in other organs. It seems evident that virus can be present in the body and excreted in the urine without the characteristic cytomegalic cells developing, or at least being too scanty to be detectable in tissues.

In conclusion, the common mode of postnatal infection is clearly oral, and in the majority of cases the CMV cells are localised to the salivary glands. However, dissemination to both the lungs and liver is not uncommon in children with kwashiorkor and is no doubt attributable to a lowering of cellular immunity. It is difficult to assess the importance of this dissemination as a factor in causing death, but in a few cases the lungs showed features of a severe viral pneumonia with haemorrhage and necrosis, a reaction apparently not previously attributed to CMV infection. Congenital infection may be acquired during birth by infection from the genital tract, but in disseminated cases showing gross pathology and congenital abnormalities, it clearly takes place via the maternal blood stream during the early stages of pregnancy. The possibility of congenital CMV infection being the cause of congenital biliary cirrhosis would seem to be worthy of further investigation.

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