Use of antisera to epithelial membrane antigen in the cyto diagnosis of malignancy in serous effusions

A TO,† DULCIE V COLEMAN* and D DEARNALEY, M ORMEROD,‡ K STEEL, AM NEVILLE *(Department of Pathology, St Mary's Hospital Medical School, Praed Street, London W2) and (Ludwig Institute for Cancer Research, and Institute for Cancer Research, Royal Marsden Hospital, Sutton, Surrey) The cytod iagnosis of malignancy in serous effusions commonly depends upon the ability of the cytopathologist to distinguish between stained non-malignant and malignant cells in the light microscope. When the malignant cells are small and characterised byisonucleosis, they are often morphologically indistinguishable from the actively proliferating mesothelial cells. This frequently results in diagnostic difficulty leading to diagnostic error. Using antisera to the Epithelial Membrane Antigen (EMA), we have applied an indirect immuno-alkaline phosphatase technique to 95% alcohol-fixed smears prepared from serous effusions in an attempt to determine whether this technique can be used to discriminate between mesothelial cells and malignant cells of epithelial origin in effusions. A total of 121 effusions from 96 patients have been investigated. We present in this paper the results of our investigation of the specificity and the sensitivity of the EMA staining in detecting cancer cells in serous effusions and our evaluation of its role in complementing the conventional morphological diagnosis of malignancy in serous effusions.

Epithelial membrane antigen: distribution and potential uses

D DEARNALEY,*, J SLOANE,‡ MG ORMEROD,‡ K STEEL,‡ AM NEVILLE* *(Ludwig Institute for Cancer Research (London Branch), Unit of Human Cancer Biology, The Royal Marsden Hospital, Sutton, Surrey) and † Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey) An epithelial membrane antigen (EMA) has been demonstrated by immunohistochemical methods on formalin-fixed, paraffin-embedded sections of human tissue, using an antiserum raised against human milk fat globule membranes (Heyderman et al., J Clin Pathol 1979;32: 35-9). This antigen is confined to, but widely distributed in epithelial tissues and tumours derived from them. Single carcinoma cells in infiltrates or metastases usually contained high concentrations of EMA, the antigen frequently being expressed in the cytoplasm as well as on cell membranes.

Applications of EMA:
1 Diagnostic tumour histopathology. The recognition of epithelial origin of anaplastic or spindle cellled tumours may be aided. The identification of minute metastatic deposits of mammary carcinoma in paraffin-embedded sections of bone marrow and skin biopsies is facilitated.
2 Diagnostic cytopathology. Techniques have been developed to prepare aspirates from human bone marrow and serous effusions for immunocytochemical staining with EMA. Its value in detecting bone marrow metastases from mammary carcinoma will be discussed. The application of EMA to cytological diagnosis of serous effusions is the subject of a further communication.
3 Cell separation. Rosetting techniques and fluorescent cell sorting are being investigated, using antiser to EMA, as a means of labelling and separating human carcinoma cells from stromal elements.

Osteoclast ultrastructure in Paget's disease (ii) its role in primary diagnosis

L HARVEY,* T GRAY,† G MACEACHERN,‡ D DOUGLAS,‡ JA KANIS,‡ RGG RUSSELL† *(Departments of Pathology and Human Metabolism and †Clinical Biochemistry, The Medical School, Beech Hill Road, Sheffield S10 2RX, and the ‡Princess Elizabeth Orthopaedic Hospital, Exeter) We have examined the ultrastructure of osteoclasts from various bone biopsies of nine patients with histological, biochemical, radiological and clinical features of Paget's disease. Electron microscopy showed the presence of complex tubulofilamentous inclusions forming whorled bundles or stacked parallel rows confined to the osteoclast nuclei. The individual filaments varied from 12-16 nm in diameter. These inclusions were present in 10-60% of the nuclei examined and occupied from 15-50% of the nuclear area. Several biopsies also contained similarly structured discrete cytoplasmic inclusions or haphazardly arranged aggregates of individual filaments or both. Such inclusions were absent from all other cell types present. Their numbers varied with the severity and activity of the disease, as judged by histological criteria and their size and appearance are suggestive of a paramyxovirus origin, possibly measles. These observations corroborate those previously reported, adding further support to the proposed viral aetiology of Paget's disease. Similar inclusions have been observed in some non-skeletal disorders of proven viral aetiology but other techniques are required to establish this conclusively for Paget's disease.

Osteoclast ultrastructure in Paget's disease (i) corroborative observations for a viral aetiology

L HARVEY,* T GRAY,* JA KANIS,† RGG RUSSELL† *(Departments of Pathology and Human Metabolism and †Clinical Biochemistry, The Medical School, Beech Hill Road, Sheffield S10 2RX) We have examined the ultrastructure of osteoclasts from various bone lesions referred to this department for initial diagnosis or review within the last 15 years. The electron microscopic specimens consisted of reprocessed, araldite re-embedded tissue retrieved from the formalin-fixed, decalcified and paraffin wax-embedded routine histology preparations. We have considered the pres-
ence of paramyxovirus type inclusion bodies (reported by five independent centres) as a diagnostic marker for Paget's disease (the specificity studies will be reported at a future date). Using this criterion five cases initially referred with a range of clinical diagnoses were re-designated as Paget's disease, resulting in the therapeutic use of diphosphonates in one instance. These five cases had various histological, biochemical, radiological or clinical anomalies, inconsistent with a diagnosis of Paget's; however further information received after the electron microscopic reappraisal supported the diagnosis in all five. This is the first known report attributing diagnostic significance to the osteoclastic inclusions. We therefore suggest electron microscopy as a standard procedure in the interpretation of all bone lesions with non-diagnostic histological or clinical features. We would emphasise the facility for the retrospective diagnosis of such lesions by the reprocessing for electron microscopy of tissue retrieved from standard histology "blocks."

Listeriosis in an obstetric hospital

J DE LOUVOS, ROSALINDE HURLEY (Queen Charlotte's Hospital for Women, London) Human listeriosis is believed by many to be more prevalent than ever, yet reported cases are still few considering the generally increased awareness of the condition. Recent evidence suggests that it is no longer a disease confined to the very young or the aged.

We report four cases from the obstetric practice of Queen Charlotte's Hospital over the last five years, ranging from the postmortem diagnosis of granulomatosis infante septica through neonatal and maternal bacteremia to carriage by mother and neonate without signs or symptoms of disease. A further case of listeric infection involving the placenta was suspected on the basis of a positive fluorescent antibody test but was not confirmed. Five of 30 sera from patients who had three or more abortions or miscarriages contained specific antibody to L monocytogenes, indicating previous infection with this organism. The need for further investigation to establish the role of the organism in repeated human abortion is emphasised.

Mean platelet volume—a step forward in haematology

C GILES (Central Pathology Laboratory, Hartshill Road, Stoke-on-Trent) The Coulter Model S-Plus counter provides not only a platelet count but also the mean platelet volume in routine blood specimens. An analysis of 5000 unselected specimens has shown that between platelet counts of 50 and 900 x 10^9/l there is an inverse relation between the count and the mean platelet volume (MPV), which falls steadily as the platelet count rises.

In over 94% of specimens from 1011 normal adults the platelet count ranged from 150 to 450 x 10^9/l and the MPV from 7-0 to 10-5 fl. This "normal" range was compared with other categories of cases.

In children aged from 6 months to 12 years the platelet count tended to be higher and the MPV smaller than in adult controls. Whilst in 1987 normal pregnant women the platelet pattern resembled that of non-pregnant adults, women with pre-eclamptic hypertension showed a trend towards fewer and larger platelets; in fully developed pre-eclampsia more than half the patients had abnormally large platelets or thrombocytopenia.

A relative or absolute thrombocytosis was found after trauma and blood loss, in iron deficiency anaemia and rheumatoid arthritis. No consistent pattern of platelet distribution was evident in patients with infection, chronic renal failure and treated malignant disease.

Effects of an aminoglycoside antibiotic on the guinea pig inner ear

I WRIGHT, RT RAMSDEN, D PAGE, WB OSWALD (Departments of Pathology and Otolaryngology, University of Manchester, Manchester M13 9PT) Engstrom elaborated Retzius' technique of examination of the osmium-fixed neuroepithelium of the inner ear and labyrinth, and refined it by the use of phase-contrast microscopy. This provided a method for assessment of cell damage or absence, and study of differing neural pathways. Guinea pigs were used to demonstrate toxicity of aminoglycoside antibiotics and diuretics. The postmortem human cochlea lacks the honeycomblike regularity of the guinea pig, and there are few studies of its pattern in disease.

Scanning electron microscopy of the whole guinea pig cochlea has been shown by Engstrom and others and provides more information than examination of selected portions by phase contrast. Human material also can be examined by this technique, yielding information on various patterns of degeneration and congenital malformation.

We present a study of toxic effects of gentamicin on aminoglycoside antibiotic on the cochlea, using phase-contrast microscopy and scanning electron microscopy.

Peliosis hepatis: case report

GB SINGH (Department of Pathology, General Hospital, Hexham, Northumberland) An unusual case of peliosis hepatis is reported. The patient had a history of lymph node enlargement in the neck, which had been treated with antituberculous therapy. It proved, however, to be due to metastatic poorly differentiated carcinoma. The origin was not traced. Treatment with radiotherapy and chemotherapy was instituted as well as Anapolon (oxythromethane). The patient died with abdominal haemorrhage due to rupture of a necrotic liver.

Carcinoma cuniculatum: a clinicopathological study of 19 cases

PH MCKEE, JD WILKINSON, MM BLACK (St Thomas's Hospital, London) Carcinoma cuniculatum is an unusual neoplasm which usually arises on the sole of the foot. Only 29 cases have been described since Aird first drew attention to this condition in 1954. We wish to report another 19 cases collected over 27 years by the late Dr I Whimster, at St Thomas's Hospital, London. All but two of these cases were in men and in 17 the age at diagnosis was greater than 40 years. The commonest site was the foot, usually the sole, but three patients had lesions elsewhere—one on the wrist, one on the finger, and one on the knee. The histology of Carcinoma cuniculatum is discussed and indirect evidence for the role of wart virus in the pathogenesis of this condition is presented.

Serum methionine and valine concentrations on nitrous oxide anaesthesia: a preliminary report

TE PARRY, JA BLACKMORE, BARBARA ROBERTS (Departments of Haematology, Llandough Hospital, Penarth, South Glamorgan Area Health Authority (Teaching) and Anaesthetics, University Hospital of Wales, Cardiff) The serum concentrations of two amino acids, methionine and valine, are known to undergo significant changes in opposite directions in pernicious
anaemia under treatment, the former rising and the latter falling. Any inactivation of vitamin B₁₂ would be expected to produce the reverse changes. Using this as a criterion, evidence for vitamin B₁₂ inactivation by nitrous oxide has been sought by assaying the two amino acids (microbiologically) at intervals in control venous line blood samples of 24 patients undergoing nitrous oxide anaesthesia. The duration of anaesthesia varied between 2 and 7½ h with a mean of 3½ h. In all cases premedication and other anaesthetic agents, together with muscle relaxants, were administered as well. The paired t test showed a highly significant difference between the two amino acid concentrations with values of p < 0.025 at 1 h; p < 0.001 at 2, 3, and 4 h; p < 0.05 at 5 h; p < 0.005 at 6 and again between 7-8 h; and p < 0.05 at 9-10 h.

Analysis of the anaesthetic records of 18 of the 24 patients showed that about 17 drugs were administered in all, but nitrous oxide and oxygen were the only agents received by all 18 cases. Thiopentone was received by 17 and halothane by 13. The single case not receiving thiopentone and the 5 not receiving halothane produced similar methionine and valine curves to the remainder. The remaining drugs could be similarly excluded. Five drugs were received by fewer than four patients and could not therefore be incriminated.

These changes are the reverse of those produced by vitamin B₁₂ in pernicious anaemia in relapse and are consistent with the inactivation of vitamin B₁₂ by nitrous oxide. This is apparent within the hour of the commencement of the anaesthetic.