J Clin Pathol 1980; **33**: 11-18

Adult pulmonary cytomegalic inclusion disease: report of a case

JD BROWNING*, IAR MORE, AND JF BOYD

From the Department of Pathology, Glasgow University and Western Infirmary, Glasgow G11 6NT, UK

SUMMARY A case is presented of pulmonary cytomegalic inclusion disease in an adult. The condition was only diagnosed post mortem. A detailed description of the inclusions and inclusion-bearing cells is given. Histochemical observations which reveal an outer and inner zone to the intranuclear inclusion body are confirmed. Electron microscopy shows the viral basis of the infection. The morphology of the virus particles is compatible with a herpesvirus infection.

In recent years, a number of reports have appeared on cytomegalovirus (CMV) infection in adults. The patients are usually secondarily immune deficient or renal transplant patients receiving immunosuppressive therapy,[1] diabetic,[2] or have received massive transfusions of fresh blood.[3] In this article we report a patient who was not known to be suffering from any of the above conditions but who experienced a very prolonged, complex, and ultimately fatal set of catastrophes after a minor urological operation. In the course of this six-week illness, 35 units of blood were transfused but none was less than five days old. It is likely that the length and severity of the illness created a situation equivalent to immune paralysis[4] and that this situation permitted the development of pulmonary cytomegalovirus inclusion disease (CID) in a hitherto healthy man. The diagnosis was not considered during life or at necropsy and became apparent only on histological study of the tissues.

Case report

Two years before his terminal admission a 62-year-old man complained of dribbling incontinence due to a simple bladder papilloma which was treated by diathermy. Follow-up included repeated cystoscopic examinations which were negative until his final admission when three small tumours were found; these were treated by diathermy. Twenty-four hours postoperatively he developed peritonism. This was treated conservatively with bladder drainage and antibiotic therapy. On the next day he collapsed. Peritonitis was diagnosed, and at laparotomy faecal peritonitis and two perforations were found, one of the posterior wall of the bladder and one of a loop of ileum. The bladder perforation was repaired and the loop of ileum was resected. Intensive supportive measures were given, including intermittent positive pressure ventilation by tracheostomy, and antibiotic therapy was continued. Over the next three weeks the patient's cardiorespiratory function caused serious concern but later improved only to be followed by left and then bilateral bronchopneumonia. Acute renal failure occurred and dialysis was required. Further, the wound dehisced, a fistula developed, and he was taken back to theatre where complete separation of the small bowel anastomosis, two further bladder vault perforations, and an abscess in the small bowel mesentery were found. After further resection of ileum an ileostomy was created. One bladder perforation was closed and the other was utilised for drainage. The mesenteric abscess was drained. Histological examination of the resected ileum showed an area of recent through-and-through necrosis with perforation and diffuse serosal reactions elsewhere with oedema and haemorrhage. No evidence was noted then, or on re-evaluation, of CID. Intensive therapy and renal dialysis were continued, but despite these measures his condition deteriorated and he died 10 days later with clinical and radiological signs of widespread bronchopneumonia.

Extensive bacteriological surveillance was carried out during the six weeks' terminal illness. *Aspergillus fumigatus* was cultured from tracheal aspirates on several occasions after the first laparotomy and was accompanied by *Pseudomonas pyocyanea* imme-

*Present address: Haematology Department, Law Hospital, Carluke, Lanarkshire ML8 5ER, UK.

Received for publication 3 September 1979

diately before the second laparotomy and in several later specimens. Urine samples yielded a mixed flora of coliforms and *Candida albicans* in significant numbers on several occasions after the diathermy procedure. The peritoneal contents draining after the first laparotomy yielded coliforms, *Ps. aeruginosa*, *Proteus* species, and *C. albicans*. Wound swabs taken after the second laparotomy yielded coliforms and *C. albicans* at regular intervals. *Proteus* species was cultured from the pus of the mesenteric abscess drained at the second laparotomy and also from one blood culture taken four days later. Numerous blood cultures before this had yielded no growth.

The patient's peripheral blood white cell count rose shortly after the first laparotomy and remained elevated until one week before death. Atypical lymphocytes were not seen in any specimen. The platelet count remained normal until one week before death. Both counts fell progressively until death, and bleeding occurred terminally from several orifices and into the skin. At no time were significant coagulation abnormalities demonstrated.

POSTMORTEM FINDINGS

External examination showed an extensive purpuric rash, a tracheostomy, arterial shunts on the right wrist and ankle, a gaping right lower paramedian abdominal wound from which pus exuded, and an ileostomy opening in the right iliac fossa.

Internally, there was diffuse loculated suppurative peritonitis but the alimentary tract showed no further perforations. Each kidney was twice the normal weight and was pale. The right renal pelvis contained clotted blood, but the left one and both ureters were normal. The bladder was inflamed, and there were four diathermy ulcers of the mucosa. The spleen was three times normal size, and dissection showed a very soft pulp.

In the thorax, extensive fibrinous pleural adhesions were found. The trachea below the tracheostomy was inflamed with multiple small 5 mm diameter ulcers. The bronchi were also inflamed, and the lungs were five to six times heavier than normal with widespread consolidation and small abscess formations in the left upper lobe. The heart was normal in size and showed posterior myocardial fibrosis with dilatation of the right ventricle and tricuspid valve. Both coronary arteries were moderately atheromatous, and both main branches of the left one showed complete occlusion. The brain was dissected after fixation and showed a deeply situated 1 cm diameter necrotic area in the white matter of the right frontal lobe.

General histological findings

Both lungs showed suppurative bronchopneumonia and abscess formations with organisation in the alveoli and interstitially. In areas, starch granulomata with florid foreign body giant cell formations indicated aspiration. Scanty isolated large cells with features characteristic of CID located most frequently in areas undergoing organisation were present (Fig. 1). No inclusion-bearing cells (IBC) were found in the bronchi or bronchioles, but rarely such a cell was found either lying free within a blood vessel or constituting part of the endothelial layer in larger blood vessels. No evidence of co-existing *Pneumocystis carinii* or *Toxoplasma gondii* infection was found. The heart showed myocardial fibrosis.

The urinary bladder showed ulceration at the site of fulguration. The surface of one slough was rich in mycelial filaments, and in the haemorrhagic submucosa a solitary intracapillary binuclear CID cell of uncertain identity was seen (Fig. 2). Both kidneys showed recent tubular necrosis at the stage of repair and focal low-grade papillitis with granulomata, and the left kidney showed acute papillitis with abscesses rich in aspergillus fungal filaments.

No CID cells were seen in the brain, pituitary, heart, liver, biliary system, pancreas, adrenal glands, or kidneys, although all organs showed individual

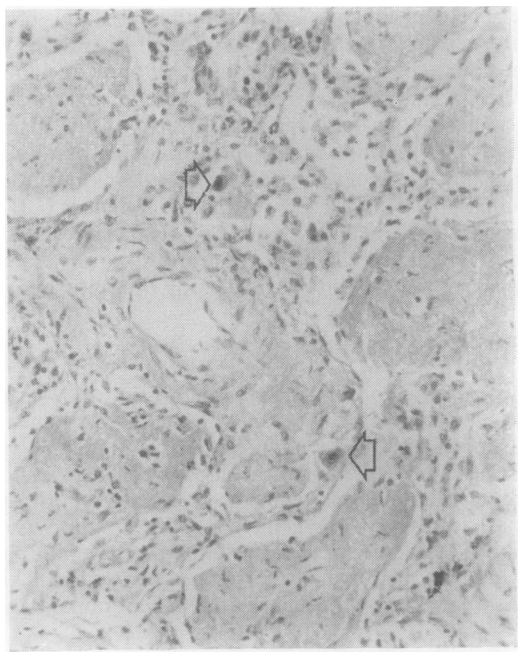

Fig. 1 *Area of organising pneumonia with scanty cytomegalic cells showing two characteristic haematoxyphilic cytomegalovirus intranuclear inclusion bodies (arrows). Haematoxylin and eosin × 150.*

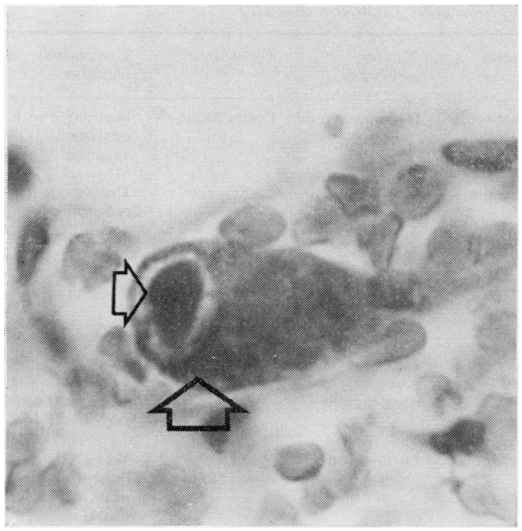

Fig. 2 *A solitary inclusion-bearing cell lies in a distended capillary of urinary bladder mucosa. An enlarged nucleus shows a central haematoxyphilic intranuclear inclusion body (small arrow) with unstained halo. A second nucleus bearing no inclusion body lies inferior to the first, to the right, and on a different plane of focus (large arrow). The cytoplasm on the right of the cell bears ill-defined granular inclusion bodies. H and E × 1500.*

cytomegalic cells without inclusion bodies. No evidence of neoplasia was found.

Cytological features of inclusion-bearing cells

In the lungs, with Mayer's haemalum and eosin stain, the cells were about twice as large as their neighbours, and the cell nuclei were also enlarged. When a nucleolus was present, this was usually eosinophilic with a haematoxyphilic rim and may be against the nuclear membrane or included within the intranuclear inclusion body (IIB). The IIB occupied the centre of the nucleus as a rule and was frankly haematoxyphilic. Numerous tiny, mostly uniform, homogeneous, refractile, eosinophilic granules were dispersed throughout the IIB, which was surrounded by an unstained 'halo' of irregular thickness. The 'halo' may be artefact or may represent unstained nucleoplasm and may contain small portions of nuclear chromatin. Most of the heterochromatin was situated peripherally in the nucleus. The cytoplasmic inclusion bodies (CIB) were easily overlooked and were not present in every cell showing a nuclear inclusion body. Each body was ill-defined and vacuolated but stained weakly with haemalum by comparison with the nuclear staining. Rarely, the affected cell was binuclear (Fig. 3) when, more often than not, only one nucleus showed an inclusion body. Multinuclear giant cells were absent. The CID cells were solitary, and seldom were more than two affected cells seen adjacent to each other. However, it was not uncommon to see cytomegalic cells without inclusions in apposition to the ones with inclusion bodies.

Histochemical features of pulmonary cytomegalic inclusion-bearing cells

Sections of lung have been stained by a variety of methods (see Table) based on those of Pearse.[5] All sections have been examined using a 2 mm (1/12 in) oil immersion objective, and 50 CID cells have been scrutinised and their details documented. A wide range of reactions to these procedures was found.

Although unaffected cells gave the expected result with the Feulgen method, namely, that polymerised (or condensed) deoxynucleic acid (DNA) stained red, the staining of the IIB showed that most have distinct inner and outer zones, the former staining red

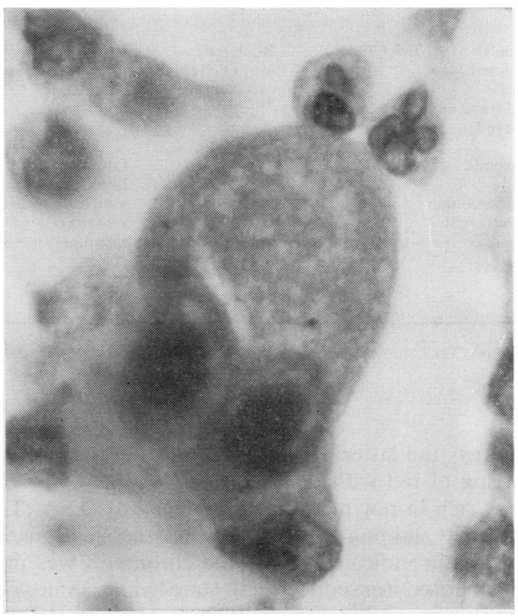

Fig. 3 *A pulmonary binuclear inclusion-bearing cell showing inclusions in both nuclei. This is uncommon in this case. The vacuolated cytoplasmic inclusions are well shown even although they are ill defined from the rim of normal cytoplasm. Two neutrophil polymorphs give an indication of the cell size. An adjacent cell (below, right) is cytomegalic when compared with the other cubical alveolar epithelial cells (top left) but is devoid of nuclear and cytoplasmic inclusions. H and E × 1500.*

Table *Histochemistry of pulmonary inclusion-bearing cells in cytomegalic inclusion disease*

Method	*Nucleolus*	*Intranuclear inclusion*	*Halo*	*Chromatin*	*Nuclear membrane*	*Cytoplasm*	*Cytoplasmic inclusion*
Mayer's haemalum and eosin	Eosinophilic centrally; haematoxyphilic peripherally	Haematoxyphilic with numerous pink refractile vacuoles	Unstained	Haematoxyphilic	Haematoxyphilic	Eosinophilic	Basophilic with unstained vacuoles
Feulgen/ Light green	Green centrally; red peripherally	Red granular or homogeneous centrally; deep blue or slate blue peripherally; unstained vacuoles in either area	Unstained	Red granular; green occasionally; both often	Red; green occasionally	Light green	Unstained; red grains occasionally
Methyl green/ Pyronin	Pink centrally; green peripherally	Green granular refractile centrally; pink homogeneous peripherally	Unstained	Green or pink or both	Pink often; green occasionally	Pink	Unstained; vacuoles; green grains occasionally
Periodic acid Schiff/ Haemalum	Orthochromic blue peripherally	Orthochromic blue; many tiny unstained vacuoles	Unstained	Orthochromic blue	Orthochromic blue	Pale blue* or purple	Weak red grains, larger granules or reticulum; unstained vacuoles and red-lined vacuoles
Alcian blue/ Chlorantine fast red	Brick red	Brick red; unstained small vacuoles	Unstained	Brick red or very pale blue	Brick red or deep blue	Delicate blue* or very pale brown	Deep blue grains, larger granules or blue reticulum; unstained vacuoles and some show blue lining
Hale's dialysed ron/ Chlorantine fast red	Brick red	Brick red; unstained small vacuoles	Unstained	Brick red	Brick red	Mostly brown*; blue areas occasionally	Deep blue grains, larger granules, reticulum, blue-lined vacuoles or unstained vacuoles
Chromic acid-methenamine silver/Light green	Green	Light green stippled centrally; darker green homogeneous peripherally	Green homogeneous with/without unstained large vacuoles	Green	Green	Green*	Delicate black grains, larger black granules, black reticulum, black-lined vacuoles or unstained vacuoles

*See text for comments on cytoplasmic membranes.

whereas the latter showed a blue colour due to the mixing of red with the green counterstain. The IIB were rich in polymerised (or condensed) DNA. The variable staining result seen in the marginated chromatin indicated that some chromatin was in a polymerised (or condensed) state while some was depolymerised (or dispersed). No unstained IIB was seen. Most CIB were unstained, but rarely a delicate Feulgen-positive peppering was seen.

The results with methyl green/pyronin staining confirmed the deductions made from the Feulgen reaction results. Unaffected cells stained conventionally, that is, polymerised DNA stained green whereas ribonucleic acid (RNA) and depolymerised DNA stained red. The CIB were mostly unstained. Rarely, a cell showed pale green grains in the CIB.

The other four staining methods stain specific components of the CIB rather than of the IIB. These methods produced a rather confusing picture in that some cells with no inclusion bodies gave similar cytoplasmic staining. Further, the inhaled starch grains stained strongly with these methods, and when the grains were intracellular some imitated nuclei with IIB, a marked halo, and specifically stained marginated chromatin. This confusion was compounded partly by the existence of a normal nucleus in these macrophages because, as explained above, the genuine binuclear CID cells commonly showed one affected nucleus and one unaffected one. It was confirmed, however, that these four methods did not stain IIB. Additionally, however, these four methods stained the cytoplasmic membranes of affected and

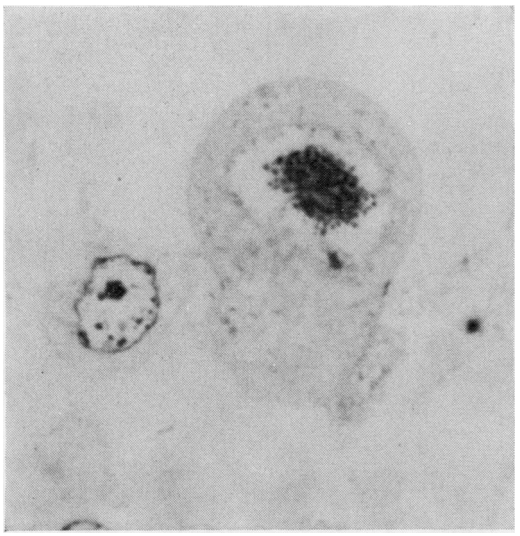

Fig. 4 *Ultrathin preparation of Araldite-embedded lung tissue showing deeply stained central intranuclear inclusion body with stippling, unstained halo, poorly stained chromatin marginated on nuclear membrane, portion of a deeply stained nucleolus below (seen better on different plane of focus), and ill-defined cytoplasmic inclusion bodies inferiorly. Note adjacent cytomegalic cell on the left without viral inclusions. Toluidine blue* ×*1500.*

unaffected cells to varying degrees. Occasionally the whole circumference was stained, at other times only varying lengths of free cell membrane. Some cytoplasmic membranes were completely smooth, others showed a beaded appearance, yet others showed a serrated pattern, others blunted spikes, and some membranes showed a frank spiky or microvillous pattern. These features were unseen on haematoxylin and eosin staining, and their significance is uncertain.

Electron microscopic features

Blocks from the formalin-fixed postmortem material were used for making the electron microscopic preparations with all the inherent problems of fixation artefact which that entails. Both thick and thin Araldite-embedded sections revaled CID cells (Figs 4 and 5). Study showed both precursor and fully formed virus particles. The latter had typical icosahedral symmetry, and both intact and disrupted virus capsids were seen. The precursor groups were seen mainly in the nucleus, while complete virus particles occurred in both nucleus and cytoplasm. Although complete particles occurred both singly and in clusters within the nucleus, they were present as only single particles in the cytoplasm. However, no section incorporated a genuine cytoplasmic inclusion body. The average diameter of complete virus particles measures 120 ± 10 nm, which, along with the morphology and distribution, is compatible with the human herpesvirus group to which cytomegalovirus belongs.

Discussion

Recent literature[6-8] points to the increasing incidence of CMV infection in association with debilitating systemic disease, increasing usage of broad-spectrum antibiotics, powerful chemotherapeutic agents, and steroids. In view of this, the course and severity of this patient's illness would make the development of CMV infection a strong possibility. Progressive infections by *Ps. pyocyanea*, *C. albicans*, and *Aspergillus* species, organisms of low pathogenicity, suggest that his resistance to infection was seriously depleted. A high index of clinical suspicion is necessary to make an antemortem or postmortem diagnosis, and the difficulties of determining whether or not infection is primary or reactivation of latent disease have been referred to by Craighead.[8] Both fresh primary infection and reactivation can arise in the presence of complement-fixing (CF) antibody, and immunosuppressed patients may not develop CF antibodies in relation to a primary infection.[1,4] Thus diagnosis during life will depend upon a combination of virus culture of tissue and/or excreta together with histological study of biopsy material. We are not certain what role this viral infection had in contributing to the patient's death.

It is now well known that CMV infection can be transmitted by transfusion of blood taken less than 34 hours previously from the donor.[3] We consider that this possible route of transmission is very unlikely in this patient because all units of blood were more than 120 hours old when transfused, and atypical lymphocytes were never noted during haematological screening.

In 1960, Symmers[9] wrote that this virus infection was very rare in adults, but Wong and Warner[6] stated that the condition occurs commonly as a terminal complication of other serious systemic disease. Five of their 14 cases were diagnosed during life, and the lungs were affected most frequently. Frequent lung involvement was also reported by Evans and Williams.[7] Symmers[9] and Wong and Warner[6] have emphasised multi-organ involvement in adults, the colon, thyroid gland, liver, and pancreas being involved in descending order of frequency. We consider that the difference between the first, and the second and third references above reflects the shift towards a more active approach to treatment of serious illnesses. Thus, seriously ill patients are kept alive longer even although some

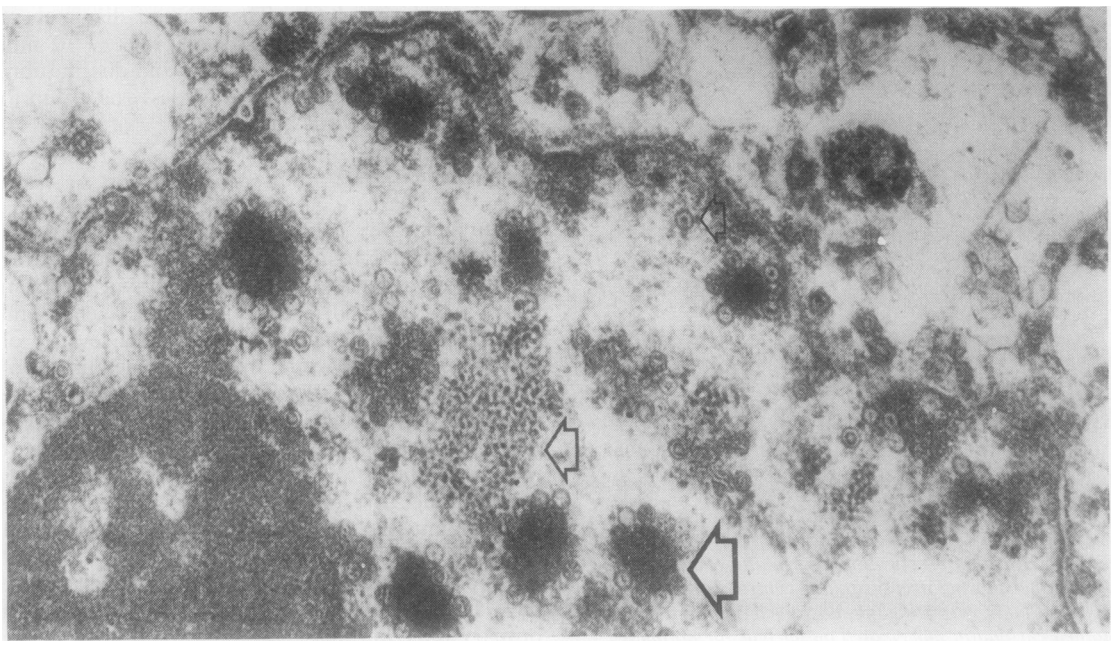

Fig. 5 *Electron micrograph of part of a pulmonary cytomegalovirus inclusion-bearing cell showing large open nucleolus at left-hand bottom corner, numerous areas of cytomegalovirus production (large arrow at one), and one area of presumed viral precursor material (medium arrow). More mature virus particles can be seen abutting the wavy nuclear membrane. Mature particles are present (top centre) in the cytoplasm. The smallest arrow identifies one of several hexagonal icosahedral virus particles. Uranyl acetate/lead citrate.* ×30 000.

may ultimately succumb. In addition, evolution of the more complete necropsy, including the histological study of macroscopically normal tissue, has contributed. Smith *et al.*[10] reported that 44 (8·8%) of 502 unselected necropsy cases yielded CMV by virus isolation from lung and kidney specimens, and virus isolation procedures were roughly six times more sensitive than conventional histology. On the other hand, we draw attention to the occurrence in many organs of cytomegalic cells without inclusion-body changes (Fig. 3). No one knows what interpretation to place on finding these cells, which are not uncommonly present in one or more organs of west of Scotland patients. Others have also made reference to cytomegaly without inclusions[7,11-13] and if the recording of such cells were to be included in any histological assessment, correlation with the results of virus isolation might become closer. Rapp *et al.*[14] showed that CMV has a long latent period of four days in human embryo lung tissue cultures, and if this observation has relevance for the natural human situation, the not uncommon finding of cytomegaly without inclusion bodies could be an indicator of infection by CMV. To add weight to the suggestion that these cells may be relevant to current CMV infection, it is noted that they also occasionally bear PAS-positive CIB, but the morphology of these bodies tends to differ from the classical CIB of CMV. Refrigerator storage of cadavers can have a deleterious effect on the success of virus isolation post mortem. This feature was investigated initially by Krugman and Goodheart,[15] and, recently, Macasaet *et al.*[16] have shown that virus isolation rates are best from fresh tissue. Abdallah *et al.*[17] reported 16 patients with pulmonary CMV infection where other pathogens including *Pneumocystis carinii* were present in 73%. Our patient showed no evidence of *Pneumocystis carinii* infection but had *Ps. pyocyanea*. After studying more than 400 CID cells we were not able to find coincident infection with *T. gondii*, as described by McGavran and Smith.[18]

There are many descriptions in the literature of the IIB and CIB of CMV infection. Not all of these agree, and the review in this case showed that there is variability in morphology and staining characteristics from cell to cell in the same patient. This is likely to be due to the inclusion bodies being at different stages of evolution. In this article the

majority results have been reported. In the lungs the alveolar lining cells are most often affected, and only rarely are bronchiolar mucosal cells involved. In the classical description of congenital CID by Goodpasture and Talbot,[19] infected cells were reported in bronchial veins and lung capillaries, and Wong and Warner[6] noted CID cells in vascular endothelium, as we have in this case. This finding tends to be forgotten. Craighead[8] reviewed CMV pulmonary disease, and his earlier reports record inclusion bodies in the endothelium, pneumocytes and macrophages of affected lungs of humans and of experimentally infected mice. Most affected cells are macrophages, and some features in mouse-infected lungs cause him to suggest that centrifugal spread from the original foci tends to occur. A similar possibility arises in our case. Tegtmeyer and Craighead[20] noted that mouse peritoneal macrophages infected by cytomegalovirus lost their capacity for phagocytosis. If this observation can be applied to human macrophages, then the ones showing both viral inclusion bodies and starch granules must have ingested the starch granules before they became infected with the virus. We agree with Wyatt *et al.*[13] that the description of the CIB by Cappell and McFarlane[12] has not been improved on. Wyatt *et al.*[13] seem to be the only authors to have noted differences between the core and the periphery of IIB, which our results confirm. This difference is difficult to interpret. We suggest that the observations by Kurnick[21] and Pearse[5] may be relevant here, namely, that the Feulgen method stains polymerised (or condensed) DNA best and depolymerised (or dispersed) DNA least or not at all, and that pyronin stains red not only the RNA of nucleoli and cytoplasm but also depolymerised (or dispersed) DNA while methyl green is readily bound by polymerised (or condensed) DNA. Generally, however, our histochemical results for both IIB and CIB agree with those of Heard *et al.*,[2] Martin and Kurtz,[22] and Evans and Williams.[7] The second authors concluded that the CIB contained acid and neutral mucopolysaccharide, with which we agree, but we would emphasise that there is great variability both in morphology and in staining response from inclusion to inclusion within the same cell as well as from cell to cell. While Martin and Kurtz[22] adapted the methenamine silver technique to electron microscopy and reported differences in the results between the periodic acid and chromic acid procedures, we support these observations at the light microscopy level. We are uncertain how to interpret the positive results that we found with the special staining procedures both in the cytoplasm of cells with no nuclear changes and also the morphological changes in the cell membranes. Very recently, some workers have had success with immunoperoxidase staining of infected tissue cultures, and Gerna *et al.*[23] reported that the reaction product was specific for the IIB but non-specific for the CIB. We have had no opportunity to adapt the immunoperoxidase method to this case.

As noted by earlier authors, we confirm that CMV has no great propensity for causing cell fusion with resulting multinuclear giant cells, which is a characteristic feature of some other human herpesviruses, for example, *H. simplex* and varicella/zoster viruses, and in this respect CMV resembles the Epstein-Barr virus. The present case is rather unusual since most binuclear CID cells in the lungs and in the bladder submucosa show only one affected nucleus. Involvement of both nuclei by IIB does occur (Fig. 3) as is also illustrated in Fig. 25 of Symmers[9] but is a minor feature in the present case. The CMV binuclear IB cells arise more frequently from fusion of two cells than from mitosis without cytoplasmic separation in a virus-infected mononuclear cell. Indeed, Craighead[8] postulates that the virus-infected cells are large because they are unable to undergo reduction division.

Many possibilities exist about the relation between the solitary CID cell in the bladder submucosa, its proximity to the site of the bladder tumours, its relation to the trauma of the recent fulguration procedure, its possible relationship to renal and urinary tract CID of the neonatal period, of infancy, and childhood, and the potential for this virus to undergo latency with reactivation in later life during adverse clinical circumstances. Answers cannot be offered to these questions.

Any remarks on the ultrastructural findings must be cautious in view of the poor fixation. The number of virus-infected cells studied has been very small. Several of the infected cells show cytoplasmic features compatible with being either pneumocytes or macrophages. Changes similar to those noted by Kanich and Craighead[24,25] have been identified, namely, attachment of capsids and nucleocapsids to the inner nuclear membrane; excurvation was difficult to determine because of potential nuclear pyknosis and fixation artefact. Vesicles bearing capsids and nucleocapsids were also seen against the nuclear membrane. Unfortunately, none of the preparations yielded cytoplasmic inclusions to allow search for the five types described in tissue culture preparations by these workers, or to confirm the results of Luse and Smith.[26] The existence of an electron-dense network in the nuclear inclusions, as reported by McAllister *et al.*,[27] McGavran and Smith,[18] and Ruebner *et al.*,[28] was confirmed, but because of autolytic and other changes, we would be hesitant in confirming the observation of Kanich and Craighead[29] that there is structurally

altered chromatin intimately associated with the developing virions.

We thank our clinical colleague for granting permission to report this case. We also thank Mr E McWilliams, FIMLS, for carrying out the histochemical tests on the lung tissue and for preparing four of the photomicrographs, and Mr D McSeveney, FIMLS, for help with the ultrastructural studies and for Figure 5. Mrs L Gilmour prepared the manuscript, and we also express our indebtedness to the MacMillan Fund of Glasgow University.

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Requests for reprints to: Dr JF Boyd, Brownlee Laboratory, Ruchill Hospital, Glasgow G20 9NB, UK.