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# The Association of Clinical Pathologists: 97th general meeting

The 97th general meeting was held at the University of Surrey, Guildford from 22 to 24 September 1976. Abstracts of the scientific communications follow.

## Localisation of surface and core antigen in the livers of hepatitis B antigen carriers

E. TAPP, D. M. JONES, AND I. W. DYMOCK (*Department of Pathology, Withington Hospital, Manchester 20*) The histological appearance of the liver in 40 asymptomatic $HB_sAg$ carriers, 20 of whom have had repeat biopsies over 3-5 years, has been correlated with the presence and distribution of surface ($HB_sAg$) and core ($HB_cAg$) antigen in the hepatic parenchyma. The purpose of this study has been to investigate the relationships between the continuing presence of virus antigen and the degree of liver damage.

Although the liver tissue was examined by electron microscopy and orcein staining, the immunoperoxidase technique was found to be superior for the demonstration of both core and surface antigen.

$HB_cAg$ staining in the nuclei of hepatocytes was found in carriers who had the more severe degrees of liver disease. On the other hand, the degree of surface antigen staining was greatest in the biopsies showing minimal lesions.

A higher incidence of progression of liver disease was found in the second liver biopsy from those carriers whose first biopsy has shown large amounts of $HB_sAg$ in the cells.

It is concluded that immunoperoxidase staining for $HB_sAg$ and $HB_cAg$ in the liver is useful in assessing the likelihood of progression of the disease in these asymptomatic carriers.

## Evaluation of the macrophage migration inhibition test in halothane induced hepatitis (halothane MIF test)

C. D. PRICE, A. R. GIBBS, AND W. JONES WILLIAMS (*Welsh National School of Medicine, Cardiff*) Many of the clinical and pathological features of halothane-induced hepatitis suggest that hypersensitivity plays a role in the pathogenesis. We have looked for evidence of delayed hypersensitivity by the use of a halothane-induced MIF test.

Lymphocytes from four of five patients who developed hepatocellular jaundice after repeated halothane anaesthesia showed a positive halothane MIF test. The fifth patient was on steroids.

Our control subjects (46) included: halothane anaesthesia without jaundice (22); obstructive and hepatocellular jaundice (9); healthy anaesthetists (5); and normal subjects without halothane exposure (10). All were halothane MIF negative.

Our results demonstrate the possible value of the halothane MIF test—as supporting a hypersensitivity factor in the pathogenesis of halothane hepatitis, as a diagnostic aid, and for the detection of patients susceptible to halothane-induced hepatitis.

## Case reports

J. L. BRENNAN (*Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry*) (1) Osteosarcoma of the vertebral body in a woman aged 22: The sudden onset of paraplegia in a previously healthy young woman was considered as possible Pott's disease, eosinophilic granuloma, or an unusual secondary tumour in such a young subject. The diagnosis was, in fact, osteosarcoma—a very rare tumour in this site unless it is preceded by Paget's disease or irradiation.

(2) Heterotopic ossification in a laryngeal haematoma: A necropsy was performed on a woman aged 34, who had died while on holiday with her husband and children on a caravan site. She had had attacks of dyspnoea treated by a local general practitioner who was not satisfied as to their origin and informed the coroner when she died. Her husband claimed that she had fallen about 25 feet down a slope but later admitted to having beaten her on at least two occasions recently.

Necropsy showed both old and recent rib fractures, various soft tissue injuries, and bilateral haemothoraces. There was a haematoma of the left vocal cord, to which her breathlessness was ascribed but this was unusually hard and histologically contained areas of osteoid in granulation tissue as well as fresh blood. At his trial the husband admitted to having struck her in the throat at least twice.

The case is interesting medicolegally, and pathologically as being an unusual site for soft tissue ossification.

## Undifferentiated carcinoma of the large intestine

N. M. GIBBS (*St. Luke's Hospital, Guildford*) Undifferentiated carcinoma of the large intestine is a rare tumour and accounts for about 0·9% of carcinomas of the large intestine. The neoplasm has the following characteristics:

1 It forms a large, ulcerated, 'barrel-shaped' tumour which does not obstruct the lumen of the bowel.

2 The tumour has a delineated border which is related to the good prognosis after surgical excision.

3 Microscopically the carcinoma is composed of sheets of cells with vesicular nuclei showing minimal pleomorphism.

It is concluded that undifferentiated carcinoma of the large intestine is a variant of adenocarcinoma and must be distinguished from poorly differentiated adenocarcinoma and malignant carcinoid tumour of the colon.

## Influence of viability on canine allograft heart valve structure and function

D. J. WHEATLEY AND C. G. A. MCGREGOR (*The Victoria Infirmary, Glasgow*) A comparative study was undertaken in

dogs of the survival of viable and non-viable allotransplanted cardiac valves which had been sterilised by antibiotic and stored. Fourteen viable and 12 non-viable valves were assessed after periods of up to eight weeks' implantation. Assessment of valve structure was made by measuring leaflet surface areas and by histological study. Pressure measurements were made across the allografted valve both at insertion and at removal. The valves which had been viable at the time of transplantation were found to undergo severe leaflet distortion and shrinkage with consequent loss of function. Non-viable valves, in contrast, showed minimal alteration in valve dimensions, and normal function was maintained. It is concluded that these findings have considerable implications in the preparation and clinical use of allograft heart valves.

**Biochemistry of catecholamine-secreting tumours: recent developments**

LINDSAY M. NELSON, M. W. WEG, AND M. SANDLER (*Queen Charlotte's Maternity Hospital, London*) A long-term study of the biochemistry of a range of catecholamine-secreting tumours has been embarked upon in the hope that pointers will emerge to a more precise classification and prognosis. Although the course of neuroblastoma is particularly difficult to predict, good prognosis may often be associated with:

1 the child being under the age of 1 year at diagnosis;
2 the tumour being partially differentiated;
3 urinary catecholamines and metabolites returning to normal after treatment;
4 urinary homovanillic acid excretion being low; and
5 no bone marrow involvement.

Even when children have a very similar history, urinary catecholamine and metabolite excretion patterns may show great variation. Using the techniques of gas chromatography and gas chromatography-mass spectrometry, we are looking for new compounds or abnormal concentrations of known compounds which might prove to be better indicators of survival than the parameters which have so far been employed.

A differential gas chromatographic urinary metadrenaline assay has been developed, which enables normetadrenaline, metadrenaline, and 3-methoxytryamine to be individually measured. The assay is particularly useful in patients with phaeochromocytoma where the type of catecholamine secreted can be monitored with greater precision than by measuring catecholamine output alone. In neuroblastoma, the urinary output of total metadrenalines (normetadrenaline plus metadrenaline) is commonly normal or raised only equivocally despite a comparatively vast urinary output of oxidatively deaminated metabolites. In the past it has not been feasible, because of technical limitations, to identify tumours associated with overproduction of trace amines (eg, phenylethanolamine, octopamine, tryamine) should they exist; latter-day methodology, however, has made it possible to measure a wide range of monoamines, their precursors, and metabolites, and the clinical benefit accruing is likely to be immense.

**Radioimmunoassay of methotrexate and its clinical application**

G. W. AHERNE, E. PIALL, V. MARKS, S. SCHACKMAN[1], G. MOULD[1], AND W. F. WHITE[1] (*Department of Biochemistry, University of Surrey, Guildford, Surrey and St. Luke's Hospital, Guildford, Surrey*[1]) The importance of measuring the concentration of methotrexate in biological fluids during treatment not only to predict the onset of toxic symptoms but also to ensure that therapeutic levels are being attained has recently been emphasised. Radioimmunoassay offers an ideal technique for measuring methotrexate in such situations.

A radioimmunoassay for methotrexate has been developed which is exquisitely sensitive (450 pg/ml). The assay uses an antiserum to methotrexate which was raised in a sheep against an ovalbumin-methotrexate conjugate prepared using a water-soluble carbodiimide. Tritiated methotrexate was used as a label and phase separation was achieved with dextran-coated charcoal. Naturally occurring folates and folinic acid (Leucovorin) did not interfere with the measurement of methotrexate.

The disappearance of methotrexate from blood of patients being treated with the drug has been followed. Results from 12 patients, each of whom received a single intravenous dose of methotrexate (200-500 mg), confirm that the drug is rapidly distributed throughout the body. The blood clearance curve of injected methotrexate could be resolved into three phases with half-lives of $15{\cdot}0 \pm 5{\cdot}4$ min, $130 \pm 260$ min, and $5{\cdot}9 \pm 3{\cdot}4$ h respectively.

Because of the high doses of methotrexate now used in treatment, the incidence of toxic symptoms may increase unless blood levels are monitored and appropriate 'rescue' steps taken. Evaluation of the pharmacokinetics of methotrexate in patients with poor renal function or slow elimination phases is also important.

**Coagulation lysis factors as markers of endothelial damage**

L. N. MCF. HARGREAVES AND A. S. TODD (*Department of Pathology, University of Dundee*) We have previously shown that factor VIII protein ($FVIII_{RA}$), like plasminogen activator (PA), is greatly increased in the blood after death, suggesting that these proteins are released as a result of endothelial damage. Measurement of $FVIII_{RA}$ have been made on routine samples from patients with acute illness and these seem to show a correlation with the likelihood of circulatory damage.

**Auto-immune haemolytic anaemia complicating infectious mononucleosis in a patient with hereditary elliptocytosis**

D. O. HO-YEN (*Ninewells Hospital, Dundee, Scotland*) A girl aged 12 years, previously in good health, presented with a two-week history of sore throat, malaise, and jaundice. She had clinical and serological evidence of infectious mononucleosis. Haematological investigations showed a leucopenia with numerous 'glandular fever cells', evidence of haemolysis, and elliptical red cells. The direct Coombs' test was positive with anti-complement but negative with anti-IgG. An IgM auto-antibody of anti-i specificity was identified, which reacted maximally at 4°C. Agglutination occurred to a titre of $\frac{1}{2}$ at 21°C with the patient's cells and $\frac{1}{4}$ at 37°C with cord cells. Significant erythrophagocytosis, maximal after chilling, was demonstrated *in vitro*. The patient's father is not anaemic, but his erythrocytes are elliptical; they show the same agglutinability by anti-i as normal circular adult red cells.

It is suggested that even though the anti-i is of cold type with low thermal amplitude, there is enough binding of complement in the peripheral vessels to promote erythrophagocytosis. The patient recovered spontaneously; her red cells are still elliptical.

**Peripheral blood films produced by the haemotrace—a preliminary evaluation**

STUART ROATH (*Department of Haematology, Faculty of Medicine, University of Southampton*) The haemotrace is a semi-automated device capable of producing a narrow stained blood film on plastic tape. This can then be examined for morphology by a suitable optical system which is part of the device. The system uses blood from a standard sequestrene container and consists of (1) a 'laying' unit which provides the initial blood trace. The correct aliquot is automatically laid on moving tape where a specially designed graphion forms the trace. Each trace is numbered; (2) an automatic staining unit through which the tape proceeds; and (3) a viewing stage in which the stained trace is transferred. This uses a conventional microscope of research grade where the film is fed 'tape recorder style' across the microscope stage. This unit is fully motorised and the movement of the film is governed by a remote control box.

Films acceptable for red cell, white cell and platelet morphology appear to be obtainable using this system. The advantages include (1) a separable laying unit for making traces in, for instance, a ward or collecting laboratory while the sample is still fresh; (2) ease of storage (and recall) via the plastic tape; (3) the cost of the tape is considerably less than that of glass slides; and (4) staining is done completely automatically using very small amounts of stain.

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## Notes