

The Association of Clinical Pathologists: 106th Scientific Meeting

The 106th scientific meeting was held at the Royal Society of Medicine, London 9-10 October 1980. Abstracts of the scientific communications follow.

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

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*(*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 cytodiagnosis of malignancy
in serous effusions currently depends upon
the ability of the cytopathologist to dis-
tinguish between stained non-malignant
and malignant cells in the light micro-
scope. When the malignant cells are small
and characterised by isonucleosis, they
are often morphologically indistinguish-
able from the actively proliferating meso-
thelial 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 tech-
nique 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 in-
vestigated. We present in this paper the
results of our investigation of the speci-
ficity and the sensitivity of the EMA
staining in detecting cancer cells in serous
effusions and our evaluation of its role in
complementing the conventional morpho-
logical diagnosis of malignancy in serous
effusions.

Epithelial membrane antigen: distribution and potential uses

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Marsden Hospital, Sutton, Surrey) An
epithelial membrane antigen (EMA) has
been demonstrated by immunohisto-
chemical 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 ex-
pressed 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 celled tumours may
be aided. The identification of minute
metastatic deposits of mammary carcino-
ma 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 stain-
ing with EMA. Its value in detecting bone
marrow metastases from mammary car-
cinoma will be discussed. The application
of EMA to cytological diagnosis of serous
effusions is the subject of a further com-
munication.

3 Cell separation. Rosetting techniques
and fluorescent cell sorting are being in-
vestigated, using antisera to EMA, as a
means of labelling and separating human
carcinoma cells from stromal elements.

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

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RUSSELL† *(*Departments of Pathology
and Human Metabolism and †Clinical
Biochemistry, The Medical School, Beech
Hill Road, Sheffield S10 2RX*) We have
examined the 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 com-
plex 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 aggre-
gates 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 ob-
served in some non-skeletal disorders of
proven viral aetiology but other tech-
niques are required to establish this
conclusively for Paget's disease.

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

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Elizabeth Orthopaedic Hospital, Exeter*)
We have examined the ultrastructure of
osteoclasts from various bone lesions re-
ferred to this department* for initial
diagnosis or review within the last 10
years. The electron microscopic specimens
consisted of reprocessed, araldite resin
embedded tissue retrieved from the
formalin-fixed, decalcified and paraffin-
wax-embedded routine histology prepa-
rations. We have considered the pre-

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 LOUVOIS, 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 granulomatous infantisepsis through neonatal and maternal bacteraemia 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 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 × 10⁹/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 × 10⁹/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 1087 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 anti-tuberculous 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 (oxymetholone). 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 serial amino acid concentrations were expressed as a percentage of the preanaesthetic level. There was a 9% fall in the serum methionine concentration within the first hour

($p < 0.001$) and a 34% drop at 6 h ($p < 0.001$). The level was still 17.5% below the preanaesthetic level between 25 and 26 from the start of the anaesthetic ($p < 0.005$). The lowest recorded fall in an individual case was 82% at 14 h. The serum valine concentrations remained unchanged for the first 3 h but were raised thereafter, but only significantly so at 4 and 6 h with a rise of 12.7% ($p < 0.025$) and 36.3% ($p < 0.05$) respectively. 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. Fewer 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.