CYTOMEGALIC INCLUSION-BODY DISEASE IN AFRICAN INFANTS

S. E. LEVIN, M.B., B.CH. (RAND), M.R.C.P. (EDIN.), D.C.H. (LOND.); and P. C. KING, M.B., B.CH. (RAND)

Department of Paediatrics, Baragwanath Hospital, and the South African Institute for Medical Research

Cytomegalic inclusion-body disease, also known as salivary-gland virus disease, is a viral infection occurring in both man and lower animals, in which characteristic morphological changes are to be found in the cells of the salivary glands and/or epithelial tissues throughout the body.

The affected cells are greatly enlarged, measuring up to 30 - 40 microns in diameter. The nucleus is similarly enlarged, containing a single acidophilic inclusion body surrounded by a clear halo and a peripheral rim of condensed chromatin material, which usually bears one or several orbital bodies. The cytoplasm is generally swollen and vacuolated, and may contain up to 15 - 20 small basophilic inclusion masses. These cellular changes were initially interpreted by Ribbert[1] and by Jesionek and Kiolemenoglou[2] as an indication of protozoal infection.

In 1921 Goodpasture and Talbot[3] coined the term 'cytomegalia infantum' to describe these characteristic giant cells in infected cases. The first suggestion that this disease might be of viral origin was made by von Glahn and Pappenheimer.[4] Further indirect evidence of the viral aetiology of the disease was supplied by Cole and Kuttner's[5] propagation of a similar disease in laboratory animals, using cell-free inoculates of the salivary-gland tissue of infected animals.

However, it was not until some 30 years later that Smith[6] and Weller *et al.*[7] independently cultured the virus in a medium of human fibroblasts. Until recently the diagnosis of the condition was made only at autopsy, but following the suggestion of Wyatt *et al.*,[8] Fetterman[9] showed that it was possible to recover the typical cytomegalic cells from the urinary sediment of an infected infant.

Epidemiology. The infection is probably endemic throughout the world, cases having been reported from the Americas, Great Britain, Europe, China,[10] Australia,[11] and South Africa.[23]

Incidence. Cytomegalic cells have been found localized to the salivary-gland tissue in 1 - 18% of all still- and live-born infants coming to autopsy, irrespective of the cause of death,[8,12] but the generalized form occurs in such patients in only 1 - 2% of cases.[8]

The following 2 case reports of generalized cytomegalic inclusion disease are believed to be the first to be described in African infants.

CASE 1

Clinical Features

This male African infant was admitted to hospital when 4½ hours old. Numerous red and purplish spots had been observed on the child immediately after delivery.

The mother had been quite well during this, her first pregnancy. Her blood Wassermann reaction was negative. She developed a conjunctivitis of the right eye 9 days before delivery.

On admission to hospital the child weighed 5 lb. 7 oz. (2,470 G.). The rectal temperature was 96°F., the pulse rate was 140 a minute, and the respirations were 50 a minute. There was marked icterus, and clinically the child appeared anaemic. There were numerous bright-red petechiae scattered all over the body, with larger purplish-blue ecchymotic areas (4 - 5 mm. in diameter) especially around the nose and mouth. Petechiae were observed in the oro- and naso-pharynx, and bilateral subconjunctival haemorrhages were present. The

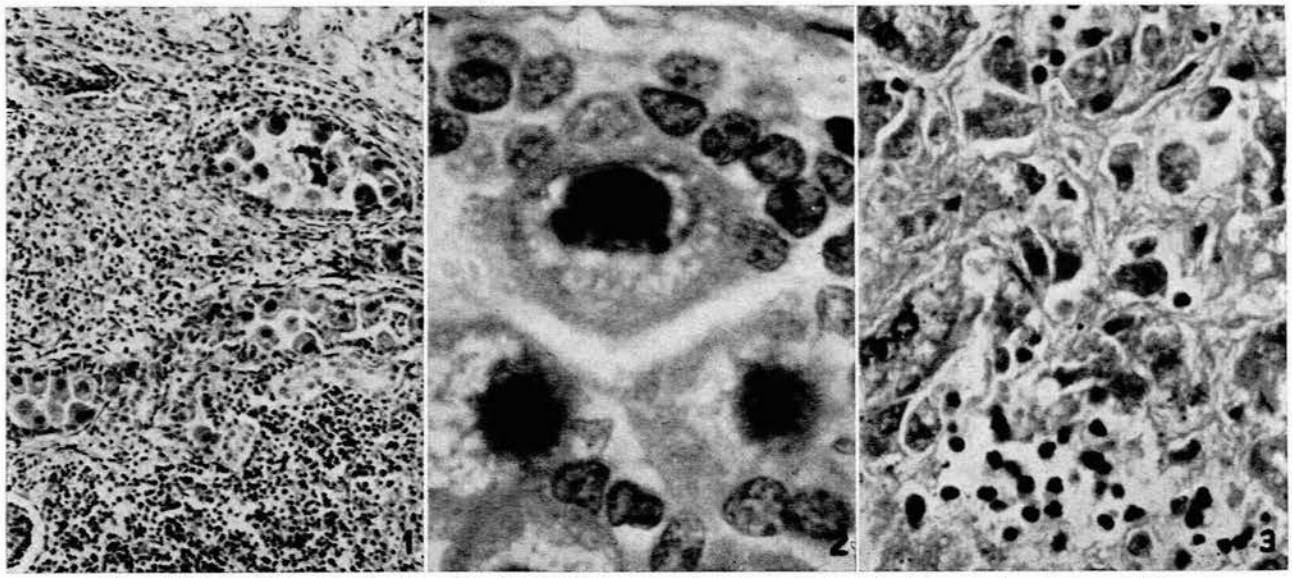

Fig. 1. Section of kidney showing large epithelial cells lining some of the convoluted tubules (haematoxylin and eosin × 120).
Fig. 2. Higher magnification of convoluted renal tubule showing typical intranuclear inclusions (haematoxylin and eosin × 1200).
Fig. 3. Section of liver showing hepatic-cell necrosis and inflammatory-cell infiltrate (haematoxylin and eosin × 480).

abdomen was moderately distended. The liver edge was palpable 5 cm. below the right costal margin, while the spleen was enlarged 4 cm. below the left costal margin.

Blood taken immediately after admission to hospital gave the following information: haemoglobin 10·6 G. per 100 ml., reticulocytes 5%, 52 nucleated erythrocytes per 100 white cells, white-cell count 12,000 per c.mm. (myelocytes 2%, metamyelocytes 9%, neutrophils 48%, lymphocytes 36%, monocytes 3%, eosinophils 1%, basophils 1%). No platelets were seen on the blood film submitted. Examination of the bone marrow showed a marked erythroid reaction, the myeloid : erythroid ratio being 1:6·5, and no megakaryocytes or platelets were seen. The prothrombin index was 31%. The serum-bilirubin level was 22·6 mg. per 100 ml., of which 15·8 mg. were direct. The mother's blood group was O Rh positive, and the infant's A Rh positive, while the infant's Coombs' test was negative. The complement fixation and Sabin-Feldman dye tests were negative for toxoplasma in both mother's and infant's blood. A single specimen of urine collected before the infant died was negative for cytomegalic cells. An exchange transfusion was carried out at 12 hours, but the infant died 46 hours after admission.

Autopsy Findings

External examination showed an intensely jaundiced African male infant with an extensive generalized purpuric eruption. Internal examination revealed marked bile-staining of the internal organs, with extensive petechial haemorrhages and ecchymoses into all the serous and mucous membranes. The lungs showed focal congestion and areas of recent intrapulmonary haemorrhage. The liver was dark-green in colour. The gallbladder and bile ducts were healthy.

The kidneys were greenish-brown in colour, and on section showed scattered areas of recent haemorrhage. The spleen was considerably enlarged and showed intense congestion. The brain weighed 300 G. and presented no abnormal features. The remaining organs showed no abnormality.

Histological Examination

Kidney. Many of the convoluted tubules were dilated and lined by large epithelial cells measuring up to 25 - 30 microns in diameter. These showed the 'owl-eye' appearance characteristically described in infections caused by the salivary-gland virus. The cytoplasm showed degenerative changes, and contained irregular granular basophilic inclusion masses (Figs. 1 and 2). The lumina of the affected tubules contained irregular masses of cellular debris and desquamated inclusion-bearing epithelial cells. A focal peritubular infiltrate of lymphocytes and occasional polymorphs was present, and was associated in some areas with interstitial haemorrhage and extramedullary haemopoiesis. Many of the tubules contained bile casts. The glomeruli and blood vessels appeared healthy.

The liver showed extensive necrosis of the parenchymal cells with distortion of the normal lobular architecture and proliferation of the interstitial fibrous tissue. Marked bile stasis was evident. The portal tracts were heavily infiltrated by acute and chronic inflammatory cells. Occasional multinucleated giant cells were also noted, together with scattered foci of polymorph infiltration and extramedullary haemopoiesis (Fig. 3). A few of the bile-duct epithelial cells showed cytomegaly and contained intranuclear and intracytoplasmic inclusions, similar to those noted in the kidney. None of the hepatic parenchymal or Kupffer cells showed evidence of cytomegalic changes.

Section of a *salivary gland* showed inclusion-bearing cells in the glandular acini.

Thyroid gland. A few of the follicular epithelial cells showed typical cytomegalic changes.

The lungs were congested and showed the presence of occasional foci of extramedullary haemopoiesis. No evidence of cytomegalic change was found in either the alveolar lining cells or the bronchial epithelium. Pneumocystis carinii was not observed.

The spleen was congested and showed marked extramedullary haemopoiesis.

Sections of *bone marrow* failed to show any evidence of megakaryocytes. The remaining organs were congested and showed foci of interstitial haemorrhage and extramedullary haemopoiesis, but no evidence of cytomegaly. Cytomegalic virus was not isolated in tissue cultures from specimens of liver, spleen, kidney and brain.

CASE 2

Clinical Features

This male African infant, aged 7 weeks, was admitted to hospital with a history of having failed to thrive since birth. In addition, vomiting and diarrhoea had developed a week before admission. The stools were blood-stained for 2 days during treatment in the outpatient department. Twitching movements of the arms were noted on the morning of admission, together with rapid respiration and inability to suck.

The pregnancy was complicated by pre-eclampsia, but the delivery was normal. The birth weight was not known, but the

mother thought that the child was losing weight. The child's weight in the outpatient department had been 4 lb. 14 oz. and this had fallen to 4 lb. 8 oz. on admission to the ward 5 days later. The rectal temperature was 104°F. and the child was dehydrated, apathetic and wasted. There was thrush of the tongue and crepitations were audible at the base of the right lung.

Blood-electrolyte estimations revealed the presence of a hypertonic dehydration (sodium 150 mEq./l., chlorides 116 mEq./l., potassium 4·3 mEq./l., carbon-dioxide content 7·9 mEq./l., urea 84 mg. per 100 ml.). The cerebrospinal fluid obtained at lumbar puncture contained no cells and the protein content was 24 mg. per 100 ml. Two rectal swabs yielded no pathogenic organisms on culture.

A blood count showed a haemoglobin level of 9·2 G. per 100 ml. on the day following admission; there were 3% reticulocytes and there were 4 normoblasts per 100 white cells. The white-cell count was 18,800 per c.mm. (neutrophils 36%, lymphocytes 62%, monocytes 2%). Platelets appeared to be present in adequate numbers. The dehydration was corrected with intravenous-fluid therapy; the thrush was treated with oral 'nystatin', 50,000 units 6 hourly, while the pneumonia was controlled by intramuscular injections of penicillin.

The child's general condition had improved greatly 1 week after admission when the blood electrolytes had returned to normal. He then passed a stool which contained altered blood and which was positive to the benzidine test. The haemoglobin level fell to 5·8 G. per 100 ml. on the 9th hospital day. The reticulocytes were then 9% (7 normoblasts per 100 white blood cells) and the platelets remained adequate. A divergent squint was observed. An enlarged spleen was palpable 1 finger below the left costal margin.

The child's weight had risen to 5 lb. 4 oz. by the 15th hospital day, while the haemoglobin had risen to 7·5 G. per 100 ml., with a reticulocyte level of 20%. Oral thrush reappeared; this was again treated with nystatin and an oral iron mixture was also prescribed. Shortly thereafter he started vomiting some of his feeds, the weight fell and he died suddenly 17 days after admission.

Autopsy Findings

External examination showed a wasted African male infant with marked pallor of the skin and mucous membranes.

Examination of the organs showed pulmonary congestion and pallor of the kidneys, but no other significant macroscopic abnormality.

Histological Examination

The lungs were congested and showed signs of early bronchopneumonia, but there was no evidence of cytomegaly or of pneumocystis carinii.

The liver and *spleen* showed numerous foci of extramedullary haemopoiesis. Sections of the *small bowel* showed no significant pathological changes. The *brain* showed congestion and isolated foci of calcification in the deeper layers of the cortex.

Kidney. Typical cytomegalic cells were seen in a few of the convoluted and collecting tubules, which were irregularly dilated and surrounded by mononuclear-cell infiltration.

Specimens of the *salivary glands* were unfortunately not removed for histological examination.

DISCUSSION

The clinical and pathological features of the generalized form of cytomegalic inclusion disease have been adequately reviewed by Wyatt *et al.*[8] and Medearis.[13] Case 1 illustrates the most commonly recognized form of the disease, namely that which occurs in the neonatal period, transmission presumably occurring transplacentally from the mother to the foetus, some time after the 3rd - 4th month of pregnancy. The affected infant is usually prematurely born, becomes jaundiced soon after birth, and has hepatosplenomegaly, purpura, thrombocytopenia and anaemia. All these features were present in case 1. In addition there may be involvement of the central nervous system. A meningo-encephalitis may be found with or without periventricular calcification, this calcification being detectable on radiological examination of the skull during life.

Apart from the salivary gland, the liver is the organ most commonly affected and shows degenerative changes ranging from fatty change through extensive necrosis to evidence of early cirrhosis. Inclusions may be found within the parenchymal cells, Kupffer cells and bile-duct epithelium. The kidney usually shows cytomegalic changes in the renal tubular epithelium with an associated peritubular inflammatory process. If the lungs are involved, cytomegalic changes are found in the alveolar septal lining cells and bronchial epithelium together with an interstitial pneumonia. Seifert and Oehme[14] have reported pneumocystis carinii in a high proportion of their patients with lung involvement. In many cases the pancreas, thyroid and brain show histological involvement, inclusion-bearing cells being rare in the intestine, ovary, pituitary and thymus. Extensive extramedullary haemopoiesis is almost an invariable accompaniment of the disease process.

A second group of infants commonly affected are those falling into the age group between 1 and 4 months. These infants may present with a chronic unresponsive pneumonia accompanied by gastro-intestinal disturbances with or without hepatosplenomegaly; the disease may also show itself as a failure to thrive.[13,14] Our second case would appear to fall within this group.

Cytomegalic inclusion disease may also be found in association with pertussis and chronic debilitating illnesses, e.g. fibrocystic disease of the pancreas and primary neoplastic diseases of the reticulo-endothelial system.[13,15] Generalized salivary-gland virus disease is extremely rare in adults.[16]

Previously, the diagnosis of cytomegalic inclusion disease was only established by postmortem examination. Increasing numbers of cases diagnosed during life have been reported, in which cytomegalic cells were recovered from centrifuged urine, cerebrospinal fluid, bronchial and/or gastric washings.[9,17-20,23]

With the introduction of recent virological techniques, salivary-gland virus has been recovered in tissue culture from the urine and liver-biopsy specimens in infected patients.[7,21,23] From these studies has emerged the concept of a group of 'cytomegalo-viruses', since the cytomegalic and salivary-gland viruses isolated from such patients appear to be antigenically heterogeneous agents.[15]

PROGRESS AND TREATMENT

Until recently the neonatal form of salivary-gland virus disease was thought to be invariably fatal. However, Weller *et al.*[22] have followed the progress of 12 infants for periods of up to 40 months of age. Mental retardation and microcephaly were frequent sequelae. It is of interest to note that chorioretinitis and cerebral calcification were found in some of the patients. In 6 of these patients a viruria persisted into the 2nd and 3rd year of life. The value of certain therapeutic measures, e.g. corticosteroids, gammaglobulin, exchange transfusions and 'terramycin', in the treatment of salivary-gland virus disease is doubtful, since survival has been reported in many untreated patients.[17,18,21]

SUMMARY

1. The history, and the clinical and pathological features of salivary-gland virus disease are briefly reviewed.

2. Two cases of generalized salivary-gland virus disease in African infants are reported. They are believed to be the first recorded instances of this disease in Africans.

3. Methods of diagnosis and treatment, and the prognosis of the condition are discussed.

We thank the Superintendent of Baragwanath Hospital for permission to publish, Drs. M. Shnier and S. Wayburne for use of case records, Dr. E. Kahn for valuable criticism, and the Director of the South African Institute for Medical Research for facilities granted. Mr. M. Ulrich produced the photomicrographs.

REFERENCES

1. Ribbert, H. (1882): Virchows Arch. path. Anat., **88,** 11 (quoted in *op. cit.*[14]).
2. Jesionek, A. and Kiolemenoglou, B. (1904): Münch. med. Wschr., **51,** 1905.
3. Goodpasture, E. W. and Talbot, F. B. (1921): Amer. J. Dis. Child., **21,** 415.
4. Von Glahn, W. C. and Pappenheimer, A. M. (1925): Amer. J. Path., **1,** 445.
5. Cole, R. and Kuttner, A. G. (1926): J. Exp. Med., **44,** 855.
6. Smith, M. G. (1956): Proc. Soc. Exp. Biol. (N.Y.), **92,** 424.
7. Weller, T. H., Macauley, J. C., Craig, J. M. and Wirth, P. (1957): *Ibid.*, **94,** 4.
8. Wyatt, J. P., Saxton, J., Lee, R. S. and Pinkerton, H. (1950): J. Pediat., **36,** 271.
9. Fetterman, G. H. (1952): Amer. J. Clin. Path., **22,** 424.
10. Nelson, J. S. and Wyatt, J. P. (1959): Medicine (Baltimore), **38,** 223.
11. Swain, I. H. F. and Osmond, R. (1959): Med. J. Aust., **2,** 647.
12. Bodian, M. (1960): Quoted by Vannier, T. (1960): Thorax, **15,** 259.
13. Medearis, D. N. jnr. (1957): Pediatrics, **19,** 467.
14. Seifert, G. and Oehme, J. (1958): Germ. Med. Monthly, **3,** 25.
15. Hanshaw, J. B. and Weller, T. H. (1961): J. Pediat., **58,** 305.
16. Symmers, W. St. C. (1960): J. Clin. Path., **13,** 1.
17. Margileth, A. M. (1955): Pediatrics, **15,** 270.
18. Birdsong, M., Smith, D. E., Mitchell, F. N. and Corey, J. H. jnr. (1956): J. Amer. Med. Assoc., **162,** 1305.
19. McElfresh, A. E. and Arey, J. B. (1957): J. Pediat., **51,** 146.
20. Blanc, W. A. (1957): Amer. J. Clin. Path., **28,** 46.
21. Kluge, R. C., Wicksman, R. S. and Weller, T. H. (1960): Pediatrics, **25,** 35.
22. Weller, T. H., Hanshaw, J. B. and Scott, D. E. (1960): Amer. J. Dis. Child., **100,** 469.
23. Gear, J., le Roux, A. F., Kessel, I. and Sichel, R. (1962): S. Afr. Med. J., **36,** 8.