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SYNOPSES OF PAPERS

Papers and posters presented at the meeting have not been refereed or reviewed by the Journal of Pathology.

Symposium:

Immunocytochemistry in Diagnostic Histopathology

TECHNIQUES AND LOGISTICS OF ROUTINE SERVICES

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Factors affecting the outcome of immunocytochemical staining for any given substance will be discussed. These include not only the specificity and affinity of the antibody used and the way it is rendered visible, but also the choice of appropriate methods of fixing and processing the specimen to produce minimum diffusion and elution and maximum preservation and availability of antigen for combination with antibody. The indirect immunoperoxidase or PAP techniques give much useful diagnostic information with paraffin sections of formalin fixed material, and with the help of new antisera and improved labelling techniques such sections may in future be adequate for most diagnostic purposes. In the meantime, if the full diagnostic potential of biopsy material is to be realised, it is the prime responsibility of the routine hospital pathologist to ensure that snap frozen blocks of unfixed tissue are prepared in appropriate cases (e.g. lymphomas) to anticipate the need to study antigens like cell surface immunoglobulin which are not readily demonstrable following formalin fixation and paraffin embedding. For rapid reporting and professional training and satisfaction immunocytochemical tests should be performed in District General Hospitals, but provision should be made to refer unstained material elsewhere for tests which are unusual or whose interpretation requires special expertise.

IMMUNOCYTOCHEMICAL DIAGNOSIS OF ANAPLASTIC TUMOURS

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Histopathologists are regularly confronted by biopsies which clearly show a malignant neoplasm, but in which the cellular origin of the tumour is not evident (e.g. the
differential diagnosis is between anaplastic carcinoma and high grade lymphoma). This problem has been exacerbated by the increasing use of endoscopic techniques, which often yield small fragments of tissue suffering from crushing artefacts. In order to resolve these difficulties, we have made use of a small panel of monoclonal antibodies directed against leucocyte- and epithelium-associated antigens. This work was initially performed on cryostat sections, but it has recently been possible to obtain antibodies of these specificities suitable for use on routinely processed paraffin embedded tissue. In an unselected series of more than 90 problem biopsies (all of which had been referred because of diagnostic uncertainty) it was possible to reach a diagnosis following immunohistological labelling in all cases. Surprisingly, the majority of tumours proved to be of lymphoid origin, suggesting that such neoplasms may be more frequent than is generally recognised. It is also of interest that only very few of the remaining non-lymphoid tumours were of non-epithelial origin (e.g. melanoma).

LYMPHOID PROLIFERATIONS
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Three fundamental questions must be answered whenever malignant lymphoma is suspected. The histopathologist must firstly determine whether the changes are the result of a benign or neoplastic proliferation of cells; secondly, a decision must be made as to whether the tumour is of lymphoid or non-lymphoid origin; thirdly, if the tumour is of lymphoid origin the cytogenetic origin of the tumour must be determined as precisely as possible so that the appropriate diagnosis and treatment can be given. Immunocytochemistry is essential if satisfactory answers are to be provided. The results from three separate studies will be described in which a variety of monoclonal and polyclonal antibodies have been used in indirect immunoperoxidase and immunalkaline phosphatase techniques. These studies have confirmed the usefulness of the monoclonal antibody technique in establishing a primary diagnosis of malignant lymphoma and preliminary data indicate that certain marker phenotypes may be associated with a better response to treatment. Successful phenotyping of lymphomas in trephine bone marrow preparations is also possible. Techniques will be described which permit the phenotyping of lymphoid cells in paraffin sections.

SKIN DISORDERS
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Immunocytochemistry in dermatology has evolved from the early use of the immunofluorescence method as a guide to the diagnosis of the blistering disorders and lupus erythematosus subsets to a refined and semi-quantitative technique. Current studies concentrate on the use of the immunoperoxidase method and interest is concentrated on the surface markers of cutaneous lymphoid infiltrates, in situ markers for the phagocyte/histiocyte series, markers of the Langerhans cell, in situ demonstration of keratins, demonstration of tumour-related antigens on malignant melanoma, and markers of vascular and lymphatic endothelial cells. The majority of the antibodies used are non-reactive in paraffin-processed and formalin-fixed tissue, but a useful subset (CALLA, NKI C3, S100, CAM 5.2 and antibodies to a\_antitrypsin and antichymotryp-
sin) do react on conventionally processed material. Such a panel is of value in the identification of the likely cell of origin of an anaplastic tumour mass. Using these techniques in mycosis fungoides it has been established that cutaneous lymphoid infiltrates bear the surface markers of the T helper subset and that Langerhans cells are numerically increased in this condition. A panel of anti-melanoma monoclonal antibodies is of value in diagnosis and there are indications that certain melanoma-related antibodies are of prognostic value. The presence of class I and class II histocompatibility antigens in tumour cells also appears to be of prognostic significance. The use of antibodies to laminin and factor VIII is of value in differentiating between channels of vascular or lymphatic origin.

RENAL DISEASE

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Immunocytochemistry is now well established as part of the diagnostic armamentarium of the renal pathologist. Problems may arise however when the immune results are read by one person and the light and electron microscopy by others. In the same way that 'all is not gold that glitters' so 'all immunocytochemical results are not immune deposits'. It is advisable to report the immunofluorescence results not only in terms of positive or negative but also in terms of their particular distribution which should be consistent with the light microscopical and electron microscopical changes. A variety of papers have been written extolling the virtues of immunoperoxidase techniques over immunofluorescence methods mainly based on the fact that the immunoperoxidase reaction product can be located more accurately within the glomerular tuft. However it is obviously essential that the basic technique should be reliable and consistent and this should be the first priority. Many of the standard patterns of linear staining and membranous nephropathy are well recognised. Similarly the pattern given by staining mesangial deposits is readily recognisable. However the patterns obtained in mesangio-capillary glomerulonephritis may be much more difficult to interpret and frequently lead to confusion. More recently it has been possible to use monoclonal antibodies to identify the structural antigens of the glomerular basement membrane such as Goodpasture antigen. It is of particular significance that this antigen is absent or shows reduced staining in Alport's syndrome thereby providing a further aid to diagnosis in this important inherited renal disease.

ROLE IN CYTOPATHOLOGY

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The development of immunochemical and hybridoma techniques which has led to a growing number of highly specific monoclonal antibodies has an obvious potential in the field of cytopathology. Monoclonal antibodies have been tested mainly on body cavity aspirations and on fine needle aspirates of solid tumours. Monoclonal antibodies to identify B- and T-lymphocytes have been of value in distinguishing lymphoma from lymphocytic reaction and there is particular interest in investigating new antibodies in the search for one which would be specific for cancer cells and others which would be specific for tumour type. In the field of cervical cytology an antibody which was specific for cells shed from intraepithelial as well as invasive cancer would have an important impact on the development of systems for automatic screening. In addition antibodies have been raised against the protein antigen of the human papilloma virus and are in
use. Similarly these techniques have been applied to the cytodiagnosis of chlamydia
trachomatis infection in smears from the genital tract and conjunctiva. In this
presentation the current position with regard to application of immunocytochemistry in
diagnostic cytopathology will be considered.

IMMUNOHISTOLOGICAL STUDY OF VASCULATURE IN
THE NORMAL PLACENTAL AND MOLAR PREGNANCY
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The classical hydatidiform mole is recognised by its typical 'bunch of grapes' appearance
due to gross distension of the chorionic villi. Consistent histological features of
hydatidiform moles are trophoblastic hyperplasia and stromal oedema. Fetal stromal
blood vessels are usually absent but are reported in up to 10% of classical hydatidiform
moles. We have investigated fetal stromal vessels by immunohistological staining using
antisera to factor VIII and blood group H, both having been reported as being useful
markers for endothelium. In a series of nonmolar placentae, with a range of gestational
ages, fetal vessels were easily identified in all cases using antisera to blood group H. Factor VIII did not prove to be a reliable marker for the identification of these small
stromal vessels. A pilot study of complete and partial hydatidiform moles showed no
staining of stromal structures in any case. As a consequence this study has been extended
to include a larger series of hydatidiform moles. The results of these studies will be
presented and discussed.

ALPHA-1 ANTI-TRYPsin, ALPHa-1 ANTi-CHYMOTRYPsin,
ACTIN AND MYOSIN IN UTERINE SARCOMAS
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In an investigation of the stromal component of normal human endometrium, we have
observed granules positive for alpha-1 anti-trypsin (α1AT) and alpha-1 antichymotryp-
sin (α1ACT) throughout the menstrual cycle and in post-menopausal endometrium.
This prompted us to investigate their presence in neoplastic endometrial stroma.
Fourteen cases of mixed Mullerian tumour (MMT) and 5 of stromal sarcoma were
examined for the presence of α1AT, α1ACT, actin and myosin, using an immuno-
peroxidase (PAP) technique. For comparison, 6 cases of leiomyosarcoma were similarly
examined and 6 cases of endometrial adenocarcinoma were examined for α1AT and
α1ACT. Eleven MMT were positive for α1AT and 13 for α1ACT. All 6 stromal
sarcomas and 4 of the 6 adenocarcinomas were positive for both. These results will be
compared with findings in leiomyosarcomas. All 6 leiomyosarcomas were positive for
myosin as were 7 of the MMT and 2 stromal sarcomas. Five leiomyosarcomas, 6 MMT
and 2 stromal sarcomas were positive for actin. These findings will be related to those in
normal endometrium. Their potential use to the pathologist and clinician in resolving
diagnostic problems will be discussed.
MINIMAL DEVIATION ADENOCARCINOMA
("ADENOMA MALIGNUM") OF ENDOCERVIX - HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY OF TWO CASES

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Minimal deviation adenocarcinoma ("adenoma malignum") is an uncommon histological variant of endocervical adenocarcinoma characterised by its exceptionally well differentiated appearance which closely resembles that of normal endocervical epithelium. Two examples of this tumour in 26 and 36 yr-old females have recently been reviewed. In both, the pathological diagnosis was initially missed because of the bland histological appearance of these tumours in biopsy material and the younger patient has subsequently died (aged 33 yr) of her disease. It seemed crucial to establish whether application of modern histochemical techniques could be of help in the diagnosis of malignancy in these lesions. To this end these cases have been examined with the use of conventional mucin and lectin histochemistry and a panel of monoclonal antibodies to epithelial membrane antigens and cytokeratins. The results of this study will be presented and compared to those obtaining in normal endocervix. The importance of recognising the characteristic histological features of this tumour variant will be emphasised.

CLASSIFICATION OF METAPLASTIC, DYSPLASTIC AND SQUAMOUS CANCER CELLS AS DETERMINED BY FLUORESCENCE OF PAPANICOLAOU (PAP) DYES

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Hyperchromatism and coarse chromatin clumping in the nucleus of dysplastic and squamous cancer cells stained by the Pap method could be the microscopical result of the interaction of several combined factors. Hyperchromatism has been ascribed to an increased content and stainability of a DNA-histone-hematin complex; coarse chromatin clumping to systematic microscopical artifact resulting from the coagulation by fixation of nuclear hydrated proteins. The acidophilic and non-acidophilic cytoplasmic response from normal to cancer cells will be related to one of the two acidic Pap dyes (EA50 or OG6) as characterized by their excitation/emission action spectra. The progressive loss of nuclear fluorescence in superficial and intermediate cells, from normal to pathological, will be related to the dedifferentiation (degree of polymerization) of nuclear keratins. With BG12 excitation the fluorescence is yellow in keratinized cells and green in non keratinized cells; with BG36 the keratinized cells are red. The aim of this cytoanalytical study is to establish the pattern of nuclear protein content and hydration in relation to hyperchromatism and coarse chromatin clumping, using the fluorescence emission spectra of acidic Pap dyes as probes. The spectral fluorescence profile of nuclear and cytoplasmic keratin in normal and abnormal squamous cells will be classified in accordance with the current concept of the histogenesis of cervical dysplasia and in situ carcinoma.

DIFFERENTIATION OF HUMAN CERVICAL EPITHELIA IN VIVO AND IN VITRO

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The relationship between glycogen accumulation and involucrin synthesis and their use as markers of keratinocyte terminal differentiation has been investigated. The studies
have been done using normal human cervical epithelial cells and cells of benign and malignant cervical lesions in vivo and in vitro. Involucrin synthesis generally began prior to glycogen accumulation and the level of expression of both markers was related to the degree of epithelial terminal differentiation. In vitro the two markers were generally coexpressed. Cultured endocervical epithelial cells were negative for these markers and were labelled by LE61 which is a marker for simple epithelia. After 14 days in culture these cells acquired a keratinocyte morphology. When actively growing endocervical keratinocytes remained LE61 positive and did not synthesise involucrin or accumulate glycogen. These cells were capable of undergoing terminal differentiation, as defined by the presence of involucrin and glycogen, when they also became LE61 negative. The relationship of this to squamous metaplasia is discussed. It is concluded that neither marker of keratinocyte terminal differentiation is superior to the other and that they have only limited diagnostic potential in discriminating between squamous metaplasia and CIN.

HISTOPATHOLOGY OF FALLOPIAN TUBE FOLLOWING STERILISATION

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This study was undertaken in order to investigate the changes in tubal morphology following sterilisation with clips and to compare the changes produced with those elicited by other forms of tubal sterilisation. Macroscopically, clipped-tubes exhibit focal thickenings of the serosa adjacent to the site of clipping, and the clips themselves are covered by a layer of connective tissue. Microscopy reveals that the tubes are completely occluded by clipping, as all that remains between the jaws of the clip is a thin strand of connective tissue. This strand and the connective tissue covering the clip itself is contiguous with the focal thickenings, which comprise vascularised fibrous tissue. The tubal segments proximal to the site of the clips show several changes in morphology. Principal amongst these are an attenuation of the epithelial folds and the presence of a partially deciliated cuboidal epithelium. In contrast, the segment distal to the site of clipping has a normal fold pattern and is lined by columnar epithelial cells with the correct proportion of ciliated cells. In comparison to other forms of sterilisation; tubal changes, inflammatory responses and the incidence of epithelial inclusions are much lower following clipping.

TRANSFERRIN RECEPTOR IN NON-MALIGNANT AND MALIGNANT HUMAN BREAST

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Transferrin receptor is a cell surface glycoprotein which is involved in the uptake of iron by cells. The receptor has been identified in malignant cells and tissues, but not in comparable resting state cells. It has been suggested that the presence of the glycoprotein is related to cell proliferation. The distribution of transferrin receptor in normal, hyperplastic, pregnant and malignant human breast tissue has been studied using a monoclonal antibody applied to frozen sections, fixed, processed tissue being unsuitable. Normal breast tissue was unreactive but in hyperplastic specimens a variable number of ducts and acini per case stained, with also a variation in intensity. There was clearly defined staining in all lobuli of pregnant breast. All carcinomas reacted in 70% of cases the majority of cells staining, but these could be divided into those with a consistent reaction and those with a variation in staining intensity. The remainder had mixtures of positive and negative cells or few cells reacting. No correlation has been found with tumour differentiation and metastatic status. The expression of transferrin receptor is not confined to breast carcinomas and may well reflect the proliferative state of breast epithelium.
BREAST CANCER METASTASIS, SURVIVAL AND CARBOHYDRATE EXPRESSION ASSOCIATED WITH LECTIN BINDING

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N-Acetyl-Galactosaminyl-α, galactosyl-, mannosyl and sialyl-group expression in breast cancer cells from 120 patients has been related to local lymph node metastasis, distant metastases and survival. Antibodies to sialyl transferase and galactosyl transferase have been used together with a panel of lectins. To quantify cells staining we have used microdensitometry, MOP, point-counting and simple visual assessment. Each sugar and enzyme investigated shows some change with progressive invasion and metastasis so that such behaviour might be predicted by examination of the primary cancer. N-Acetyl-Galactosaminyl-expression (Helix pomatia lectin binding) shows a close association with metastasis so that in the absence of axillary lymph node examination it may be possible to stratify patients for treatment on the basis of markers in the primary. Sequential changes in carbohydrate expression associated with tumour progression suggest replacement of low malignancy cells by cells with higher metastatic behaviour (clonal selection?), and metastases commonly show carbohydrate expression which reflects the dominant cell population of the primary. But patients with a breast cancer which has metastasised do not inevitably progress to widespread disease or early mortality; thus metastases behave in a variable manner, although less so than do primaries. By relating to metastases, disease free interval and survival to the expression of carbohydrate groups we may increase our understanding of the biology of breast cancer and select more appropriate treatment for individual patients.

IMMUNOHISTOCHEMICAL LOCALISATION OF COLLAGENS AND FIBRONECTIN IN HUMAN BREAST NEOPLASMS

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44 biopsies from neoplastic, hyperplastic and normal human breast were studied for localisation of collagens and fibronectin. Affinity purified antihuman type I, III and IV collagens and antifibronectin were utilised by the indirect immunoperoxidase technique on fixed and paraffin embedded sections. Almost all cancer cells of ductal or lobular origin were positively stained for the three collagen types apart from the medullary carcinoma. The benign, hyperplastic and normal epithelial cells were negative. The patterns of staining in the cancer cells in the various neoplastic lesions varied from mainly focal in the intraduct carcinomas to more diffuse type in the infiltrative malignancies. Fibronectin staining was inconsistent. The importance of localisation of type IV collagen antigen in the neoplastic cells as well as its diffuse localisation in the stroma concomitantly with type I and III collagen and other connective tissue components will be discussed.

IMMUNOHISTOCHEMICAL STAINING OF SYNOVIAL SARCOMAS WITH EPITHELIUM-SPECIFIC ANTIBODIES

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We have studied the immunohistochemical staining pattern of 17 cases of synovial sarcoma using epithelium-specific antibodies. These were compared with 20 cases of
giant cell tumour of tendon sheath (benign synovioma), 5 examples of normal synovium and examples of a variety of other spindle cell sarcomas. Sections were stained by an immunoperoxidase method using three monoclonal antibodies. Two of these, CAM 5.2 and LP 34, recognise intracellular cytokeratin proteins. The third, HMFG-2, recognises an epithelial membrane-specific oligosaccharide. Sixteen synovial sarcomas showed staining of the epithelial component with at least one antibody. Cells lining blood filled spaces within the tumours also stained. The large majority of cells in the spindle cell component did not stain, but occasional cells did show staining. No staining was demonstrated in normal synovium nor in giant cell tumours of tendon sheath. A minority of other spindle cell sarcomas contained cells which stained positively but in a qualitatively different fashion, easily distinguishable from the type of staining seen in synovial sarcomas. These results show that immunohistochemistry may be helpful in the diagnosis of synovial sarcoma, and add weight to the proposition that these neoplasms do not arise from synovium.

EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) ANTIGENS IN MELANOCYTES, NEVOCELLSULAR NEVI AND MALIGNANT MELANOMAS

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Changes in expression of MHC antigens HLA-A,B,C and la have been described associated with malignant transformation and with tumor-related cellular immune response, especially in nevomelanocytic lesions. We have studied normal skin (i.e. melanocytes), nevocellular nevi, primary cutaneous melanomas and melanoma metastases immunohistochemically with monoclonal antibodies against framework determinants of the MHC molecules. In addition, immuno-electronmicroscopical immunoperoxidase and immunogold techniques were applied in order to visualize different antigens on the same cell. The results indicate that normal melanocytes express HLA-A,B,C, and do not express la, nevocellular nevi do not, or in very low amounts, express HLA-A,B,C and do not express la, whereas marked, but variable, expression of HLA-A,B,C and la is found in melanomas. The proportion of tumor cells staining for HLA-A,B,C is higher in primary than in metastatic melanomas, whereas it is the reverse for la. Three expression patterns can be observed in melanomas: a) high HLA-A,B,C and low la; b) low HLA-A,B,C and low la; c) high HLA-A,B,C and high la. Low expression of HLA-A,B,C or high expression of la may be associated with a high grade of malignancy in malignant melanoma.

DISTRIBUTION OF ENOLASE ISOENZYMES, GLIAL FIBRILLARY ACIDIC PROTEIN AND NEUROFILAMENT PROTEIN IN 38 CEREBRAL NEOPLASMS USING THE PEROXIDASE-ANTIPEROXIDASE TECHNIQUE

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Monoclonal antibodies to glial fibrillary acidic protein (GFAP) and the 68 KD neurofilament subunit and polyclonal antibodies for alpha and gamma enolase were used to stain paraffin sections from 38 cerebral neoplasms using the peroxidase-antiperoxidase technique. The neoplasms studied comprised 10 astrocytomas (grades I-
IV), 9 subependymal gliomas (4 from patients with tuberose sclerosis), 12 giant cell glioblastomas, 4 gangliogliomas and 3 ganglieneuromas. GFAP was present in some cells from all astrocytomas, subependymal gliomas and gangliogliomas, and in 7 giant cell glioblastomas and 2 ganglieneuromas. Positive staining for neurofilament protein was found in only 1 ganglieneuroma and 3 gangliogliomas. Alpha enolase was found in many cells from all groups of neoplasms and gamma enolase was present in all gangliogliomas and ganglieneuromas and in 10 giant cell glioblastomas, 8 subependymal gliomas and 3 astrocytomas. Reactive astrocytes from 7 examples of non-neoplastic gliosis were also studied and in 3 cases occasional cells were found to contain gamma enolase when adjacent to areas of cerebral necrosis. In astrocytomas, gamma enolase was seen in some gemistocytic cells and in pleomorphic cells from high grade neoplasms. This may represent uptake of gamma enolase from adjacent degenerate neurones; however, the presence of GFAP, alpha and gamma enolase in morphologically similar neoplastic cells raises the possibility of a stem cell origin for some types of cerebral neoplasms.

OESTROGEN AND PROGESTERONE RECEPTORS IN HUMAN MENINGIOMAS: MEASUREMENT OF SPECIFIC RECEPTORS BY AN ISOELECTRIC FOCUSING TECHNIQUE

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Oestrogen receptors (ER) and progesterone receptors (PR) were measured in 25 meningiomas from 24 patients (9 males, 15 females) using cryostat sections and an isoelectric focusing technique. This method is based upon the observation that soluble receptors will readily diffuse from a cryostat section placed in an aqueous medium. Competitive binding studies on control tissues confirmed the specificity of the assay procedures. Significant PR binding (>10 fmol/mg protein) was present in 12 tumours (5 males, 7 females). No specific ER binding was detected in any of the tumours. Histology of adjacent near-fascimile sections of the assayed samples found no correlation between histological subtype and receptor status. Both groups of patients included cases of recurrent neoplasms. No significant difference was found between the mean ages of the groups or the menopausal status of the females in either group. One premenopausal female with a PR positive tumour also had a PR negative meningioma of larger size and was from a family in which the females suffered from multiple meningiomas. The results confirm that human meningiomas possess specific PR binding apparently in the absence of specific ER binding and suggest that antihormonal chemotherapy may be of value in selected patients with recurrent or inoperable lesions.

ERRORS IN FROZEN-SECTION DIAGNOSIS: RETROSPECTIVE AUDIT

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Happily most frozen section diagnoses are correct. However the errors and deferred diagnoses give rise for concern. We have reviewed a 10-year consecutive series of 1,000 such assessments. Despite the intense efforts made in the last 20 yr to improve awareness of infrequent morphological problems, it remains depressingly true that misinterpretation is an important cause of error in frozen-section diagnosis. The diagnoses issued at the time of intraoperative frozen-section examination have been compared with subsequent paraffin-section diagnoses. The discrepancies (accounting for approximately
3% of cases) were divided into clinically significant differences (all false-negative diagnosis of malignancy), deferred diagnosis: awaiting subsequent paraffin sections, and, clinically unimportant differences in diagnosis. The sources of these misdiagnoses were categorised as being due to the focal nature of the lesion, technical errors in section preparation and pathologist’s misinterpretation. These sources of error were further divided into avoidable and unavoidable types. The sources of these errors and their analysis will be discussed.

**CIGARETTE SMOKE INDUCED INJURY OF RAT PERITONEAL MESOTHELIAL, CELLS AND HUMAN ENDOTHELIAL CELLS**

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Mesothelial cells harvested by collagenase digestion of rat omentum, and endothelial cells obtained from human umbilical vein have been cultured. The mesothelial cells were used after being passaged 2–4 times, but the endothelial cells were used as soon as confluence had been obtained. Non-smoking male volunteers donated 1 ml of blood before and after smoking 2 cigarettes each yielding 19 mg tar and 1.5 mg nicotine, at a rate of 1 inhalation every 15 s. After washing the cells with phosphate buffered saline (PBS), separate dishes of cells were incubated with platelet poor plasma obtained from pre- and post-smoking blood samples. After 30 min incubation, the plasma was removed, the cells washed with PBS, and then covered with fresh medium. Mesothelial cells have been examined by scanning electron microscopy (SEM) at 30 min, 1 h, 2 h, 4 h, 8 h, and 20 h after being covered with fresh plasma. Endothelial cells have only been examined at 20 h. SEM examination of the mesothelial cells shows little or no damage when plasma obtained from pre-smoking blood is used. With post-smoking samples, extensive blebbing and vacuolation of the cells was apparent at 8 h and was even greater by 20 h. These results have been consistent with 10 volunteers. Only 2 volunteers have been examined using endothelial cells. At 20 h after treatment with post-smoking plasma, few endothelial cells remained attached to the culture surface. These results indicate that following cigarette smoking, cytotoxic factors are present in plasma.

**EFFECTS OF NICOTINE ON ULTRASTRUCTURE OF RAT THORACIC AORTA AND ITS ABILITY TO PRODUCE PROSTACYCLIN**

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Nicotine was administered to 200–250 g male Sprague-Dawley rats using both single subcutaneous injections and implanted infusion pumps. Four groups of 4 rats received nicotine injections at the following doses – 1 mg/kg, 2.5 mg/kg, 10 mg/kg and 20 mg/kg. An equal number of rats were given saline injections as a control. 40 min after receiving the injection, half the rats from each group were sacrificed and the thoracic aortas removed, from which samples free from connective tissue were incubated in 50 mM Tris buffer, pH 7.4, at 22°C on a gentle rocking platform. Subsamples were removed at 4 min intervals and assayed for 6-keto PGF₁α production by radioimmunoassay. The remaining rats were, following anaesthesia, exsanguinated with Kreb's Ringer solution then perfused for 20 min with 2.5% gluteraldehyde in cacodylate buffer, pH 7.2. The aortas were carefully removed and examined by scanning and transmission electron microscopy. Plasma nicotine levels of 425 ng/ml were maintained in 18 rats for 7 days using infusion.
pumps. Eight of the animals were used for prostacyclin studies and the remainder prepared for morphological examination as described above. An equal number of animals received saline as control. The results indicate that a single dose of nicotine has no significant effect on the morphology or prostacyclin production of endothelial cells, but that continuous infusion of nicotine significantly depresses prostacyclin production and results in limited endothelial cell damage.

IMMUNOCYTOCHEMICAL DEMONSTRATION OF MACROPHAGES IN HUMAN Atherosclerosis

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A conspicuous component of early human atherosclerotic plaques and of the periphery of some more advanced lesions is the large, lipid-laden 'foam cell'. Such cells often also contain numerous granules and ring-like structures of ceroid pigment, composed in part of oxidized lipids. The origin of these 'foam cells' is still contentious, some authors inclining to the view that they are derived from smooth muscle cells while others believe them to be of monocytic origin. The present study, using 4 rat monoclonal antibodies directed against T200 (anti-leukocyte common antigen), HLA-Class II molecules, macrophage cytoplasm and smooth muscle cells suggests that the 'foam cells' are monocytic origin. The presence and structure of ceroid within these cells lends some support to the suggestion that the macrophage's oxidative mechanisms are active within the developing plaque and may be involved in the progression of the disease.

NAKED EYE ASSESSMENT OF CORONARY ARTERIAL STENOSIS

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Assessment of coronary arterial stenosis is made by radiologists at angiography and pathologists at autopsy. Both groups define severe stenosis in similar but not identical ways. The purpose of this study was to examine how a group of pathologists assessed coronary arterial stenosis and to see if agreement between these pathologists could be improved by the use of a diagram proforma. 16 pathologists of widely differing experience were asked to assess the degree of stenosis in 25 slices of coronary arteries obtained at autopsy. Mathematical derived, computer drawn diagrams illustrating varying degrees of arterial stenosis, each accompanied by a percentage value for luminal area occlusion, were prepared. Using these diagrams the same 16 pathologists were asked to reassess the 25 arterial slices. Measurements were then made on elastic van Gieson stained paraffin sections of the 25 arteries. The three sets of results obtained were compared. There was good inter observer agreement in the assessment of coronary arterial stenoses which were less than 30% and greater than 70% of luminal area occlusion. Inter observer agreement was poor where the degree of coronary arterial stenosis was between 30% and 70% but with stenoses of this order the use of the prepared diagrams improved inter observer agreement.
SMALL VESSEL CHANGES IN CLAUDICATING PATIENTS - MORPHOLOGICAL AND MORPHOMETRIC STUDY
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Patients identified as having claudication in the calf muscles on exercise were taken from post mortem cases. Skin samples from the toes of 4 claudicating patients were compared with similar samples from 14 age matched patients with no evidence of large vessel vascular disease or history of claudication. Morphometric analysis showed significantly higher luminal occlusion of dermal and subcutaneous vessels by intima in claudicating as opposed to non-claudicating patients. (t-test: 0.01<p>0.02). No changes in the medial component were identified. Similar size distributions of vessels were seen in each group. Morphological assessment showed that in the non-claudicating group there are a few vessels exhibiting either cellular or hyaline intimal proliferation with luminal occlusion. In the claudicating group identical morphological changes were observed but these affected many more vessels. Hyaline change in the walls of arterioles were seen in both groups of patients. These hyalinised vessels showed no luminal occlusion. The findings suggest that symptoms of claudication are associated with a quantitative increase in occlusion of small vessels in the skin and subcutaneous tissues. Studies performed previously on limbs amputated for critical ischaemia have shown more severe but morphologically identical small vessel occlusive disease when compared to this group of claudicating patients. It is possible that there is a spectrum of small vessel disease in the elderly which differs quantitatively but not qualitatively depending on the severity of proximal large vessel occlusion.

SEEDING DACRON ARTERIAL PROSTHESES WITH PERITONEAL MESOTHELIAL CELLS
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A study has been undertaken in dogs in which the abdominal aortas below the renal arteries were replaced with Dacron arterial prostheses, with and without prior seeding of mesothelial cells, which were harvested from omentum at the time of surgery. Control animals were sacrificed at 2 wk, one at 4 wk and one at 6 wk. Of the dogs in which seeded grafts had been implanted, one was sacrificed at 2 wk, three at 4 wk and two at 6 wk. Segments of graft were taken for electron microscopy and with the grafts removed at 2 and 6 wk the remainder of the graft was incubated in Tris buffer from which aliquots were removed at 4 min intervals over a 20 min period for TXB₂ and 6-keto PGF₁α production. A 50% cellular covering of the graft surface at 2 wk and a confluent cellular layer at 4 wk, apart from the regions close to the anastomoses, was apparent using electron microscopy. 6-keto PGF₁α production was 36% in comparison with normal aorta at 2 wk in the seeded grafts, and as much as 54% in comparison by 6 wk. Considerable TXB₂ production was detectable in the control grafts, but no 6-keto PGF₁α was measurable in the 2 wk grafts although by 6 wk production by non-seeded grafts was 5% in comparison with aorta. These results suggest that grafts seeded with mesothelial cells have a less thrombogenic surface.

MYOCARDITIS IN ENDOMYOCARDIAL BIOPSIES
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Myocarditis is notoriously difficult to diagnose, especially in small endomyocardial tissue
samples obtained by biopsy. In 250 patients suspected to suffer from myocarditis this clinical suspicion was confirmed in approximately half these patients. The majority of the remaining cases showed a non-specifically hypertrophied dilated myocardium. In an additional group of 1,156 patients suspected with dilated cardiomyopathy undergoing biopsy examination, 288 showed evidence of myocarditis. Examination of biopsy tissue has permitted a definition to be formulated. Myocarditis is defined as the presence of inflammatory cells in the myocardium with evidence of fraying of adjacent myocardial fibres but without concomitant sequential fibre necrosis. Sequential biopsies on patients undergoing treatment with corticosteroids and immunosuppressive agents have made it possible to distinguish healing and healed stages. This grouping is dependent on the location of the inflammatory cells in the widened interstitium and collagen tissue. Virological and immunological studies have suggested that in a substantial number of cases with dilated cardiomyopathy an infectious-immune mechanism is operative.

ENDOCRINE NATURE OF OESOPHAGEAL AND LARYNGEAL SMALL CELL CARCINOMA DEMONSTRATED BY IMMUNOHISTOCHEMISTRY WITH ANTIBODIES TO NEURON SPECIFIC ENOLASE

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We have previously reported the endocrine nature of small cell carcinoma of the lung as shown by immunoreactivity for neuron-specific enolase – a marker for mammalian nerves and endocrine cells. Small cell undifferentiated (oat cell) carcinoma of the oesophagus or larynx is an uncommon tumour which has similar morphological features to its counterpart in other sites. We therefore investigated, using the same techniques, the possibility that the oesophageal and laryngeal neoplasms may also be endocrine in nature. Sections from 22 formalin-fixed and wax-embedded tumours were immunostained using antisera to neuron-specific enolase and to regulatory peptides including bombesin, adrenocorticotropic hormone, neuropeptide, calcitonin and pancreatic polypeptide. Twenty of the 21 oesophageal cases and the one laryngeal case were immunoreactive for NSE, showing a range of staining intensity from weak to intense. Immunostaining was often strongest at the periphery of the tumour and in nests or isolated neoplastic cells outside the main tumour bulk. Of the regulatory peptides, immunoreactivity was obtained with antisera to adrenocorticotropic hormone (1 case), calcitonin (3 cases) and bombesin (1 case). These results suggest that small cell carcinoma of the oesophagus does indeed have an endocrine character, and that immunostaining with anti-NSE may be useful for the distinction of this tumour from other neoplasms at this site.

ATYPICAL CARCINOID TUMOURS OF LUNG: HISTOLOGICAL FEATURES AND RESPONSE WITH NEURONE SPECIFIC ENOLASE TECHNIQUE

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26 atypical carcinoid tumours of the lung were identified from a review of 815 consecutive thoracotomies carried out over a 5 year period in Edinburgh. The histological features of atypical carcinoid tumours include large cell (compared to classical carcinoid tumours) with pink granular cytoplasm and well defined oval or round nuclei. The tumours characteristically show rosettes, ribbons or trabeculae. Necrosis,
often extensive, was common and mitoses easily identified. These appearances have similarities to features seen in intermediate type of small cell carcinoma, large cell undifferentiated carcinoma and classical carcinoid tumour. Those cases with a florid trabecular pattern or frequent rosettes may be mistaken for adenocarcinoma. We have applied the polyclonal neuron specific enolase technique to formalin fixed tissue of 22 of the 26 cases, of which 19 gave positive results confirming a relationship of the group described with classical carcinoid tumours and small cell carcinoma. About one-third of carcinomas showing adenoc or squamo differentiation also gave positive results with this technique. The importance of making the diagnosis of atypical carcinoid tumour is the good prognosis after surgery, 77% 5-yr survival for stage I tumours.

IMMUNOSTAINING OF HUMAN LUNG TUMOURS USING A PANEL OF MONOCLONAL ANTIBODIES

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Immunocytochemistry has become established as a diagnostic tool in human tumours. Monoclonal antibodies to intermediate filaments and other tumour markers are becoming widely available. It is important to establish the staining pattern of these antibodies in neoplastic tissue. We investigated human lung tumours to determine the immunostaining of the malignant cells with five epithelial monoclonal antibodies. Tissue was obtained from formalin fixed paraffin embedded blocks of 8 small cell carcinomas, 10 adenocarcinomas and 10 squamous cell carcinomas. Monoclonal antibodies AuA1, HMFG1, HMFG2, 5.2 and LP34 were applied to the sections and the indirect immunoperoxidase technique used. Results show that all three histological tumour types were positive for AuA1, 5.2, HMFG1 and HMFG2. The depth of immunostaining and number of cells stained varied. All small cell carcinomas were negative for LP34 while all the adenocarcinomas and squamous cell carcinomas were positive. In summary, small cell carcinomas, adenocarcinomas and squamous cell carcinomas of the lung share common epithelial antigens while the negative staining of small cell carcinomas with LP34 makes this a useful negative marker in the differential diagnosis of this tumour type. In addition the preservation of the antigens in formalin fixed tissue makes it possible to apply these antibodies to routinely fixed tumours.

HUMAN LUNG TUMOURS: A CORRELATION OF ANTIGENIC PROFILE WITH HISTOLOGICAL TYPE

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54 human lung tumours have been immunostained with a large panel of monoclonal antibodies including reagents against cytokeratins, prekeratins, EMA, CEA and neural antigens. These results have been compared with the histological types of tumour using the current WHO classification scheme. The most striking finding of this study was the considerable overlap between antigenic profile and histological type of tumour. This suggests that there may be a greater similarity between different histological categories of lung tumour than has hitherto been assumed. Secondly it was evident that immunostaining highlighted areas of different morphology within many tumours emphasising the heterogeneous differentiation patterns seen in many lung tumours. The present study supports the viewpoint that lung tumours arise from a common stem cell and that these neoplasms represent a single tumour with a tendency to differentiate along one or more pathways.
ULTRASTRUCTURE OF CLEAR CELL CARCINOMA OF LUNG
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Clear cell carcinoma of the lung is defined by the World Health Organisation on light microscopic grounds as an undifferentiated large cell tumour composed of water-clear or foamy cells. Widespread clear cell change is not unusual in squamous, adeno- and large cell undifferentiated carcinomas, and it is therefore not clear whether tumours composed entirely of clear cells should be regarded as an entity. We have studied the ultrastructure of six pulmonary tumours diagnosed as clear cell carcinoma by light microscopy. Three showed the characteristics of adenocarcinoma, in two there was squamous differentiation, and one consisted of undifferentiated large cells. The 'clear cell' appearance resulted from large quantities of glycogen in the cytoplasm. It is concluded that so-called clear cell carcinomas of lung are a heterogeneous group of neoplasms, having in common only intracytoplasmic accumulations of glycogen.

EPITHELIAL MARKERS IN PLEURAL MESOTHELIOMAS. A COMPARISON WITH PRIMARY LUNG CARCINOMA
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The distribution of carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA), and a cytokeratin (CAM 5.2), has been studied in autopsy material from 26 pleural mesotheliomas of mixed epithelial and sarcomatoid types, and in surgically resected specimens of 40 primary lung tumours – 10 squamous cell carcinomas, 10 adenocarcinomas, 10 large cell and 10 small cell anaplastic carcinomas. Monoclonal antibodies and an indirect immunoperoxidase technique were used on paraffin-embedded formalin-fixed material, without enzyme digestion. With the monoclonal antibody to CEA, which does not recognise the shared Normal Cross-reacting Antigen (NCA) determinant, all the mesotheliomas were negative, while 9/10 squamous, 6/10 adenocarcinomas, 7/10 large cell, and 5/10 oat cell carcinomas were positive. EMA was positive in 23/26 mesotheliomas, 9/10 squamous, 10/10 adenocarcinomas, 10/10 large cell, and 7/10 oat cell carcinomas. Cytokeratin was demonstrated in 26/27 mesotheliomas, 9/10 squamous, 9/10 adenocarcinomas, 8/10 large cell, and 7/10 small cell carcinomas. This work confirms previous reports that the presence of CEA excludes a diagnosis of mesothelioma. The stain for cytokeratin was more useful than EMA for demonstrating small foci of epithelial cells, and the epithelial nature of some of the spindle cells in sarcomatoid mesotheliomas. All three markers were useful in demonstrating foci of squamous or adenocarcinomatous differentiation in the lung carcinomas, not easily detected in routine H & E preparations.

IS SUDDEN INFANT DEATH SYNDROME (SIDS) CAUSED BY COMMON BACTERIAL TOXINS?
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In a previous presentation to this society we proposed that viral infections of the upper respiratory tract may lead to the overgrowth of common toxin-producing bacteria and cause severe toxicaemia in childhood. In this paper we present evidence to show that this is a possible cause of SIDS: 1) There is epidemiological evidence that viral infections predispose to SIDS. 2) We have found a moderate or heavy growth of Staph.
pyogenes (6 cases), E. Coli (4 cases) and Group B Streptococci (3 cases) in the nasopharyngeal secretions of 16 cases of SIDS. 3) Minimal morphological findings post mortem are consistent with toxin induced disease. 4) Maximal incidence during sleep can be explained by pooling of nasopharyngeal secretions. 5) We have developed a mathematical model based on this hypothesis which closely predicts the age distribution of SIDS, which is the single most consistent and characteristic feature of the disease. The model assumes an exponential fall of incidence, with a half life determined by the probability of encountering the putative toxins, modified in the first four months by the protective effect of maternal IgG. For bacterial toxins that 50% of the population meet in any two month period the model predicts an age distribution close to that observed in England and Wales in 1982 (n=1268). The model leads to predictions of the age distribution of SIDS in premature infants that can be tested.

THICKNESS OF ALVEOLAR CAPILLARY WALL IN GUINEA PIGS AT HIGH AND LOW ALTITUDE

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The thickness of the pulmonary air-blood barrier was compared between 7 guinea pigs from La Raya in Peru at an altitude of 4,200 m and 7 from sea level. Lung tissue was prepared for electron microscopy and selected areas photographed at a magnification of 5,000× using a systematic random sampling technique. The arithmetic mean thickness of the alveolar-capillary wall was calculated from the ratio of its volume to surface area using the integrated test system devised by Weibel. The harmonic mean thickness was determined by direct measurement of the thickness of the alveolar-capillary wall with a ruler. The latter measurement provides a more realistic measure of the thickness of the barrier with regard to its resistance to diffusion of gases. The average arithmetic mean thicknesses of the alveolar-capillary wall in the high and low altitude animals were 0.45 μm, and 0.70 μm respectively; the average harmonic mean thicknesses were 0.29 μm and 0.45 μm respectively. The thinner alveolar-capillary wall in the high-altitude animals may be a feature of adaptation, enhancing oxygen uptake from the alveoli and hence reducing the oxygen deficit at tissue level.

PREPROGLUCAGON COMPONENT PEPTIDES IN NORMAL HUMAN PANCREAS AND IN GLUCAGONOMAS: LOCALISATION USING ULTRASTRUCTURAL IMMUNOCYTOCHEMISTRY

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In the mammalian pancreatic preproglucagon, glicentin, a 69 amino acid peptide which contains glucagon within its structure, is extended at its C-terminal by two other glucagon-like peptides (GLP, and GLP,). In this study we have investigated the distribution of GLP, and GLP, within human normal pancreatic islet A cells. Previously described topographic segregation of pancreatic glucagon from its immediate pro-molecule, glicentin, has been reinvestigated in the light of our findings. Rabbit polyclonal antisera raised to glucagon (16-29), glicentin, GLP,(1-19 and 1-37) and GLP,(1-33) were used. Adsorption controls revealed no cross-reactivity between any heterologous antigen-antibody combination, except anti-glucagon(16-29) which was fully adsorbed by 5 nmol GLP,/ml diluted antiserum. Our findings confirm that glicentin is restricted to the argyrophilic halo of normal A cell granules. Conversely, GLP, and GLP, are localised within the core of alpha granules. The finding that anti-glucagon(16-29) serum cross-reacts extensively with GLP, suggests that GLP, rather than pancreatic
glucagon is topographically segregated from glicentin in the alpha granules. Furthermore, our study demonstrates that GLP₁ and GLP₂ antisera may be used as reliable markers of glucagon-containing cells in normal pancreas.

USE OF MULTIPLE MARKERS IN HISTOPATHOLOGICAL DIAGNOSIS OF ENDOCRINE TUMOURS

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Histological diagnosis of endocrine tumours can no longer be based solely on the detection of the active peptide or amine produced by the tumour. Lack of peptide, for example, may be due to its being stored in a precursor form. Thus, investigation of a number of different markers is warranted, not only those for active products and their precursors but also for storage granules. An immunocytochemical and histochemical study was made of 7 cases of islet cell tumours producing pancreatic glucagon. The cases were selected randomly from the files and the tissues selected had various types and qualities of fixation. The markers employed were antisera to pancreatic glucagon, to two peptides present in pre-pro-glucagon, GLP₁ and GLP₂, and to pancreatic polypeptide, which is frequent in pancreatic tumours. General markers for endocrine cells were antisera to chromogranin A, a protein in polypeptide storage granules, neuron specific enolase, an enzyme marker, and Grimelius' silver impregnation. Only 5 of the cases contained pancreatic glucagon but all cases showed immunoreactivity, of varying density, for at least one of the markers used. Thus, investigation of these cases of glucagonoma reinforced the premise that multiple markers must be employed in the immunocytochemical diagnosis of tumours.

C CELL POPULATIONS IN THYROID:
A POST MORTEM STUDY

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120 thyroids from coroner's autopsy cases were examined for C Cell populations. The glands were fixed in formalin, weighed and a standard block was taken from the mid point of each lobe. Age and sex of the patients were recorded. Sections were then stained using anti-calcitonin antibody (PAP method) and with H & E. The numbers of C cells in the sections from each lobe were measured semi-quantitatively (0 to 1+, 2+, 3+, 4+). Electron microscopy was also attempted in cases with dense populations. There were two main findings. Firstly, the proportion of men with larger numbers of C cells (3+ and 4+) was significantly greater than women (P=0.05). This may be related to the known preponderance of males in the sporadic type of medullary carcinoma. Secondly, a correlation of age with increasing numbers of C cells has previously been suggested. This was not confirmed in our study. There was no significant increase in mean age with numbers of C cells. Baseline studies on C cell populations may be relevant to the assessment of C cell hyperplasia in the individual case.
FOLLICULAR CELL MORPHOLOGY IN RELATION TO LYMPHOCYTE SUBSETS AND HLA-DR EXPRESSION IN GRAVES' DISEASE

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Frozen section immunocytochemistry has been of fundamental importance in elucidating the functional character of lymphocytes infiltrating the thyroid in Graves' Disease. More recently, it has helped to demonstrate an aberrant expression of HLA-DR antigen on the follicular cells. From the data accumulated so far, it is suggested that both the presence of lymphocytic subsets and HLA-DR expression are highly focal in their distribution. Preliminary examination of the relationship between follicular cell morphology, HLA-DR expression in follicular cells and lymphoid infiltration has confirmed the focal distribution and shows that the follicular cells either expressing the HLA-DR antigen and/or in the vicinity of lymphoid aggregates (consisting predominantly of T-helper and B-cell lymphocytes) consistently have hyperplastic morphology: large plump cells forming many crowded small follicles. Objective image analysis of this observation however has been hampered by the variably poor morphology of the unfixed acetone-dried frozen sections. We have developed a method based on the use of a dextran protectant (8.6% dextran, 18,000 mol. wt. average, dissolved in 1 mM Tris-HCl, 0.1M NaCl, pH 7.5 buffer) which significantly overcomes this handicap. Results obtained using this novel method on Graves' Disease thyroids and human tonsil material will be presented to critically evaluate its usefulness.

CHARACTERISING GROWTH FACTOR CONTROL OF THYROID EPITHELIAL CELL PROLIFERATION

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To study control of cell proliferation in a differentiated epithelial tissue we have used as a model a primary thyroid culture which retains the ultrastructural and functional characteristics of the in vivo gland. Rat thyroids are dispersed with collagenase (200 U/ml) plus dispase (2.0 mg/ml) to give a preparation of >95% epithelial cells in sheet/follicular form. This is cultured in RPMI 1640 medium in agarose-coated wells to prevent both monolayer formation and fibroblast growth. Growth was assessed by 3-H thymidine incorporation and autoradiography. In the standard supplemented medium there is modest TSH independent growth and the TSH response is limited. We have explored the role of growth factors by supplementing serum free medium with various combinations of somatostatin, transferrin, insulin, hydrocortisone or fetal calf serum. No proliferation occurred in the presence of transferrin and somatostatin with or without TSH. The addition of insulin alone at low concentration (800 ng/ml) did not allow growth but the further addition of TSH led to a dramatic stimulation of 3-H thymidine incorporation. Insulin is thus shown to be permissive for the epithelial cell growth response to TSH. It is hoped that clearer understanding of the controls exerted by growth factors will lead to a deeper understanding of the mechanisms of tumour formation.

GRANULAR PERIPOLAR CELL OF THE HUMAN GLOMERULUS - AN ULTRASTRUCTURAL STUDY

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We have recently confirmed the existence of a granular cell at the hilum of the human glomerulus. It is often very sparse, being found in as few as 3% of glomeruli. In this
study we examined serial sections of small resin embedded blocks of tissue from seven histologically normal kidneys. We found granular peripolar cells in three of them. They were situated in the reflection of Bowman's capsule round the vascular pole between the parietal and visceral epithelial cells to which they are joined by tight junctions. Their granules had the ultrastructural features of secretory granules, having a granular substructure and a limiting outer membrane; their diameter ranged from 400–2,150 nm (mean 1,050 nm). The peripolar cells also contained the cellular apparatus of protein synthesis and secretion, but these were not well developed. It is considered that the ultrastructural features of the human peripolar cell, reported here for the first time, are consistent with it being a secretory cell.

**GRANULAR PERIPOLAR CELL OF HUMAN GLOMERULUS AND ITS RELATIONSHIP TO RENIN-ANGIOTENSIN SYSTEM**

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In 1979 a new cell was discovered in the mammalian kidney. It is a glomerular epithelial cell which contains secretory granules and discharges them into the glomerular filtrate. We report here the first study of this cell in the human kidney. We studied serial sections of histologically normal renal cortex until at least 30 glomeruli from all layers had been examined. We found granular peripolar cells in all of six kidneys. They were present in 3–28% of glomeruli and were situated in the reflection of Bowman's capsule round the vascular pole. We studied the tinctorial properties of the granules and compared their staining reactions with the granular renin containing cells of the JGA which were often present in the same section. We mapped the distribution of peripolar cells and renin containing cells in the renal cortex and showed both were more often present in glomeruli from the superficial cortex. In addition, there was usually a close relationship between granular peripolar cells and renin containing cells in individual JGAs. However, using an antibody to pure human renin and the peroxidase-antiperoxidase technique, the granules of the peripolar cell contained no immunoreactive renin. The significance of these observations will be discussed.

**PROTEINASE INHIBITORS IN KIDNEY AND ITS TUMOURS**

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The proteinase inhibitors alpha-1 antitrypsin (A1AT) and alpha-1 antichymotrypsin (A1ACT) identified by immunocytochemical methods have been widely used as histiocytic markers in diagnostic histopathology. These substances are also known to be present in non-lymphoid organs including the kidney. Using polyclonal antisera raised against A1AT and A1ACT and the immunoperoxidase method on formalin fixed paraffin embedded tissue we have studied the distribution of these antigens during renal embryogenesis and in renal tumours. Ten foetal and five adult kidneys were studied. A1AT and A1ACT were found in different cell types during embryogenesis. They were found in the cytoplasm of the cells of the proximal tubule during the maturation stage of development, in some cells in the undifferentiated blastema and in primitive mesenchymal cells adjacent to the metanephric blastema. In the adult kidney the antigens were found in proximal tubular epithelium, but not in differentiated mesenchymal cells. Ten cases of childhood nephroblastoma and twenty adult renal carcinomas were studied using the same techniques. The antigens were found in blastemal cells in nephroblastoma and in epithelial cells in renal carcinoma. The significance of the distribution of
proteinase inhibitor-containing cells in renal tumours will be interpreted with reference to their distribution during the development of the kidney.

INTERSTITIAL INFLAMMATORY CELLS IN TYPE I MEMBRANO proliferative GLOMERULONEPHRITIS

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In Type I membranoproliferative glomerulonephritis interstitial inflammatory cells are usually present but their nature has not been well documented. Four renal biopsies were examined by conventional microscopy, histochemical and immunohistochemical techniques to identify and quantify the numbers of different cells in the infiltrate over the whole area of sections of each biopsy. In these biopsies the predominant cell was the T lymphocyte with 287 cells/mm² and a Helper/Suppressor ratio of approximately 2:1. The numbers of histiocytes, polymorphs, mast cells, and plasma cells were 29, 24, 10 and 10 cells/mm² respectively. An occasional eosinophil was present. Two cases of endocapillary proliferative glomerulonephritis were examined for comparison. The predominant cell was also the T lymphocyte with 327 cells/mm² and an H/S ratio of 2:1. There were, however, larger numbers of plasma cells, histiocytes and polymorphs being 134, 103 and 180 cells/mm² respectively. Small numbers of mast cells were present and only an occasional eosinophil was seen. These results suggest active immunological processes in the interstitium and that they appear to differ in these two morphological types of glomerulonephritis. Further characterization of the interstitial cell population and its role in disease progression is necessary.

PLASMA CELLS IN ACUTE HEPATITIS

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Plasma cells, common in chronic hepatitis, are also seen in the liver in acute hepatitis. Liver biopsies from 92 patients with acute hepatitis (24 type A, 16 type B, 27 presumed non-A, non-B and 25 drug-induced) were studied by conventional microscopy and immunoperoxidase staining for IgG, IgA and IgM in paraffin sections. Immunoglobulin(Ig)-containing cells with plasma cell morphology were mainly found in portal tracts. The total in each section was divided by the number of portal tracts to give an index of their population density. Plasma cells were found in 77 of the 92 biopsies, and IgG-containing cells in 74. IgG and IgA-containing cells were most abundant in acute hepatitis with bridging necrosis as compared with the classical form (p<0.05), and least abundant in drug-induced as compared with viral hepatitis (p<0.05). Among the viral groups the largest number of IgG-containing cells was found in type non-A, non-B, while IgA-containing cells were most abundant in type A. These differences were not statistically significant. It is concluded that Ig-producing plasma cells are common in acute viral hepatitis, and presumably play a part in antibody-dependent immune reactions in this disease.
VENO-OCCCLUSIVE LESIONS IN ALCOHOLIC LIVER DISEASE
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In a retrospective autopsy study, Goodman and Ishak (1982) noted a high prevalence of venous lesions in alcoholic liver disease. They described three distinct lesions; (i) phlebosclerosis, which was characterised by perivenular and pericellular fibrosis in zone 3 of the hepatic acinus, (ii) veno occlusive lesions, which were characterised by intimal proliferation and intravenous fibrosis and (iii) a lymphocytic phlebitis involving terminal hepatic venules and interlobular veins. We have examined 256 percutaneous liver biopsies and 50 autopsy livers from patients with alcoholic liver disease for the presence of such lesions using sections stained with Orcein and Elastica van Gieson stains. Phlebosclerosis was noted in all but 2 cases of alcoholic hepatitis and in all cases of established cirrhosis. Veno occlusive lesions were found in 25/256 (9.8%) of biopsies and 11/50 (22%) of autopsy livers. Phlebitis was seen in 10/256 (3.9%) of biopsies and in 2/50 (4%) of autopsy livers. The prevalence of veno occlusive lesions and phlebitis in our study is significantly less than that noted by Goodman and Ishak.

ASSESSMENT OF HISTOLOGICAL DIAGNOSIS OF REFLUX OESOPHAGITIS
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The purpose of the study was to assess the value of histological parameters in the diagnosis of reflux oesophagitis. 56 adult patients with epigastric pain or evidence of oesophagitis were examined endoscopically and suction biopsies obtained 5cm proximal to the oesophagogastric junction. On the basis of symptoms and endoscopy, patients were divided into 3 groups. 12 had no reflux symptoms or endoscopic abnormality and these constituted a control group. 20 patients with reflux symptoms but no endoscopic abnormality were designated Group 1 and 24 with symptoms and endoscopic evidence of oesophagitis Group 2. Biopsies were interpreted independently by two pathologists without prior knowledge of clinical or endoscopic findings. Various histological parameters considered relevant to oesophagitis were assessed including basal zone thickness, height of intraepithelial papillae, vascular dilatation and inflammatory cell infiltration. No histological parameter was sufficiently sensitive to detect all cases with endoscopic evidence of oesophagitis. Specificity was also limited and there was some overlap in the histological findings in the three groups. Correlation between pathologists was fairly good but interpretation of basal zone thickness was a source of disagreement. Reasons for the limited accuracy of histological diagnosis of oesophagitis will be discussed.

CELL-MEDIATED IMMUNITY TO CANDIDA ALBICANS IN PEPTIC ULCER PATIENTS
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Gastric and duodenal ulcers are often contaminated with Candida albicans and it has been suggested that in such cases Candida may aggravate and perpetuate the disease. Patients with clinically significant mucosal Candida infections usually show impaired cell-mediated immunity. We have investigated 31 peptic ulcer patients for cell-mediated
responses to Candida albicans (whole yeasts) and compared them with normal subjects by skin testing, leucocyte migration inhibition (LMT) and lymphocyte transformation responses (LIT). There was no significant difference between patients and controls in skin reactivity or in the LMT. However, transformation responses were greatly reduced in the patient group, a large number of whom failed to respond \((p<0.002)\). Cross-serum experiments using normal donor lymphocytes and sera from non-responding patients demonstrated a serum suppressive factor in only 3 out of 9 subjects suggesting that in the majority of non-responding patients the defect was cell-mediated. Since immune responses are under the control of HLA-DR genes, we studied the distribution of HLA-DR antigens in both subject groups in relation to their response to Candida albicans. The incidence of HLA-DR 5 was increased among all non-responders and was significantly higher in patients compared with controls \((p<0.01)\).

GASTRITIS, PEPTIC ULCER AND C. PYLORIDIS

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‘Campylobacter pyloridis’, only recently identified, may be implicated in peptic ulceration. This is a histological (including scanning microscopy) and microbiological analysis of 54 sets of antral biopsies from 50 patients endoscoped for upper gastrointestinal complaints to study this suggested association. The organism was cultured in 16 of the 20 \((80\%)\) antral biopsies from patients with acute duodenal ulcer, 4 of 7 \((57\%)\) with a chronic gastric ulcer, 3 of 9 with a healed peptic ulcer, 1 of 2 with gastritis only and was absent in 2 patients with pernicious anaemia. It was present in 5 of 14 \((36\%)\) ‘control’ patients with dyspepsia but normal endoscopy. All five had histological gastritis. Histology demonstrated the organism in 71% of the 42 biopsies with gastritis, colonization ceased at sites of intestinal metaplasia. Inflammatory activity (polymorphs within epithelium) was of no influence. It was found in only 1 of 12 patients with normal mucosa. Scanning microscopy and culture agreed on all but 2 occasions and the organism was invariably observed beneath the mucous layer. In the 4 patients with repeat biopsies colonization persisted despite the ulcer healing. Clearly ‘C. pyloridis’ is associated with gastritis and in particular duodenal ulceration. From its position it seems protected by the gastric mucus layer and exposed by metaplasia. If primarily involved in the pathogenesis of peptic ulcer disease the study has implications for the ‘control’ carriers and relapse rates in persistent carriers following ulcer healing.

GASTRIC DYSPLASIA, ITS VALUE AS AN EARLY PRECANCEROUS LESION

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Part of the prevention of gastric cancer depends on knowing if gastric dysplasia is a progressive lesion. Gastric biopsies ‘SNOP-coded’ dysplastic or atypical were re-assessed for the years 1976-80 and the outcome determined. Established criteria for dysplasia were used and a recently suggested classification into high-grade, low-grade, ‘probably negative’, ‘probably positive’ and definitely negative. There were 981 biopsies and 38 had been coded atypical or dysplastic. After review 5 were designated high-grade dysplasia. All had proven cancer within one year. In 8 the dysplasia was low-grade. 3 had proven cancer within one year, in 1 progression to cancer occurred in three years. 1 was lost to follow-up and in 3 subsequent biopsies were normal. 17 biopsies were classified ‘probably negative’ and 8 negative. 5 from these two groups had a cancer proven, 4
within one year and 1 after four years. A carcinoma diagnosed within one year of initial biopsy was assumed a sampling error. All but 1 patient with dysplasia, 2 overall, fell into this category. Within these limits no evidence emerged that dysplasia is slowly progressive and appears a late marker for gastric cancer if gastro-intestinal symptoms have developed. The study also suggests better criteria are needed to select out a group with low-grade dysplasia (3 of 8) that seems to regress.

GASTRIC MUCOSAL ENDOCRINE CELL PROLIFERATION IN PERNICIOUS ANAEMIA
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Pernicious anaemia is associated with gastric carcinoma, endocrine cell hyperplasia and carcinoid tumour. Endoscopic biopsies from seven consecutive patients with proven pernicious anaemia showed small intestinal metaplasia with varying degrees of inflammation, fibrosis and expansion of the lamina propria. NSE and PGP 9.5 (neuroendocrine markers) immunostaining revealed a proliferation of endocrine cells (EC) in the epithelium and lamina propria. Clumps of EC in the latter were composed of two cell types, either small or large. Islands of both cell types were represented in five specimens; epithelial EC tended to be large and in one case small EC appeared to be related to a nerve in the lamina propria. Some EC showed gastrin, 5-HT, VIP and substance P immunoreactivity. Ultrastructurally nine types of neurosecretory granules were found within EC. The finding of intestinal type EC functionally appropriate to the type of epithelium in the vicinity suggests that the metaplastic change occurs in the stem cells of the affected area. Lamina propria EC could derive from the epithelium by ‘budding’ or from EC of the lamina propria plexus. Endocrine tumours associated with pernicious anaemia tend to be multifocal and carcinoid tumours of the stomach, even in association with pernicious anaemia are infrequent. Our findings of endocrine cell proliferations in all seven cases of our unselected series of biopsies of patients with pernicious anaemia indicate that this is possibly an adaptive change that occurs frequently and provides the basis on which carcinoids less frequently develop.

RELATIONSHIP OF INTESTINAL METAPLASIA AND GASTRIC CARCINOMA
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The prognosis of gastric carcinoma has remained essentially unchanged over the last 25 years despite advances in endoscopic and radiological techniques aimed at earlier diagnosis. The identification of intestinal metaplasia in stomachs with the intestinal type of carcinoma has led to the hypothesis that metaplasia of gastric epithelium to intestinal type epithelium represents a pre-neoplastic change in the mucosa. Mucin histochemical studies have identified sub-types of intestinal metaplasia of which the Type IIB of Jass (sulphomucin predominating) appears to be specifically related to intestinal type carcinoma. The argument remains as to whether intestinal metaplasia is a preneoplastic condition or whether it represents an essentially non-specific change in gastric mucosa in which there is severe mucosal disturbance associated with malignancy. The results confirm the presence of Type IIB metaplasia in gastric mucosa adjacent to gastric carcinomas of the intestinal type. The amount of Type IIB metaplasia has been
quantified and shown to be a small proportion of the total metaplasia present. The usefulness of identifying this form of metaplasia in diagnostic pathology will be discussed.

**DISTRIBUTION OF CATHEPSIN D IN NORMAL AND NEOPLASTIC TISSUES**

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Cathepsin D is an aspartic proteinase with optimal pH 3.5. It is a lysosomal enzyme and is known to be present in a variety of tissues, including gastric mucosa, although its distribution within these tissues has not been fully determined. We have purified cathepsin D from homogenised human spleen and have prepared an antiserum to it. Using the immunoperoxidase (PAP) method on formalin-fixed paraffin-embedded tissue, we have studied the distribution of cathepsin D in a variety of normal and neoplastic tissues. Its presence is not limited to cells of macrophage origin and its distribution is not the same as that of lysosome. In the stomach it occurs in mucus neck cells and, somewhat surprisingly, in parietal cells. In the liver it is present in hepatocytes as well as Kupffer cells. In the large bowel it is found in mucosal epithelial cells. In addition it occurs in carcinomas of all tissues studied both in neoplastic epithelial cells and in stromal cells.

**MUCINOUS GASTROINTESTINAL CARCINOMAS: STUDY USING LECTIN HISTOCHEMISTRY AND IMMUNOPEROXIDASE DETECTION OF BLOOD GROUP ANTIGENS**

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The histochemical characteristics of secreted mucin in 38 mucinous (colloid) carcinomas of the gastrointestinal tract has been studied. The objective was to test whether differences exist between the mucins secreted by gastric and colorectal carcinomas which could be used in diagnostic histopathology. Two techniques which identify peripheral carbohydrates in glycoproteins (lectin histochemistry and blood group immunohistochemistry) have been used. The results were compared with those obtained using several conventional histochemical mucin stains. Overall the results with the different techniques were similar in gastric and colorectal carcinomas and no clearcut subdivisions emerged. Lectin staining of mucin did not correlate with the presence of blood group antigens and in particular staining with Peanut lectin did not match with the presence of Thomsen-Friedenreich antigen.

**HLA-DR EXPRESSION IN SMALL BOWEL IN COELIAC DISEASE**

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In the normal small bowel HLA-DR expression in epithelium is limited to the villi, the crypt epithelium being negative. In coeliac disease HLA-DR is expressed in the crypt cells. The antigen expression take two forms – a cytoplasmic granule and a linear staining of the cell border. Class II MHC antigen expression has been shown in other tissues to be associated with various cellular immune reactions – the putative mediator being gamma or immune interferon. The induction of this antigen in coeliac disease provides further support for the immune nature of this disease.
DEFICIENCY OF SERUM PREGNANCY ASSOCIATED
\(\alpha_2\) GLYCOPROTEIN (\(\alpha_2\) PAG) AND GASTROINTESTINAL DISEASE

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Pregnancy associated \(\alpha_2\) glycoprotein (\(\alpha_2\) PAG) is a high molecular glycoprotein which occurs in the serum of all normal individuals and during pregnancy and following oestrogen administration there are marked increases in its concentration in the serum. The biological function of \(\alpha_2\) PAG is as yet unknown but there is a growing body of evidence to suggest that it has immunosuppressive properties both in vitro and in vivo. In recent studies we have demonstrated that IgA producing plasma cells also have the ability to produce \(\alpha_2\) PAG. Furthermore, \(\alpha_2\) PAG has been identified in similar secretions to secretory IgA. Using a sensitive Elisa technique serum \(\alpha_2\) PAG concentrations have been measured in patients with various forms of gastrointestinal disease. Very low serum concentrations of \(\alpha_2\) PAG have been detected in patients with a variety of diseases including irritable bowel syndrome, coeliac disease, dermatitis herpetiformis, almost all the patients showing very low levels being male. In view of the novel association with IgA it is tempting to speculate that low levels (or deficiency) of \(\alpha_2\) PAG may be implicated in the aetiology of some forms of gastrointestinal disease.

COMPARATIVE STUDY OF DNA FLOW CYTOMETRY ON FIXED EMBEDDED AND FRESH TISSUE FROM HUMAN LYMPHOMAS

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The ploidy (DNA content) and fraction of tumour cells in the DNA-synthetic (S) phase of the cell cycle can be estimated using DNA flow cytometry. These measurements have been shown to be of prognostic significance in a variety of human tumours. Although usually applied to fresh cell suspensions, recently DNA flow cytometry has been reported to be possible using fixed paraffin embedded histopathological material. This offers considerable practical clinical advantages if validated. We have applied a slightly modified version of the technique to a pilot series of human lymphomas and metastatic carcinomas. Results obtained using conventional flow cytometry of fresh tissue have been compared with those obtained using thick sections from fixed embedded material. The sections are dewaxed, enzymatically disaggregated and the DNA stained with the fluorochrome DAPI. The results show that high quality DNA histograms can be obtained from histopathological material although discrepancies may be observed between the estimates of cells in S phase using the two techniques.

HISTOPATHOLOGICAL SPECTRUM OF MALIGNANT LYMPHOMA IN SUB-SAHARAN AFRICA

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Over an 18 mth period, we received 85 malignant lymphomas out of 2,000 specimens sent from 8 countries in sub-Saharan Africa, all fixed in formalin. Other than Burkitt's lymphoma, most of the lymphoma biopsies were nodal. Standard lymph node stains were used, with immunocytochemistry (leukocyte common antigen, HLA-DR, alpha-1 antitrypsin, Leu M1, low MW cytokeratin) and chloroacetate esterase histochemistry in
selected cases. The Kiel classification was used for the non-Hodgkin's lymphomas. The diagnoses were:
Hodgkin's disease: 8 cases; 6/8 mixed cellularity type.
Centroblastic/centrocytic (FCC) lymphoma: 37 cases; diffuse pattern 32, follicular 2, follicular and diffuse 3 cases.
Burkitt's lymphoma: 11 cases.
Lymphoblastic lymphoma: 10 cases.
Histiocytic lymphoma: 5 cases, including 2 with malignant histiocytosis in liver.
T-cell lymphoma (diagnosed on morphology and absence of Leu M1 staining in pleomorphic cells): 4 cases.
Others: undifferentiated lymphoma 6; lymphocytic lymphoma 2; plasmacytoma 2 cases.

We believe these figures are representative; over this period we received 63 cases of tuberculous lymphadenitis and 32 cases of metastatic nasopharyngeal carcinoma in neck nodes. Notable differences from malignant lymphoma in developed countries are: 1) the infrequency of Hodgkin's disease (9.4% of total); and 2) the infrequency of follicular pattern FCC lymphoma.

SPINAL CORD COMPRESSION AS FIRST PRESENTATION OF LYMPHOMA
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This paper reviews our experience in Southampton over the last 3 yr with a group of 14 patients, ranging in age from 5 to 87 yr, who have presented to the Wessex Neurological Centre with signs and symptoms of spinal cord compression. From laminectomy biopsy specimens using a combination of conventional histological techniques we have diagnosed lymphoma in each case. A panel of monoclonal and polyclonal antibodies have been particularly useful in distinguishing lymphoma from other primary and metastatic tumours, and also in classifying the neoplasms. The cases described are 9 B-cell follicle centre cell lymphomas, 3 T-cell lymphomas and one histiocytic and one lymphoblastic lymphoma. In each case cord compression had been the first presentation of the patient's lymphoma. Subsequent investigations have shown some of these cases to have disseminated neoplasia. In others however the disease appears to be localised in the spine. Clinical and survival data are also presented.

DIAGNOSTIC IMMUNOHISTOCHEMISTRY ON ROUTINE SURGICAL PARAFFIN SECTIONS
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IHC was performed with a panel of monoclonal and polyclonal antibodies on material referred because of diagnostic uncertainty using conventional methods. The principal problem was the typing of anaplastic tumours by tissue of origin. The leucocyte common antigen monoclonal PD7/26 and the cytokeratin monoclonal CAM5.2 proved particularly useful. CAM5.2 was more effective than the epithelial membrane antigen monoclonal HMFG2, as only 15/25 (60%) carcinomas positive with CAM5.2 were also positive with HMFG2. Of the anaplastic tumours, IHC showed that about half were carcinomas, 1/3 lymphomas, and 1/8 were unclassifiable. In this group, the provisional conventional diagnoses were only slightly more frequently concordant than they were discordant with the IHC results. Other problems studied were the typing of lymphomas and lymphadenopathies, determination of the organ of origin of carcinomas, and
confirmation of diagnoses of melanoma, mesothelioma, chorconcarcinoma and ovarian
tumours. With all these groups, IHC on paraffin sections gave useful information,
particularly in the recognition of prostatic tumours. We conclude that IHC has a major
contribution to make to routine surgical pathology.

SMOOTH MUSCLE PROLIFERATION IN
SUPERFICIAL LYMPH NODES
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The hilar region of superficial lymph nodes was studied to determine the nature of an
unusual muscular proliferation, previously noted in an axillary lymph node removed at
mastectomy. Two groups of nodes were examined. The first comprised 66 normal or
reactive nodes from 50 patients (31 inguinal, 19 axilla) and the second comprised 344
axillary lymph nodes removed at mastectomy for breast carcinoma from 80 patients.
Smooth muscle proliferation in the nodal hilum, distinct from vessels, was seen in 18/50
(36%) normal or reactive cases and 14/80 (17.5%) mastectomy cases. More inguinal
(14/31, 49%) than axillary (4/9, 21%) nodes were affected, and significantly more
affected normal or reactive nodes were from males (p<0.01). In individual cases where
several nodes had been removed, a tendency for more than one node to be involved was
noted. The smooth muscle proliferation was not related to age or to the presence of
metastatic carcinoma. The degree of smooth muscle proliferation was variable. However
90% of cases with marked lesions were accompanied by a highly vascular hilum. This
association, and the predominance of lesions within inguinal nodes which are especially
susceptible to ascending infections, suggest that the smooth muscle proliferation in the
hilum of lymph nodes may result from previous lymphadenitis.

STRUCTURE OF BASEMENT MEMBRANE AND ASSOCIATED
STRUCTURE IN HIGH ENDOHELIAL VENULES AND ITS
RELATION TO LYMPHOCYTIC MIGRATION
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High endothelial venules, whether present in lymph nodes or granulomata, characteristi-
cally show very thick and elaborate ‘basement membranes’. The nature of this structure
has been investigated in normal and pathological lymph nodes by means of a panel of
lectins and by monoclonal antibodies directed against laminin and fibronectin, using
avidin-biotin-peroxidase and immunoperoxidase methods. Three distinct layers can be
recognised; a true basement membrane underlying the endothelium, a thick perivascular
sheath which contains fibrillar and amorphous components and an external layer which
is locally continuous with the reticulin meshwork of the nodes. The thickness and
chemical composition of the perivascular sheath depends upon the height of the
endothelial cells and the numbers of lymphocytes in transit between them. There is,
first, a shift from high-mannose to complex saccharide sequences and then a fall in the
density of both N-linked and O-linked types. Migrating lymphocytes are often seen
against the basement membrane or within the sheath, suggesting that the penetration of
these may be a rate-limiting process in lymphocytic migration. We have found that many
lymphocytes contain aryl sulphotase, N-acetyl hexosaminidase and β-glucuronidase and
so might degrade glycosaminoglycans in these structures.
LYMPH NODE HEV IN REACTIVE AND LYMPHOMATOUS
LYMPH NODES

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Lymph node high endothelial venules (HEV) are vessels specialised for promoting lymphocyte migration from blood into the nodal parenchyma. They can usually be recognised by their characteristic morphology which has been likened to epithelium-lined ducts. There is, however, increasing evidence from animal studies that vessels which function as HEV exhibit considerable morphological diversity which can be related to alterations in lymphocyte flux across their walls. Although it is not possible to measure transmural lymphocyte migration directly in human HEV we have devised a quantitative method for examining the relationship between the morphology of HEV and the number of lymphocytes apparently in transit across their walls. This method has been employed in a study of HEV in reactive lymph nodes and nodes involved in non-Hodgkin's lymphoma. There are no qualitative differences between similar HEV in reactive and lymphomatous nodes but a distinct variation in the number, morphology and distribution of these vessels was found in the different lymphomas. These parameters varied with the type of tumour and the density of 'reactive' lymphocytes within it.

LANGERHANS CELL IN LYMPHOID AND
NON-LYMPHOID TISSUES

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Using the T6 antibody with an indirect immunoperoxidase technique we have analysed the distribution of Langerhans cells (LC) in 53 lymph nodes and 62 non-lymphoid tumours. The lymph nodes exhibited reactive changes (13), primary (24) or secondary neoplasia (8) and 8 were from patients with mycosis fungoides (MF). The tumours were squamous carcinomas of the cervix (32), lung (3), anus (1) and 26 non-squamous tumours. LC were identified in 31/36 squamous carcinomas. The number of LC did not correlate with the degree of squamous differentiation. 7/26 non-squamous tumours contained LC. LC were found in groups on the edge of each sinus of reactive nodes. Numbers were increased in MF and dermatopathic lymphadenitis. All but two nodes containing primary lymphoid tumours were devoid of LC. In normal areas of nodes containing metastatic tumour, there were residual LC. We propose that squamous epithelium induces T6 expression in LC by a factor independent of squamous differentiation. Tumours other than squamous type may induce T6 expression. Under normal circumstances as LC move from the epidermis to regional lymph node, loss of 'squamous influence' leads to progressive loss of T6 antigen.

DISTRIBUTION OF DEFINED CARBOHYDRATE ANTIGENIC
DETERMINANT SHOWN BY MONOCLONAL ANTIBODY AGF 4.48

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The mouse monoclonal antibody, AGF 4.48, was raised against a human promyeloid cell line, HL60. The antibody was used in an immunoperoxidase study of formalin fixed, paraffin embedded human organs. Several tissues as well as myeloid cells reacted with AGF 4.48 in a distribution that could not have been predicted. The antigenic determinant recognised by the antibody was shown to be carbohydrate and to be a
trisaccharide, 3-fucosyl-N-acetyl-lactosamine. In antibody-binding studies it has been shown that this trisaccharide can be obscured by sialic acid residues. Pre-treatment of sections with neuraminidase altered the distribution of reactivity of AGF 4.48 with many tissues. It is clear that some other monoclonal antibodies raised against different cells by different workers recognise the same antigenic determinant, 3-fucosyl-N-acetyl-lactosamine.

7th C. L. Oakley Lecture
The Use of Normal and Malignant Keratinocytes to Study Differentiation and Transformation

THE USE OF NORMAL AND MALIGNANT KERATINOCYTES TO STUDY DIFFERENTIATION AND TRANSFORMATION
B. Gusterson

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Malignant cells express a broad spectrum of phenotypic patterns, some of which reflect the histogenesis of the tumour while others may be markers of neoplasia. This lecture will consider the value of keratinocyte cultures and established squamous carcinoma cell lines as a representative system to study factors that control differentiation and to identify markers of the early events in the neoplastic process. A combination of biochemical analysis of cytokeratins, and the production of monoclonal antibodies specific for the epidermal growth factor receptor and basal cell specific glycoproteins have provided the reagents to dissect keratinocyte systems and to study how these parameters are altered by changes in the in vitro and in vivo environment. The significance of the increased expression of epidermal growth factor receptors in squamous cell carcinomas will be discussed and in particular the relevance of this finding to epidermoid malignancies and to the concept of gene amplification as a mechanism involved in the evolution of malignancy.

BIOPSY SEMINAR – COLORECTAL PATHOLOGY
A. B. Price and I. C. Talbot

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A series of cases with relevant clinical information will be available for study throughout the meeting. These will be presented and discussed.

EXPRESSION OF INTESTINAL MUCUS ANTIGENS IN EPITHELIAL ATYPIA IN ULCERATIVE COLITIS
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Dysplasia is the acceptable morphological marker of premalignant change, and yet little is known of the preceding steps, and because no precise criteria exists for its characterisation, a new classification of epithelial atypia in inflammatory bowel disease, has been proposed to include an ‘indefinite’ category. Cytological atypia and impairment of differentiation are often associated, and are expressed by alterations of function. Mucus associated antigens (LIMA and SIMA) obtained from normal large bowel.
mucosa and from colonic mucoid carcinoma respectively, as well as routine mucin histochemistry were used to probe functional atypia and abnormal antigenic expression in epithelial atypia in ulcerative colitis. The material consisted of mucosa from resected rectal stumps with carcinoma from 8 patients, who had had total colectomy and ileorectal anastomosis for ulcerative colitis. We established profiles for: (a) dysplasia and carcinoma and (b) for normal mucosa controls. Histological normal areas or areas of 'indefinite' atypia may show foci of pattern (a). This altered phenotype, probably reflects degrees of differentiation, and may help to recognise and understand a preneoplastic phase and assess the risk of malignancy in the colonic epithelium. More detailed characterisation of non-neoplastic reactive epithelium is however needed before any definite conclusion can be taken.

INFLAMMATORY CELL POPULATION OF THE COLONIC MUCOSA IN INFLAMMATORY BOWEL DISEASE: AN IMMUNOHISTOCHEMICAL STUDY

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Inflammatory bowel disease represents a difficult differential diagnosis on endoscopic biopsies. The categories of Crohn's disease, ulcerative colitis and indeterminate colitis are readily recognised on colectomy specimens but the small size of endoscopic biopsies often precludes a diagnosis more specific than 'inflammatory bowel disease'. Various studies have looked at the types of lymphoid cells present using immunofluorescence techniques and attempted to determine patterns of immunoglobulin production in Crohn's disease and ulcerative colitis. In this study we have examined blind a series of colonic biopsies using the avidin-biotin complex technique for the localisation of IgG, A, M, K, λ and J chain in the cells of the lamina propria. Preliminary results suggest that the pattern of staining may aid the differential diagnosis of this group of conditions.

HLA-DR EXPRESSION IN HUMAN COLONIC ADENOCARCINOMA CELL LINE

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The HCA-7 cell line was isolated from a well differentiated mucoid adenocarcinoma of the colon. The cells have an epithelial morphology, synthesise carcinoembryonic antigen and form adenocarcinomas in nude mice. HCA-7 cells synthesise HLA-DR which is predominately detected in the cytoplasm. Stimulation with lymphocyte-conditioned medium and recombinant gamma interferon results in enhanced synthesis of HLA-DR and appearance of the antigen at the cell surface. Maximal stimulation of HLA-DR synthesis was achieved after 48 hr in the presence of 50μ/ml recombinant gamma interferon. After this stimulation, cytoplasmic HLA-DR was increased two fold while cell surface HLA-DR was increased 19-fold. Immunocytochemical staining for HLA-DR showed it to be confined to focal areas of the monolayer, similar to the patchy expression of HLA-DR observed in sections of human colonic adenocarcinomas.

A COMPARISON OF CRYPT-CELL PROLIFERATION IN RAT COLONIC MUCOSA IN VIVO AND IN VITRO

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The successful development of a long-term organ-culture system has made it possible to perform experiments on rat colonic mucosa in vitro. To interpret these experiments it is
necessary to compare proliferative parameters in vitro with those in vivo, since fundamental changes in these parameters, due to trauma or the withdrawal of trophic factors, may occur when the mucosa is cultured. Morphological observations of crypts in a stable phase of growth in vitro suggests that they may be reduced in size. In the present study stathmokinetic experiments were performed to estimate cell birth-rate. To obtain the in vivo estimate, 22 rats were given vincristine and killed at 15 min intervals thereafter. For the in vitro estimate cultures of colonic mucosa were established, and on the seventh day vincristine was added and explants sampled at 15 min intervals. Half of the cultures had been maintained in a standard medium and the remainder in a supplemented medium. Mitotic and labelling index distributions were also investigated. The in vivo birth rate ($7.8 \pm 0.8$ cells/1,000 cells/h) and the in vitro birth-rate ($7.7 \pm 0.5$ cells/1,000 cells/h) were found to be similar. This has implications for the useful application of in vivo studies in man where in vivo data is difficult to obtain.

EFFECTS OF INTRAVENOUS AND INTRAGASTRIC UROGASTRONE-EGF ON THE PROLIFERATION OF EPITHELIAL TRACT IN PARENTERALLY RED RATS

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Rats were maintained on total parenteral nutrition (TPN) so that intestinal epithelial cell proliferation was reduced to a steady state basal level and to lessen the effects of luminal nutrition and endogenous secretions. The wet weight of the stomach, small intestine and colon and the mucosal crypt cell production rate (CCPR) of these tissues were significantly decreased ($P<0.01$ to $0.001$) after 10 days on an isocaloric TPN diet when compared to orally fed controls. Continuous infusion of 15 μg per rat per day of recombinant beta urogastrone, a dose below that needed to inhibit gastric acid secretion, significantly increased ($P<0.01$ to $0.001$) both the weights of the various sections of the intestine and the CCPR of various sites in the intestine ($P<0.05$ to $0.001$). Increasing doses of urogastrone progressively elevated the 2-hr collection of metaphases and the weights of the intestine, with the colon showing the most pronounced effects. Intravenous infusion of urogastrone was also effective in restoring cell proliferation when it was infused after the intestine had become hypoproliferative. 15 μg per rat per day of urogastrone administered via an intragastric cannulae thrice daily had no significant effect on intestinal weight or CCPR, neither did the luminal administration of higher doses of urogastrone (150 and 300 μg/rat/day) have any significant effect on intestinal weight or 2-hr metaphase collection. It is proposed that one of the in vivo actions of urogastrone-EGF is the maintenance of gastrointestinal growth and that this occurs primarily via a systemic mechanism.

IMMUNOHISTOCHEMICAL DEMONSTRATION OF IGA AND SECRETORY COMPONENT IN RELATION TO EPITHELIAL CELL DIFFERENTIATION IN NORMAL COLORECTAL MUCOSA AND METAPLASTIC POLYP

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The metaplastic polyp is not regarded as precancerous, but recent studies have shown that this lesion expresses multiple cancer-associated phenotypes. Reduced staining for epithelial IgA and secretory component (SC) is known to accompany loss of differentiation within neoplasms of the colorectum. IgA and SC were demonstrated within 30 metaplastic polyps by an indirect immunoperoxidase technique. Staining intensities were
assessed semiquantitatively in the extreme crypt base, lower crypt, upper crypt and surface epithelium for metaplastic polyps and adjoining normal mucosa. The crypt base cells and also the surface epithelial cells stained with similar intensity in both types of epithelium. However, the expected increase in staining characterising normal lower and upper crypt columnar cells and reduction in staining associated with the switch from crypt to surface columnar cell was not observed in the metaplastic polyp. Metaplastic crypt columnar cells showed significantly reduced staining for both IgA and SC as compared to their normal counterparts. There was also a significant reduction in the number of IgA secreting plasma cells in the lamina propria of the metaplastic polyp. These findings are consistent with the concept of a premature switch to mature surface cell characteristics within the metaplastic polyp.

CLINICOPATHOLOGICAL SIGNIFICANCE AND CELLULAR DISTRIBUTION OF ras FAMILY ONCOGENE EXPRESSION IN NORMAL AND NEOPLASTIC COLORECTUM

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We have previously demonstrated a variably elevated expression of ras family oncogenes in a series of polyps and carcinomata (n=12) of the colorectum and have now correlated this with conventional staging criteria (Dukes’ staging and tumour differentiation) and clinical outcome with particular reference to the development of metastatic disease. No relationship was evident between these parameters suggesting that although abnormal expression of ras oncogenes may be critical in the development of malignancy, variations in the level of their expression do not appear to be related to clinically evident phenotypic differences. Study of the distribution of the ras encoded p21 protein using the monoclonal antibody Y13-259 shows this protein to be apparently homogeneously distributed in all cells in both adenomatous polyps and carcinomata and throughout the normal foetal and adult colorectal mucosal population. This suggests that the presence of this protein is a feature of normal cellular metabolism in certain cell types and is not restricted to those involved in cell proliferation. It thus appears that neither cells at different stages of carcinogenesis nor variants of a malignant phenotype can be identified by these means.

SPARING OF ENTEROCHROMAFFIN CELLS IN GRAFT VERSUS HOST DISEASE OF THE LARGE BOWEL

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Graft versus host disease affecting the large bowel causes destruction of the crypt epithelium. In 10 out of 14 biopsies from 13 cases of gut GVHD there was selective sparing of enterochromaffin cells. As a consequence of this, small clumps and individual enterochromaffin cells are to be seen formerly occupied by the destroyed crypt epithelium. The reason for this phenomenon is unclear. There are a priori reasons for it to be anticipated that a similar phenomenon could be seen after ionising irradiation and cytotoxic drug therapy. Thus care must be taken if the phenomenon is to be employed for diagnosis shortly after exposure to such forms of therapy.
DETECTION OF ENTEROPATHOGENIC VIRUSES IN PARAFFIN EMBEDDED INTESTINAL TISSUES OF CALVES BY IMMUNOPEROXIDASE

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Diarrhoea in neonatal calves may be caused by viruses. Their excretion in diarrhoeic faeces does not prove a causal association because they may be excreted by normal animals. In determining the circumstances under which viruses may cause diarrhoea it would be useful to know the extent of infection and structural damage required to cause diarrhoea and the importance of mixed infections. An immunoperoxidase method used to detect virus antigen in association with characteristic lesions has permitted a better understanding of pathogenic processes. Rotavirus-infected enterocytes and lesions were detected in twelve clinically normal calves naturally infected with rotavirus and killed between days 1 and 4 of excretion; both predominated in the upper small intestine and although the lesions were severe in some calves they did not appear to have been sufficiently extensive to cause diarrhoea. Results of a study of outbreaks of diarrhoea indicated that mixed infections with bovine coronavirus and rotavirus were significantly associated with disease (p<0.001). Immunoperoxidase staining of viral antigen detected, in some of these calves, an anterior small intestine enteropathology associated with rotavirus and an enteropathology of the posterior small intestine and colon associated with coronavirus. The combined infection had produced an extensive enteropathology and diarrhoea.

PATHOLOGY OF DYSENTERY IN CALVES CAUSED BY AN ENTEROPATHOGENIC ESCHERICHIA COLI

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A dysentery syndrome was recognised amongst the Institute’s calves which was reproduced experimentally in germ-free and conventional calves with an atypical Escherichia coli (S102-9) isolated from affected calves. Calves passed copious red blood in the faeces and developed diarrhoea. Walls of the colon and rectum were thickened and the mucosa was reddened and covered by an exudate containing blood clots. Bacteria were seen closely adherent to the luminal surface of enterocytes, often in cup-shaped depressions or on cytoplasmic pedestals. Microvilli were distorted, disorientated or absent. There was exfoliation of infected enterocytes and mild acute inflammation of the underlying lamina. In two of five calves with natural disease the adherent bacteria did not stain by the immunoperoxidase method with antiserum raised against E. coli (S102-9). This indicated that there was possibly more than one bacterial cause of the syndrome. Lesions in experimentally infected calves and pigs were indistinguishable from those produced by some E. coli which are enteropathogenic for man, rabbits and pigs. Strains of E. coli designated as enteropathogenic are those which do not produce enterotoxins or penetrate and multiply in enterocytes; the disease in calves may provide a model in which pathogenic mechanisms, which are poorly understood, may be studied.

PREVALENCE OF ANGIODYSPLASIA AT POST-MORTEM

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Caeaca taken at routine post-mortem, from adults without history of gastro-intestinal haemorrhage or other bowel disease, were injected with barium sulphate suspension with gelatin. Out of 57 cases (to date), angiodysplasia was found in four. In one case there was a 'large' lesion and in three others there were 'small' lesions, multiple in one. Angiodysplasia may be much commoner than is usually realised.
VISUALISATION OF SILVER ENHANCEMENT OF IMMUNOGOLD STAINING BY ELECTRON MICROSCOPY

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Immunogold enhanced with silver provides a very sensitive and specific label for peptide immunocytocytochemistry. However, there is some variability which we can now explain in order to optimise the method. Wax and resin sections of pig anterior pituitary were incubated with growth hormone antiserum followed by goat anti-rabbit immunoglobulin conjugated to colloidal gold. Silver enhancement was carried out with silver lactate and hydroquinone in citrate buffer (pH 3.5), followed by fixing in photographic fixer. After examination by light microscopy and subsequent re-embedding in resin, ultrathin sections for electron microscopy were cut perpendicular to the original plane of sectioning. Empirically smaller colloidal gold gives stronger staining. By electron microscopy large gold particles (40nm) are seen to attract no more silver than small ones (5nm) which, being closer together, produce a denser silver deposit, giving best staining. The size of the silver deposits increases with time; after 6-8 min they start to agglomerate, which emphasises stain density where there is much gold. After more than 10 min the same effect occurs where there is less gold, increasing the apparent 'background' staining. The effect of permeabilisation and etching on staining and stain penetration were also investigated. Thus we can explain and optimise variations which occur with different staining conditions by this useful technique.

DIFFERENCES BETWEEN CELL COMPARTMENTS IN EPIDERMIS AND ORAL EPITHELIUM REVEALED BY ANTIKERATIN MONOClonAL ANTIBODIES

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Immunocytochemical studies with monoclonal antibodies to keratin-associated determinants have shown differential binding to various compartments in the epidermis. These antibodies appear specific for the basal layer, suprabasal layers or simple, non-stratified appendage epithelium. As oral mucosa has a wide range of stratified epithelia whose maturation is often disturbed in disease the present study was undertaken to screen fresh frozen sections of normal oral mucosa from several sites in 6 patients using a panel of monoclonal antibodies with the following compartment specificity in epidermis: 601 and 421 for basal cells, LHP1, 2, 3 and 5 for suprabasal cells, LP2K, LP3K and 5.2 for simple epithelia and LP34 for all epithelial cells. A combination of fluorescence and peroxidase methods were used. There were several major differences from skin. Although the ortho- and parakeratinized gingival epithelium showed a pattern of binding closer to that of epidermis, in non-cornified buccal epithelium 'basal' antibody 601 consistently bound to suprabasal cells only. 421, however, bound only to basal layers. In contrast to epidermis, LP2K, LP3K and 5.2 stained short stretches of basal cells, particularly in non-cornified areas. 5.2, LP2K, LP3K and 601 also revealed single intensely staining cells in the basal and parabasal layers of ortho- and parakeratinized zones. They were rare or absent in non-cornified epithelium. LHP1 and LHP2 were more uniform in cornified areas but patchy or absent in non-cornified epithelium. Although the structural basis for these differences has yet to be determined, such screening is essential before the antibodies can be used to analyse pathological changes in oral mucosa.

CHOLESTEROL DEPOSITION IN HUMAN ODONTogenic CYSTS

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Sequential collection of 200 odontogenic cysts over 2 yr has allowed a detailed histochemical and microscopical study of cholesterol deposits occurring in the walls of some cysts. Lipid-laden foamy macrophages were present in most cholesterol-containing cysts and ultrastructure revealed that many of these cells contained intracellular cholesterol-like clefts. Some foamy
cells also contained erythrocytes undergoing digestion. Intracellular haemosiderin was detected in all cholesterol granulomata. Extracellular cholesterol clefts were usually associated with foreign body giant cells but were also seen lying in necrotic debris and sometimes appeared to have been extruded from foamy cells. Chromatography and lipid histochemistry confirmed that crystals present in some cyst walls were cholesterol. The foamy cells were shown to contain cholesterol esters. Hydrolases demonstrated lysosomes in both giant cells and foam cells. The study suggests that red cell breakdown within foamy cells contributes to the cholesterol ester formation and that the esterification system may be overloaded if many erythrocytes have to be handled when the blood supply is inadequate for normal cholesterol removal. Intracellular crystallisation and subsequent extrusion may occur. Foamy cell breakdown also leads to extracellular crystal formation. Ineffective digestion of large crystals by giant cells results in persisting granulomata.

BASOPHILS AND MAST CELLS IN ATOPIC DERMATITIS

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Four patients suffering from atopic dermatitis but in remission at the time of the tests, were patch tested by repeated application of a purified antigen (extracted from the house dust mite D. pteronyssinus) to mildly abraded skin, over a period of 10 days. Punch biopsies were taken from the test sites at 2 days, 6 days and 10 days and from a control (saline) site at 2 days. The tissues were processed for light and electron microscopy by particular techniques designed to demonstrate the numbers and morphology of mast cells and basophil leucocytes. Cell counts demonstrated that there was a rise in numbers of basophils and eosinophils at 2 days followed by a fall. In contrast, the numbers of mast cells rose during the course of the test to reach a maximum at 6–10 days. Morphometric measurements were made on electron micrographs of mast cell granules from one case in order to try and determine whether a different population of mast cells had been recruited to the site. The results do not support the suggestion that mucosal mast cells are recruited to the skin in this situation. The relevance of the findings to the pathogenesis of atopic dermatitis will be discussed.

INHIBITION BY CYCLOSPORIN OF IgE PRODUCTION AND CYCLOPHOSPHAMIDE-INDUCED EOSINOPHILIA IN RATS IMMUNIZED WITH NON-PARASITE ANTIGENS

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Administration of cyclosporin A (CS-A; 25 mg/kg daily) to rats from the time of immunization with ovalbumin (OVA) in complete Freund's adjuvant abolished the production of anti-OVA antibodies, including IgE. Cyclophosphamide (Cy; 150 mg/kg) given two days before immunization also inhibited specific antibody production but at the same time induced a striking eosinophilia. Combined administration of both drugs resulted in the inhibition by CS-A of the Cy-induced eosinophilia. The results suggest that IgE synthesis and eosinophil proliferation may be under the control of separate T cell subsets. This rat model may prove useful in studies on the regulation of eosinophil production and the role of these cells in disease processes.
DETECTION OF ANTI-Ro AUTOANTIBODIES IN PATIENTS WITH POLYMYOSITIS

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Polymyositis is an inflammatory myopathy of unknown aetiology in which defective immunoregulation has been demonstrated. Numerous immunological abnormalities occur, including evidence of an immune complex disorder and the presence of a large variety of autoantibodies, especially to nuclear constituents. We report here a search for antibodies to the Ro small ribonucleoprotein particles, in a large series of cases. The Ro antigen has only recently been characterized and shown to be present in its highest concentration in mammalian heart and brain. Anti-Ro antibodies occur in several other connective tissue disorders where they may delineate a particular patient subgroup. Although no pathogenetic effect has as yet been established, these autoantibodies are undoubtedly specifically associated with cardiac damage in infants born to mothers who possess them: 34 of 41 babies with complete congenital heart block had mothers with anti-Ro antibodies. Since up to 70% of cases of polymyositis have cardiac involvement, sometimes confined entirely to the conducting tissue, we examined the sera of our 50 patients. 46% were positive for anti-Ro autoantibodies, supporting the view that this antibody may indeed have a directly damaging effect on the heart. The result also documents yet another immunological abnormality in polymyositis.

ABH BLOOD GROUP ANTIGENS IN SENSORY NEURONES

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Until recently it has not been known that neural tissue may carry ABH antigens. We have examined posterior root ganglia, spinal cord and other tissues (formalin fixed and paraffin embedded) from primates (marmosets, baboons and man). An immunofluorescence technique was used, sometimes utilizing double labelling, in order to demonstrate the presence of ABH antigens. They were found in a proportion of small and intermediate sized neurones, the positivity extending distally in the sensory nerve and proximally as far as the substantia gelatinosa of the spinal cord. The neuronal expression correlated with the ABO phenotype in the three species but was independent of secretor and Lewis genes. The percentage of positive cells was different in the three species, as was the efficiency of conversion of H to A and B. It seems likely that a functional subset of posterior root ganglion cells carry these antigens. Examination of other parts of the nervous system and other tissues indicate that these antigens are present in primary sensory neurones and their branches.

VIP-IMMUNOREACTIVE NERVE CHANGES IN EYE AND URINARY BLADDER OF DIABETIC CHINESE HAMSTERS

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Neuropathy in diabetes is frequently associated with the eye and urinary bladder, although the aetiology of this condition is still unclear. VIP-immunoreactive nerves are present in most peripheral tissues, where they are often involved in pathological conditions. We investigated the possible changes of VIP-immunoreactivity in the diabetic Chinese hamster model, particularly in eye and urinary bladder. Tissues from pre-diabetic and fully diabetic animals from three different sublines (XA, AH and AC) and from non-diabetic age-matched controls were investigated by immunocytochemistry and radioimmunoassay. In normal eye, VIP-immunoreactive nerves are primarily
associated with choroidal blood vessels; in all lines of diabetic hamsters choroidal VIP-immunoreactivity was found to be greatly enhanced, while in pre-diabetic animals the increase was less prominent. In the bladder of non-diabetic hamsters, VIP-immunoreactive nerves are mainly found in the muscle layer; in all diabetic sublines, the number and immunostaining of these fibres appeared to be increased. VIP levels were increased in most of the diabetic and pre-diabetic groups. The parallel immunocytochemistry and radioimmunoassay results show that changes of VIP immunoreactivity occur early in the onset of diabetes, and are enhanced with time course. These data suggest that the elevated VIP immunoreactivity in experimental animals might be an initial factor leading to more severe ocular and urinary disorders.

LIPID TRANSPORT BY HUMAN GALLBLADDER

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Despite the economic cost of cholecystitis and the ready availability of the tissue little is known of the (patho)physiology of the gallbladder other than its ability to concentrate bile. We have found that it is possible to investigate some of these processes in operative material obtained freshly from theatre using an Ussing Chamber. This apparatus consists essentially of two compartments perfused with physiological saline which are separated by an interposed disc of mucous membrane and is used in physiological studies of secretion. We have shown by electron microscopy that gallbladder epithelium survives in an Ussing Chamber at least 2 hr compared with the unperfused control. Isotopically labeled lipids are absorbed from the mucosal surface and secreted through the muscle coat to the serosal surface but not in the reverse direction. Metabolic poisons inhibit the process. Uptake of lipids is enhanced by ATP and cholecystokinin and inhibited by colchicine, a known inhibitor of secretion. Addition of lipoprotein to the serosal chamber increases transport more than twofold. This is associated with characteristic ultrastructural changes in the epithelial cells. Lipoproteins in bile may play a role in cholesterol lithiasis.

AUTOMATED MEASUREMENT OF SPERM MORPHOLOGY BY IMAGE ANALYSIS

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There is evidence that treatment with sulphasalazine causes changes in the morphology of sperm heads. However sufficient statistics on the shapes and sizes of normal heads are not currently available. This paper presents quantitative data on the shapes and sizes of sperm heads from subjects who at routine investigation had been assessed as normal with respect to count, morphology and motility on at least two occasions. Measurements were made on Papanicolaou stained preparations of two samples of semen from 20 subjects. The boundaries of 400 heads from each sample were detected with a semi-automatic method using the Magiscan 1 Image Analyser. The area and three shape measurements were computed for each head. Each slide was subsequently examined independently by two observers who noted the percentage of abnormal forms. In addition the shapes of 100 detected head boundaries from one sample from each subject were printed and individually scored for abnormality on a scale from 1 to 4. This allowed a direct comparison of subjective abnormality with objective measurements. An abnormality index was constructed using the computed measurements which was compared with the percentage abnormality on the remaining samples. The distribution of sizes and shapes are presented showing inter and intra subject and observer variability.
ALUMINIUM-INDUCED OSTEOMALACIA – EFFECT OF PRE-EXISTING HYPERPARATHYROIDISM

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‘Aluminium bone disease’, occurring in haemodialysis patients with chronic renal failure has usually been described as consisting of osteomalacia without secondary hyperparathyroidism, the mineralisation deficit being sometimes of a focal nature. Because aluminium accumulates in parathyroid tissue and suppresses parathyroid hormone secretion in vitro, it has been proposed that aluminium-induced (relative) hypoparathyroidism plays an important role in the development of osteomalacia. In this paper we present 13 aluminium-intoxicated haemodialysis patients with osteomalacia varying in severity from focal to extensive distribution of thick osteoid along bone trabeculae. 9 patients (group 1) suffered a gradual onset of chronic renal failure and of these 7 had biochemical and radiological evidence of hyperparathyroidism before dialysis. 4 patients (group 2) had a relatively acute onset of chronic renal failure and were euparathyroid before dialysis. Within each group the extent of the osteomalacia tended to increase with duration of exposure to aluminium. However, the patients in group 1 developed osteomalacia at a much faster rate than those of group 2. These results indicate that, with plasma aluminium levels sufficient to produce clinical toxicity, hyperparathyroidism at the onset of dialysis does not protect against osteomalacia and in fact contributes to its rapid progression by providing a large trabecular osteoid surface capable of binding aluminum.

CANCELLOUS BONE ALUMINIUM IN HAEMODIALYSIS PATIENTS – FACTORS GOVERNING ITS QUANTITY AND DISTRIBUTION

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In Edinburgh, as in several other dialysis centres, haemodialysis patients with osteomalacia do not differ in total bone aluminium content from those with osteitis fibrosa. The aluminium in osteomalacia is concentrated at the calcified bone-osteoid interfaces whereas in osteitis fibrosa aluminium is distributed diffusely throughout the bone and usually cannot be demonstrated histochemically. In a series of 15 aluminium-intoxicated haemodialysis patients with osteomalacia, 11 of whom had superimposed mild to moderate osteitis fibrosa, cancellous bone aluminium content correlated with the total exposure of the bone to aluminium (correlation=0.49 p<0.01). Using the total osteoid surface as a reflection of the mean osteoid surface during exposure to aluminium, the correlation between cancellous bone aluminium content and total exposure of osteoid surfaces to aluminium was much stronger (correlation=0.62, p<0.001). This result is consistent with the hypothesis that the calcified bone-osteoid interface behaves as a sink for aluminium, thereby providing a mechanism for patients with subtoxic plasma aluminium levels and osteitis fibrosa, having a large rapidly turning over osteoid surface, to incorporate significant amounts of apparently 'invisible' aluminium into bone. Only where there is defective mineralisation of osteoid, with a (relatively) stable calcified bone-osteoid interface, can sufficient aluminium accumulate to be demonstrable by histochemistry.

MULTIPLE ALUMINIUM LINES IN BONE AND HISTOLOGICAL HEALING OF ALUMINIUM-RELATED OSTEOMALACIA

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There is now convincing evidence that aluminium causes osteomalacia in some patients with chronic renal failure. Using electron probe x-ray microanalysis and histochemical staining we have previously shown that Al accumulates at the calcification front in bone
where it appears to inhibit mineralisation although the mechanism of action remains unclear. In many cases one or more Al lines may be seen within calcified trabeculae but to date the presence of the second line has not been satisfactorily explained. Bone biopsy specimens from 6 cases of healing Al-related osteomalacia were studied using the electron probe and histochemical staining. Calcification was observed within the osteoid in all cases varying from small deposits around osteoid osteocytes to large areas of diffuse calcification. Lower Ca/P ratios were found in mineralisation nuclei in these sites than in the underlying fully calcified bone. Toluidine blue staining of this diffuse calcification within osteoid was less intense than the staining of underlying calcified matrix. Al was detectable with the electron probe and histochemical staining at the osteoid/calcified bone interface but not in the diffuse calcification within osteoid. It is suggested that the mineralisation defect in Al-related osteomalacia heals within the osteoid by a diffuse periosteocytic calcification which extends down to the aluminium at the cement line and up towards the lamina limitans at the trabecular surface. It is also suggested that within thick osteoid seams a new normal calcification front may be established during this healing process– further Al incorporation at this stage could thus lead to the appearance of a second Al line within the trabecula.

FOCAL OSTEOMALACIA – DISTINCTIVE HISTOLOGICAL ENTITY UNRELATED TO VITAMIN D DEFICIENCY

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In vitamin D-related osteomalacia, the combination of progressive bone resorption and formation plus inhibition of mineralisation lead to a diffuse distribution of thickened osteoid seams along bone trabeculae. In contrast, we have noted that a focal distribution of osteoid is characteristic of some cases of osteomalacia due to aluminium toxicity in chronic renal failure and to therapy with the diphosphonate, disodium etidronate, in Paget's disease. Transiliac bone biopsy specimens from 5 cases of each of these types of osteomalacia were assessed using histomorphometry. In aluminium and diphosphonate-related osteomalacia the extent of thickened osteoid seams (>4 lamellae) along bone trabeculae was significantly lower and the extent of fully calcified trabecular surfaces significantly higher than in vitamin D-related cases highlighting the focal distribution of osteoid. It is suggested that the osteomalacia in both of these conditions is focal because progressive accumulation of osteoid along trabecular surfaces is prevented due to suppression of bone resorption and the subsequent reduction in formation. In Al-related osteomalacia osteoclastic resorption is inhibited by hypercalcaemia which develops due to the combined effects of Al blocking the uptake of calcium into bone, the addition of calcium to the dialysis fluid, and vitamin D therapy. In disodium etidronate-related osteomalacia osteoclastic resorption is directly inhibited by the diphosphonate although the precise mechanism remains unclear.

IMMUNOHISTOLOGICAL STUDY OF OSTEOCLASTS AND MULTINUCLEATE CELLS OF GIANT CELL TUMOUR OF BONE

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Multinucleated cells of the giant cell tumour of bone and osteoclasts have several common morphological and functional characteristics and may share origin from a similar precursor cell. Osteoclasts are known to be derived from a circulating mononuclear precursor cell of bone marrow origin. The identity of this mononuclear precursor is unknown but the most likely candidate is the mononuclear phagocyte. Using a panel of monoclonal antibodies against a variety of macrophage and non-macrophage associated components, the immunohistological staining of multinucleated cells derived from a giant cell tumour and osteoclasts from long bones of human foetuses was assessed. Only EBM-11, an antibody reacting with monocytes and a wide spectrum of
tissue macrophages stained both osteoclasts and giant cells. Both osteoblasts and mononuclear stromal cells of the tumour did not react. This indicates that osteoblasts and giant cells are antigenically related and that they may share origin from a similar precursor cell, the mononuclear phagocyte.

HISTOMORPHOMETRIC PARAMETERS OF OSTEOCLASTS AND THEIR CORRELATION WITH RESORPTIVE ACTIVITY IN BONE BIOPSIES EXHIBITING ACTIVE PAGET'S DISEASE OF BONE

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Paget's disease of bone results from a primary osteoclastic dysfunction causing an undefined period of massive, uncontrolled osteolysis. The Pagetic osteoclast is traditionally described as much larger and more nucleated than its ordinary physiological counterpart. Though quantitative validation of this subjective observation seems unnecessary, strict comparison of Pagetic giant cells to those present in other lesions containing similarly non-physiological multinucleate cells (e.g. tumours, other metabolic disorders, reactive lesions) is otherwise prevented. Recent publications have also suggested a correlation between the nuclearity of bone macrophages and their lytic activity. We have therefore measured several parameters of Pagetic osteoclasts in bone biopsies exhibiting active Paget's disease using a computerised digitisation system (Videoplan – Kontron). These have included: perimeter, area, maximum optical diameter, form factor(s), nucleiklast. These have then been correlated with the Rough Crenated surface measured in the biopsies, as a parameter of resorptive activity. Results have established true values for the various parameters of Pagetic osteoclasts of use in comparative studies. They have also shown osteoclast density in the biopsy (cells/mm²) is significantly correlated with the measured R.C.S. No correlation has, however, been demonstrated between the other osteoclast parameters, including nuclei/clast, and the R.C.S. This raises several questions relating to individual cell resorption and the proposed viral etiology of this disease.

HISTOMORPHOMETRIC COMPARISON OF GIANT CELLS PRESENT IN GIANT CELL GRANULOMA, OSTEOCLASTOMA AND PAGET'S DISEASE OF BONE

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The differential diagnosis of giant cell containing lesions of jaws and long bones is still marred by the absence of definitive histological criteria. At least two previous quantitative studies of giant cell parameters in jaw and long bone giant cell lesions have had directly conflicting results. Equally the functional type (i.e. resorptive, reactive, tumourous) histogenetic origin and ultrastructural features of the giant cells present in these lesions add to our problems in understanding their true nature. We have used a semi-computerised digitisation system (Videoplan – Kontron) to investigate pre-selected parameters of the giant cells present in giant cell granuloma (GCG) and osteoclastoma (GCT) pertinent to their differential diagnosis. The giant cells present in bone biopsies from a series of patients with active stage 2 Paget's disease of bone were also included and act as an example of functionally resorptive giant cells (osteoclasts). The latter were also of interest in relation to the traditional concept of the Pagetic Osteoclast, its size, nuclearity and activity and with regard to the proposed viral etiology of the condition. Results have revealed significant differences between giant cells of GCG and GCT, Paget's disease and GCT but not between GGC and Paget's disease. These may be of diagnostic significance. The study also revealed the probable presence of at least 2 giant cell types in Osteoclastoma.
AMYLOID IN AGEING ARTICULAR CARTILAGE
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Amyloid was sought in the hyaline articular cartilage from the femoral heads (FH) and condyles (FC) of 34 hospital postmortem cases aged 50–94 yr and in 16 acetabula (AC) and tibial condyles (TC). Congo red (CR) and thioflavine T (TT) stains were used on formalin-fixed, paraffin sections which were also tested with potassium permanganate. CR sections, examined with polarised light, gave positive reactions in 15/16 AC, 29/34 FH, 30/34 FC and 15/16 TC. TT yielded a blue-green fluorescence in UV light in 26/34 FH and 24/34 FC. Amyloid was recognised as discrete linear patches close to the articular surfaces, around chondrocytes and chondrones and near the clefts of fibrillated tissue; in several cases more diffuse deposits extended into zone II cartilage. FC cartilage from 6 cases was reacted by the PAP immunoperoxidase method for amyloid P: all were positive. Characteristic 7–10nm microfibrils were shown by EM at sites corresponding to the CR-positive material. The chemical identity of the amyloid found in the articular cartilage of older individuals and its effects on the mechanical function of this tissue remain subject to discussion.

PHASE CONTRAST AND TRANSMISSION ELECTRON MICROSCOPICAL STUDY OF INTERACTION BETWEEN HYDROXYAPATITE CRYSTAL AGGREGATES AND MONOLAYER CULTURES OF MURINE PERITONEAL MACROPHAGE
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Commercially available hydroxyapatite crystals (HAC) were ground briefly and added to 200ml of phosphate buffered saline (pH 7.2, 0.001M phosphate). The mixture was sonicated for 3 min and left undisturbed overnight at 4°C. Large aggregates of HAC settled at the bottom of the flask leaving small aggregates (0.4μm-3.0μm diameter) in suspension. Both types of aggregate (120μg/300μl) were added to monolayer cultures of murine (C57) peritoneal macrophages. Duplicate cultures of cells with and without added aggregate were maintained and examined by phase contrast light microscopy 4, 20, 24 and 50 h, 7, 9 and 14 days after the aggregates. These were also added to cultures which had been established on milipore filters and recovered 4, 10 and 60 min, 24 and 48 h after addition. These were studied by transmission electron microscopy (TEM). Both phase contrast and TE microscopy showed rapid endophagocytosis of the aggregates by macrophages resulting in marked cellular enlargement and some disaggregation of the crystals into smaller intraphago-lysosomal structures. There was some decrease in cell numbers but not significantly so in comparison to control cultures. These studies show that macrophages can tolerate the endophagocytosis of large quantities of HAC without significant cellular damage.

PATHOLOGY OF EAR AND TEMPORAL BONE
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The study of the pathology of the ear and temporal bone has been hampered by the lack of a suitable technique other than serial sectioning of the whole decalcified temporal bone in celloidin, which is too difficult, too time consuming and is unsuitable for special histopathological methods. A microslicing method is presented in which slices of the fixed temporal bone are cut on a special slicing machine which does not damage the delicate structures of that bone. After slicing, material is prepared for paraffin wax, celloidin or plastic embedding and sections are produced. The organ of Corti is very susceptible to post mortem autolysis and damage during decalcification. To prevent this, perfusion of the perilymphatic space with fixative is required as soon after death as possible. Microslicing of the temporal bone is then performed and surface preparations of suitably prepared organ of Corti may be examined. A range of pathological processes affecting the middle and internal ears will be demonstrated in which the microslicing method has been used.
Symposium:
Implication of Molecular Biology for Pathology

METHODS OF MOLECULAR BIOLOGY
J. Scott
Clinical Research Centre, Harrow, Middlesex

Introduction of DNA structure, and DNA and genomic cloning, screening of DNA libraries, Southern blotting, chromosomal localization of genes and in-situ hybridization to RNA in tissue sections. The talk will be illustrated with examples from growth factor genes.

MOLECULAR PATHOLOGY OF SOME INHERITED BLOOD DISEASES
D. J. Weatherall
Nuffield Department of Medicine, Oxford University, Oxford

During the last few years it has been possible to isolate, clone and sequence different human genes from patients with single gene disorders. Although most of this work has been carried out in the haemoglobin field there has been some recent progress in elucidating the molecular basis for other single gene disorders. The pattern which is emerging is one of striking molecular heterogeneity. It is becoming apparent that many different defects can produce a very similar clinical phenotype. This phenomenon will be illustrated by describing recent progress in analysing the molecular basis of thalassaemia and related disorders of haemoglobin synthesis. The practical problems which this heterogeneity raises, particularly as regards using recombinant DNA technology for prenatal diagnosis, will be summarised.

DNA HYBRIDIZATION TECHNIQUES ON CERVICAL SMEARS
Alan D. B. Malcolm
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Cervical scrapings from 78 women attending VD, family planning and colposcopy clinics were assayed for human papillomavirus type 6 (HPV-6) by DNA hybridisation. The results of hybridisation were compared with the clinical, colposcopic and cytologic findings. Fifty per cent of women with genital warts gave positive results with the HPV probe, 0-10.5% of women with normal cervices were positive for wart virus DNA, depending on the source of sample. Thus no viral DNA was detected in scrapings from well women, whereas 10.5% of scrapings from VD clinic patients were positive. These findings indicate that this technique can be used to detect wart virus infection where previously none was suspected. HPV-6 DNA was detected in cervical scrapings from women with CIN both before and after treatment. Indeed where viral DNA persisted after laser therapy, it was associated with local recurrence of neoplasia. This non-invasive technique has enabled us to screen for HPV infection and to identify two groups of women who have a high risk of developing CIN and for whom close, cytologic and colposcopic surveillance is indicated. A simplified DNA hybridisation technique will be described which will simplify routine screening of large numbers of samples.

ONCOGENES
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The enormous effort spent over the last ten years aimed at understanding how RNA tumour viruses cause cancer in animals has finally paid off with respect to the disease in
humans. Although the direct role of viruses in human cancer is far from clear, the genes involved in the development of tumours in animals have recently been shown to be prime candidates for critical steps in normal cellular growth. Lesions in and around these genes are expected therefore to have major biological consequences possibly leading to at least some of the steps involved in human carcinogenesis.

CHRONIC HEPATITIS B INFECTION: PATHOGENESIS AND APPROACHES TO TREATMENT

H. C. Thomas

London

GENE REARRANGEMENT IN MALIGNANT LYMPHOPROLIFERATIVE DISEASE

N. T. J. O'Connor

Nuffield Department of Medicine, University of Oxford, Oxford

The genes in B cells which code for immunoglobulin (Ig) heavy and light chains undergo rearrangement (involving excision of portions of the gene) before Ig is synthesised. To demonstrate the pattern of Ig gene arrangement in a lymph node biopsy, DNA is extracted and analysed by Southern hybridisation using heavy and light chain-specific DNA probes. DNA from normal tissue shows a non-rearranged (germline) pattern, while a monoclonal B-cell proliferation yields one or more new fragments, differing in size from the germline fragment(s). Of particular value is the fact that polyclonal B cell proliferations produce numerous fragments of varying size which in practice are too diffuse to be seen. The method can therefore simultaneously demonstrate the B cell nature of a lymphoproliferative process and also reveal its monoclonal nature. Recently the gene coding for the beta chain of the antigen receptor on T-cells have been identified, and shown to rearrange in a similar fashion to Ig genes. A panel of DNA probes thus exists which offers a means of resolving many of the diagnostic problems confronting the pathologist in the field of lymphoproliferative disorders.

GENE LOCALISATION BY IN SITU HYBRIDISATION OF BIOTINYLATED PROBES: SEX DETERMINATION USING A Y SPECIFIC DNA PROBE

J. Burns, J. A. Jonasson, V. Chan and J. O'D. McGee

University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital and Department of Clinical Genetics, The Churchill Hospital, Oxford

In situ hybridisation procedures for gene and or message localisation in cells was introduced about 15 yr ago. Those techniques which are dependent on radioactive probes are time-consuming, and resolution is compromised by the presence of a photographic emulsion over the biological sample. Biotinylated probes remove some of these limitations but present techniques are less sensitive than those employing radioactive probes. For these reasons we set out to develop a technique for gene/mRNA localisation in cells and tissues which was sensitive enough to identify rare gene and mRNA species rapidly. A Y specific DNA probe (present at 2,000 copies on the Y chromosome) and male and female cells together, has been used as a model system in which to achieve this. The Y marker was nick translated in the presence of UTP - biotin; the degree of biotin base substitution was approximately 30%. The biotinylated Y (in 50% formamide, 5% dextran sulphate and 2xSSC) was hybridised to WBC's, chorionic villi and amniotic cells at 37°C for 16 hr. The biotinylated probe was then localised by an indirect immunohistochemical procedure and the signal amplified by silver precipitation. It was shown that 78–92% of all male WBC contain a single spot in interphase nuclei which is labelled with this probe. This spot probably corresponds to the Y body detected by other methods. Unlike the latter, however, the preparations made by this technique are permanent. Chorionic villi (6 wk gestation) and amnion cells were also correctly sexed. In a double blind study 10 males and 10 females were also correctly sexed by this procedure. The method is also applicable to sexing of cells in tissue sections.
GENE LOCALISATION BY IN SITU HYBRIDISATION OF BIOTINYLATED PROBES: CHROMOSOMAL GENE ASSIGNMENT
J. Burns, J. A. Jonasson, V. Chan and J. O'D. McGee

University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital and Department of Clinical Genetics, The Churchill Hospital, Oxford

As a necessary proof of the specificity and sensitivity of the technique described in the preceding abstract it would follow that genes could be assigned to specific chromosomes. Using this technique we have examined the chromosomal location of a Y specific marker, the H-ras-1 and alphafoetoprotein genes on human WBCs. The probes used were a genomic Y fragment, the mutant H-ras-1 probe and pA5 a c DNA probe to rat alphafoetoprotein. The Y probe localised to 2 sites in the long arm of Y, the telomeric regions of 13, 14, 15, 21 and 22 and a few other sites. The H-ras-1 probe localised to chromosome 11 (p14), 1(p31; q31), 3 (q26), 4 (p14) and 5 (p14, q32). The alphafoetoprotein probe localised to chromosomal 4 (q13, q28). These results confirm that these genes are present on chromosome Y, 11 and 4 respectively but also show that there are loci on other chromosomes which hybridise with these probes. This technique is entirely compatible with regular chromosome banding techniques after in situ hybridisation. The data show that the technique is capable of identifying the chromosomal location of genes which are present in low copy number (e.g., alphafoetoprotein is reputed to be a single copy gene). The chromosome smears are ready for analysis within 24 hr as opposed to weeks using autoradiographic techniques.

GENE LOCALISATION BY IN SITU HYBRIDISATION OF BIOTINYLATED PROBES: LOCALISATION OF A Y SPECIFIC MARKER TO THE Y CHROMOSOME BY SCANNING ELECTRON MICROSCOPY
D. J. P. Ferguson, D. Harrison, J. Burns, J. A. Jonasson, V. Chan and J. O'D. McGee

University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital and Department of Clinical Genetics, The Churchill Hospital, Oxford

WBCs chromosomes smears and interphase cells were hybridised with a biotinylated Y specific marker. The labelled probe was localised by goat anti-biotin, peroxidase labelled rabbit anti-goat IgG and peroxidase reacted with DAB H₂O₂. No reaction product was visible at this stage, but after silver intensification 100% of Y chromosomes were labelled as well as Y bodies in interphase nuclei. After carbon or gold coating SEM clearly demonstrated that this probe was localised to 2 locations on the long arm of the Y chromosome and also to several autosomes. Because of the resolution of SEM, and the fact that whole intact chromosomes can be viewed, this procedure enables quite precise gene assignment on chromosomes.

GENE/mRNA LOCALISATION BY IN SITU HYBRIDISATION OF BIOTINYLATED PROBES: DEMONSTRATION OF THE RAS AND C-MYC GENES IN HUMAN TUMOURS
J. Burns, S. Taylor, V. Chan, K. A. Fleming and J. O'D. McGee

University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford

The H-ras-1 probe and c-myc genomic probes were biotinylated by standard procedures. These were hybridised to frozen sections of 2 breast cancers, 1 thymoma and 1 clear cell cancer of kidney. The malignant epithelium, and non-malignant stromal cells in both breast cancers labelled with the ras probe. This probe reacted weakly, if at all, with the thymoma but reacted not only with the malignant but also supporting stromal cells in the clear cell cancer of kidney. The c-myc probe reacted only with the malignant cells in the thymoma. This technique therefore enables one to localise oncogenes in human tumours. It is of interest that stromal cells in 3 of these malignant tumours bind the ras probe. This simply means that members of the ras gene family are present within these cells and does not indicate that the mutant H-ras gene is present in stromal cells. Theoretically, therefore, it should be possible to determine which oncogene and its message is localised to human cancer cells in intact tissue.
USE OF *IN SITU* HYBRIDISATION HISTOCHEMISTRY FOR LIGHT AND ELECTRON MICROSCOPICAL LOCALISATION OF PEPTIDE BIOSYNTHETIC SITES

I. M. Varndell,1 J. Wharton,1 K. L. Sikri,1 C. D. Minth,2 A. F. Gazdar,1 H. K. Oie,1 J. D. Minna,2 J. E. Dixon,2 S. R. Bloom3 and J. M. Polak1

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The immunocytochemical localisation of stored, or structural, products gives little indication of where, or how actively, peptide biosynthesis is occurring. In *situ* hybridisation histochemistry may be adopted for this purpose. Using a cDNA probe directed to rat pre-pro-somatostatin (ppS) mRNA, we have visualised sites of somatostatin synthesis at both light and electron microscope levels. The tissue was a somatostatin-producing cell line (RIN-14B) originally derived from a rat insulinoma. The cells were grown on plastic coverslips and fixed with 1% glutaraldehyde (pH 7.2; 1 hr). After thorough washing the cells were incubated with cDNA probe. The ppS probe was composed of a 450 base pair single-stranded cDNA linked into a mp18 bacteriophage vector made double-stranded with the incorporation of biotin-dUTP. Hybridisation sites were visualised using an avidin-biotin-peroxidase complex (ABC) procedure with osmium intensification. Some coverslips were embedded in Araldite following a standard electron microscopical procedure. Ultrathin sections were cut and were viewed in a T.E.M. without counterstaining. Intracytoplasmic reaction product deposits were readily visible in most cells, though to a varying degree of intensity. At the ultrastructural level electron-dense precipitate was observed along short lengths of endoplasmic reticulum membrane, often closely associated with ribosomes. No reaction products were visualised in the cisternae. The results show that cDNA-mRNA hybridisation, with an ABC detection system, is a sensitive method for demonstrating the precise site of peptide biosynthesis.

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IN *SITU* HYBRIDIZATION AS A TOOL IN PATHOLOGY – THE INVESTIGATION OF HERPES SIMPLEX VIRUS INFECTION

G. B. Clements and Fiona Jamieson

Institute of Virology, Church Street, Glasgow

The availability of cloned restriction endonuclease fragments from the herpes simplex virus (HSV) genome provides a well characterized source of DNA probes for *in situ* hybridization. Transcription of HSV during the lytic cycle follows a cascade through from immediate early to early and then to late times after infection and commencement of transcription. Individual regions of the genome fall into particular categories there being qualitative differences between immediate early and early and quantitative differences between early and late transcription. Nick translation-labelled tritiated HSV DNA fragments cloned into PBR322 have been used to examine the pattern of transcription at the level of individual cells after infection of BHK 21/C13 cells in culture by *in situ* hybridization. The appearance of transcripts from various regions of the HSV genome follows a time course as predicted from previous studies using other approaches on bulk preparations of RNA but there is heterogeneity in labelling between individual cells. Results will also be presented on transcription in individual neurons during reactivation of latent HSV from mouse dorsal root ganglia as determined by *in situ* hybridization. These studies illustrate the potential of *in situ* hybridization as a technique in the investigation of pathological processes.
Posters and Demonstrations

HUMAN PAPILLOMA - VIRAL CYTOPATHIC EFFECT IN LARYNX
Ingle Wright

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Human Papilloma Virus produces characteristic cytopathic effects in squamous epithelium. Some HPV types are associated with different lesions, some at least of which are precancerous. Although laryngeal papillomata have long been associated with viral bodies, no previous attribution to virus has been made in other laryngeal disease. The cytopathic effects are squamous hyperplasia, keratosis or hyperkeratosis, acanthosis, koilocytosis and binucleation or multinucelation. Macroscopically, whether focal hyperplasia of the mouth (Heck's disease), condyloma plana, or common wart, there is a thickened epithelium, with rounded downgrowths. In the larynx, similar appearances are seen in the lesions known as keratitis laryngis. The simple keratitis with or without dysplasia always puzzles pathologists, as surgeons relate that 'stripping the mucosa' may result in cure, if the patient refrains from smoking, drinking and exposure to dusts. Other lesions in other sites, middle ear and mastoid, uvula and nasal cavity may present similar cytopathic effects suggesting human papilloma virus. These are illustrated, as are also any accompanying columnar epithelia. Similar appearances may be found in verrucaous carcinoma, the imitator of non-neoplastic squamous epithelium with remorseless enlargement and destruction of neighbouring tissues.

NEUROENDOCRINE DIFFERENTIATION IN CARCINOMA OF LUNG
J. Rode,1 A. P. Dhillon,1 D. P. Dhillon,2 E. Moss,1 R. J. Thompson,3 S. G. Spiro1 and B. Corrin2
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Pulmonary small cell carcinoma (SCC) is a neuroendocrine type of tumour with particular therapeutic and prognostic connotations. The expression of neuron specific enolase (NSE), PGP9.5 (neuroendocrine markers) and S100 protein (glial marker) was studied by immunohistochemistry in endobronchial biopsy and lung tumour specimens to see if these features have any diagnostic or prognostic implication. Twenty consecutive Zamboni-fixed biopsy specimens of tumour showed 12 cases of SCC (7 positive for NSE, 6 for PG9.5 and 5 for S100 protein). NSE positive cases had a mean survival of 9.1 mth and NSE-negative cases, 3.9 mth. There was no difference in survival between cases staining or not for PG9.5 or S100 protein. All 8 cases of non-SCC stained for all three markers. Formalin-fixed resection specimens of lung tumours were examined. Of 6 SCC, 3 showed staining for NSE, 3 for PG9.5 and 1 for S100 protein. Of 25 non-SCC, 10 showed staining for NSE, 12 for PG9.5 and 6 for S100 protein. Immunostaining for NSE, PG9.5 and S100 protein is of little value in distinguishing SCC and non-SCC. Staining for NSE in SCC may indicate prolonged survival but further study is necessary to establish this. The results of this study emphasise the essential heterogeneity of lung carcinoma and the frequent functional tendency of this tumour to express neuroendocrine features regardless of its apparent histological type.

IMMUNOHISTOCHEMICAL DEMONSTRATION OF ARGININE-VASOPRESSIN IN SMALL (OAT) CELL CARCINOMA OF BRONCHUS
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The clinical syndrome of inappropriate secretion of anti-diuretic hormone (SIADH) has been well described in association with carcinoma of the bronchus. However, the syndrome is also seen in a variety of non-malignant conditions and the view that SIADH associated with malignancy is due to the ‘ectopic’ production of arginine vasopressin
(AVP) by tumour, has recently been challenged. With the use of bio- or radio-immunoassay the levels of AVP reported in these tumours are generally very low. The aims of this study were (i) to demonstrate AVP within bronchial small cell carcinoma cells with the use of the immunoperoxidase technique (PAP) and (ii) to determine the incidence of this phenomenon in clinically unselected cases. An antibody to AVP was produced which showed greater specificity for AVP than oxytocin. Normal human and Wistar rat pituitaries were used as positive control tissues and Brattleboro rat (with inherent diabetes insipidus) pituitaries were used as negative control material. Formalin-fixed pneumonectomy specimens (n=32) containing small cell carcinoma were examined. Three cases showed cells with a strongly positive reaction for AVP. These findings do not of course exclude the possibility that neurophysins produced by tumour tissue are actively binding circulating AVP.

PULMONARY METASTASIS
Julie Crow, G. Slavin and L. Kreeb
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A combined histological and radiological study has been performed on lungs obtained at autopsy to record the frequency and patterns of pulmonary metastasis in 56 patients dying with a malignant neoplasm. Thirty of the 56 (54%) had pulmonary metastases but in only 19 of these were the lesions detected on radiographs of 1-2cm thick lung slices. Metastatic disease most commonly involved the pleura and the outer third of the lung fields. In 9 cases only histological examination was able to identify metastases and the patterns of growth were much more variable than expected, making differentiation from non-neoplastic lesions difficult in some cases. The findings indicate that pulmonary metastasis is likely to be underestimated in the clinical situation even with the aid of CAT scans.

DISTRIBUTION OF GALANIN-LIKE IMMUNOREACTIVITY IN THE RESPIRATORY TRACT OF PIG
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Galanin was originally isolated from the porcine intestine and was later found in the central nervous system and gastrointestinal wall of other mammals including man and rat. Galanin is known to cause dose-dependent contraction of muscle preparations from the rat and to induce a mild and sustained hyperglycemia in dog. Using a specific antiserum to porcine galanin, we have mapped the distribution of galanin-like immunoreactivity in different anatomical areas of the porcine respiratory tract. Fine, varicose nerve fibres containing galanin-like immunoreactivity were seen in the nasal mucosa, in close association with seromucous glands and blood vessels, and occasionally beneath the epithelium. The trachea and principal bronchi also harboured a moderate number of galanin-positive nerve fibres, which were more numerous in the upper airway. These fibres occurred predominantly in airway smooth muscle. Single fibres were observed around seromucous glands and in the adventitia of the blood vessels in the submucosa. A small number of galanin-immunoreactive cells were found in the adventitia of the tracheobronchial wall. Galanin-immunoreactivity was rarely found in the lung tissue. The occurrence of galanin-immunoreactivity in the porcine respiratory tract suggests a possible regulatory role on the functions of the respiratory system. Its discovery in this tissue adds to the increasing number of peptides found in the mammalian respiratory system.

SUDDEN INFANT DEATH SYNDROME (SIDS) AND COMMON BACTERIAL TOXINS: A MATHEMATICAL MODEL
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A mathematical model is presented which is based on the proposition that common toxins produced by bacteria growing in the nasopharynx following a viral infection are a
possible cause of SIDS. Consider common bacterial toxins that 50% of the population meet in any period of x days. Assume that when infants encounter the toxins for the first time the majority become immune, but a small fraction, which is constant, die. Then the % of the population who are susceptible and the number who die will follow an exponential curve with a half life of x days. However since the toxins are common infants will be protected in the early weeks of life by maternal IgG and the probability of SIDS will rise as maternal IgG falls. It is argued that the rise in probability will follow a cumulative frequency distribution function of the random variable which is the amount of toxin absorbed from the nasopharynx in potential cases of SIDS. Then the predicted number of cases of SIDS at any time (t) equals the expected number from the exponential curve at t, multiplied by the probability of SIDS for the maternal IgG level at t. For x=50 days the model produces a close fit to the observed number of cases of SIDS in England and Wales in 1982 (n=1,268).

RAPID DIAGNOSIS OF PNEUMONIA DUE TO PNEUMOCYSTIS CARINII BY BRONCHOALVEOLAR LAVAGE: REPORT OF THIRTEEN CASES
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Pulmonary infiltrates are common in immunosuppressed patients, have a varied aetiology and carry a high mortality. The potential for recovery is increased by early institution of specific treatment. We report the rapid diagnosis of 13 cases of opportunistic pneumonia due to Pneumocystis carinii by cytological examination of bronchoalveolar lavage fluid. Twelve patients were on therapeutic immunosuppression for renal disease or following transplant and one had acute lymphoblastic leukaemia. With the Papanicolaou stain characteristic ‘honeycomb debris’ was present in the fluid and within this diagnostic cystic forms of Pneumocystis carinii were identified by Gram and Grocott techniques. Ten patients recovered. In two of the three that died intranuclear viral inclusions due to cytomegalovirus and Herpes simplex virus were also seen in the bronchoalveolar lavage fluid. The cytological findings in pneumonia due to Pneumocystis carinii are confirmed by histological and ultrastructural study.

PATHOLOGICAL AND IMMUNOLOGICAL RESPONSE OF CALVES TO MYCOPLASMA BOVIS
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Mycoplasma bovis has been shown to produce clinical pneumonia in gnotobiotic calves following intratracheal inoculation. At post mortem 14 or 27 days after inoculation macroscopic lesions involved 5 to 37% of the lung. Stained sections of lung tissue revealed focal areas of necrosis in the lung parenchyma that were surrounded by infiltrating leucocytes. These cells were mostly macrophages and lymphocytes. Lung sections were examined for mycoplasmal antigen by immunoperoxidase staining. M. bovis antigen was demonstrated in the necrotic areas, particularly around the periphery at the interface with the infiltrating leucocytes. Immunoperoxidase staining of lung sections demonstrated IgG1 and IgG2 producing cells amongst the leucocyte accumulations. The lesion in the lung appears to be partly due to a toxic effect of M. bovis and partly to the hosts immune response. The lesion varies from the cuffing pneumonia induced by other mycoplasmas such as M. pneumoniae and M. pulmonis that localise primarily in the ciliated epithelium lining the lower respiratory tract.

TECHNIQUE FOR SIMULTANEOUS MICROSCOPICAL OBSERVATION AND MECHANICAL TESTING OF CONNECTIVE TISSUE
Patricia O'Connor and D. L. Gardner
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Even distribution of load across a joint and low friction during movement are two requirements of normal articular cartilage; these functions are disordered in diseases
such as osteoarthrosis. To achieve these mechanical functions throughout a range of activities requires a complex, heterogeneous structure in which the distribution of collagen and proteoglycan and the size, shape and distribution of chondrocytes vary with spatial location. Using Nomarski differential interference contrast microscopy, fresh, unfixed and unstained 100 μm thick slices of cartilage can be observed during mechanical loading. When a compressive load is applied to the articular surface the cartilage deforms to become thinner in the area under load. The amount of deformation is not uniform throughout the cartilage thickness; it is greatest immediately beneath the surface and least in the middle zones, with the deep zones compressing to an intermediate extent. This variation in compressive strain correlates with known variations in glycosaminoglycan distribution. Work is underway to make direct comparisons between the mechanical response and the distribution of proteoglycans as seen in transmission electron microscopy by cupromeronic blue staining and in light microscopy by alcoholic toluidine blue staining.

CARTILAGE DEGRADATION IN ARTHRITIS: A COMPARISON OF IN VIVO AND IN VITRO MODELS

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Loss of cartilage proteoglycan is of central importance to the pathogenesis of joint destruction in rheumatoid arthritis. We have compared in vivo and in vitro systems with respect to their effects on cartilage matrix proteoglycan. Two types of cartilage were employed: rat femoral epiphyseal cartilage, and bovine nasal septum (prepared as discs). Cartilage was implanted into rats, either subcutaneously or into a previously created air pouch (with or without superadded inflammation). Cartilage was also cultured, alone or together with pouch tissue. Our results show that rat epiphyseal cartilage lost proteoglycan over a ten day period, in vivo and in vitro: the depletion was less marked in vivo, and was inhibited by the presence of inflammation. Culture of the bovine nasal cartilage led to accumulation of proteoglycan in the culture medium, without corresponding loss from the tissue. Similarly, implantation of bovine cartilage subcutaneously or into the air pouch resulted in a gain of proteoglycan which was enhanced by co-existent inflammation. The contrast between the responses seen with the two kinds of cartilage may have several causes, and illustrates the value of comparative studies, and the need for care in evaluating any test system of cartilage behaviour. Our results also suggest that inflammation may have a role in helping to protect cartilage against matrix degradation — if confirmed, this finding would have significant implications for anti-inflammatory therapy in rheumatic diseases.

HOW TO REMOVE TEMPORAL BONE AT NECROPSY AND EXAMINE IT IN HISTOPATHOLOGY LABORATORY

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The removal of the temporal bone at necropsy is illustrated. Further processing in the histopathology laboratory is carried out by microslicing it on a special machine at two or three mm. in a horizontal plane, preparing radiographs of each slice and then embedding in paraffin, celloidin or plastic. Examples of otitis media, paraganglioma, eighth nerve Schwannoma, malformations and otosclerosis in temporal bones prepared by this method will be shown.

IMMUNOPEROXIDASE PRACTICAL FOR STUDENTS

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In the Southampton Medical School, general pathology, which includes basic microbiology and immunology, is taught throughout the first year. Lectures and practical classes on neoplasia come just before the examination in the summer term, well after the completion of the immunology component. To provide variety in the practical classes, to illustrate fundamental principles of the antigen antibody reaction and to emphasize the
role of immunocytochemistry in diagnosis, we have devised an immunoperoxidase technique that can be completed by students within 2 hr. Students were provided with two separate dewaxed paraffin sections. One was of a large intestinal adenocarcinoma and included some normal mucosa. The other contained sections of secondary mammary and secondary prostatic adenocarcinoma. These were stained with commercial primary antibodies to CEA and prostatic acid phosphatase respectively. An indirect peroxidase technique was used with antibody incubation times of 30 min. The whole procedure was performed in plastic petri dishes. DAB was used as chromogen, the sections were counterstained with haemotoxylin and mounted in an aqueous medium. Students were asked to note the distribution of CEA in normal and neoplastic large intestine and to determine which of the two secondary deposits in the second slide came from prostate. At least two-thirds of the students produced good results. The poster will illustrate the equipment necessary for the practical and photomicrographs of slides actually prepared by students. Copies of the practical class sheet detailing the method will be available.

**GUT ENDOCRINE CELLS IN COELIAC DISEASE ESTIMATED BY IMMUNOCYTOCHEMISTRY USING MONOCLONAL ANTIBODY TO CHROMOGRAFIN**

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Abnormalities of gut and pancreatic endocrine responses, as well as changes in the numbers of different types of endocrine cells, have been previously reported in coeliac disease. However, no estimation has been made of the total numbers of gut endocrine cells in well defined groups of coeliacs. In the present study, a monoclonal antibody to chromogranin, a marker for polypeptide-containing endocrine cells, was used to quantify cell numbers in endoscopic jejunal biopsies from 3 groups of coeliacs: a) 9 with active disease, b) 10 on a gluten-free diet, and c) 8 receiving gluten challenge. These were compared with d) 5 normal controls. Quantitative estimation of endocrine cell density (cells/mm²) was made using an image analyser (IBAS - Reichert Jung). The results were: group a) 84.8±36.7, group b) 41.7±13.9, group c) 82.5±34.1 and group d) 42.1±27.0 cells/mm². Thus there is a significant (P=0.005) increment in endocrine cell density in coeliacs with active disease (groups a and c) compared with normal controls and this condition is resolved in treated coeliacs.

**DELINEATION OF NUCLEI FOR IMAGE ANALYSIS**

C. Sowter and R. Jagoe

Clinical Research Centre, Harrow, and Department of Histopathology, St. Bartholomew's Hospital, London EC1

Nuclei for image analysis may be defined by drawing the nuclear boundary using a 'light pen' or cursor. This method is tedious and prone to inconsistency. Total automation is a difficult if not impossible task for most histological material. We describe a method for the semi-automatic boundary detection of hepatocytes. Two points on the longest axis of the nucleus to be measured are indicated with the light pen. A circle with this diameter defines the area of search. This area is scanned along radial spokes to produce a histogram of grey level values from which a threshold value for the rejection of the background is calculated. Points lying within the search area and along the spokes are selected as boundary candidate points by an algorithm which uses the grey level values and changes in grey level gradients of both strength and direction. A tracking algorithm attempts to make linkages between the candidate points and all chains of connected points are stored. From this data a 'best' nuclear boundary is displayed on the monitor for assessment by the operator who accepts or rejects the image. This method has been used in a study of liver cell nuclei and was successful in delineating approx. 85% of normal hepatocyte nuclei and 70% of nuclei from hepatomas. It has been used as the basis for successful automatic delineation of spermatozoa.
HISTOLOGICAL MORPHOMETRY OF ORAL MUCOSA:
SOME STRUCTURAL ALTERATIONS INDUCED BY TOPICAL
ADMINISTRATION OF RETINOL PALMITATE

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Retinoids are powerful modulators of epithelial differentiation which may have potential as antineoplastic agents. We are currently evaluating the effect of retinol palmitate (RP) on hamster cheek pouch mucosa subjected to applications of the chemical carcinogen DBMA using light and electron microscopical morphometry. In this report, we describe some histological characteristics of our model subjected to thrice weekly applications of RP in corn oil. Untreated and corn oil-treated pouches were used as controls. RP-treated animals were sacrificed after 2, 4, 6 and 10 wk of treatment, processed for histology and sections were quantified using a MOP AM03 semi-automatic image analysing system interfaced with a magneto-restrictive digitising tablet. Parameters selected for quantification were epithelial thickness (T), ratio of epithelium to lamina propria (EP/LP) and the volume (Vv) and length (Lv) densities, number per unit area (Nv) and mean transverse sectional area (A) of blood vessels within the lamina propria. A number of significant alterations were detected which were not evident on routine histological examination. When compared with untreated mucosa, values for T were reduced in the corn oil group but were generally elevated in RP groups, whereas the EP/LP ratio was reduced in the corn oil group and elevated in all RP groups. All data for vascular parameters (Vv, Nv, Lv and A) were elevated in treated groups and showed similar trends, with the corn oil control group usually possessing the highest value. We conclude that in the hamster cheek pouch mucosa, retinol palmitate induces a mild epithelial hyperplasia and a substantial increase in the volume of the blood vascular network. This increase in vascularity is produced by increases in both vessel frequency and dilatation. Morphometry is thus a valuable technique for quantifying certain tissue responses to experimental treatments.

MORPHOMETRIC ANALYSIS OF DERMAL INFLAMMATORY INFILTRATE IN CONTROL SKIN AND CUTANEOUS T CELL LYMPHOMAS;
NUCLEAR CONTOUR INDEX AS DIAGNOSTIC AID FOR DETECTION OF MYCOSIS FUNGOIDES

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Mycosis fungoides is a malignant lesion of T cells that primarily affects the skin and the early diagnosis of these lesions greatly improves prognosis. A number of techniques have been used to assist in early diagnosis, including cytogenetics, monoclonal antibody markers and morphometry. Ultrastructural analyses of the nuclear contour index (NCI) have yielded variable results and of the techniques so far evaluated, this seems to have the most useful potential application. In the present report, we describe the results of our analyses of the NCI in a number of control patients (normal N; lichen planus LP and pityriasis lichenoides PL) and in patients with various stages of mycosis fungoides (MF I-V). Following processing of skin biopsies for ultrastructural examination, electron micrographs were obtained from dermal infiltrates and quantified on a MOP AM03 (Kontron) semi-automatic image analysing system. Nuclear perimeters (P) and areas (A) from lymphoid cells were determined. Langerhans cells, small lymphocytes and histiocytes were excluded from the analysis. The NCI was calculated using the formula NCI = P/V. Values of NCI were obtained as follows: N = 4.49; LP = 5.80; PL = 4.49; MF I = 5.06; MF II = 6.68; MF III = 7.05; MF IV = 7.08; MF V = 7.49. When control and MF data were pooled, mean values of 5.27 and 6.70 were obtained for control and MF groups respectively. From our preliminary studies, there would appear to be some diagnostic potential for the numerical characterisation of MF cells in the skin. The progress of the disease is accompanied by a progressive increase in NCI although the use of this single parameter in isolation may not be useful since our data revealed overlap between MF stage I and the infiltrate of lichen planus lesions. The application of multiparameter analysis by determining for example nuclear cytoplasmic ratios and cell contour index in addition to NCI might provide better separation between control and early MF groups.
ULTRASTRUCTURAL QUANTIFICATION OF LYMPHOID CELLS IN MYCOSIS FUNGOIDES: NUCLEAR-CYTOPLASMIC RATIO, NUCLEAR VOLUME DENSITY AND NUCLEAR DIAMETER

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Of several approaches which have been used to assist in the early diagnosis of cutaneous T cell lymphomas, morphometry has been reported to be of potential value. In particular, the atypical infiltrating lymphoid cell with its cerebriform nuclear morphology has attracted attention and a number of research groups have attempted to define an objective indicator of nuclear shape. The commonest shape factor evaluated has been termed the nuclear contour index (see accompanying demonstration). In this preliminary report we hypothesise that there are other morphometric parameters characterising the atypical lymphocytic cells which might have some practical value. Biopsies of skin from normal control subjects and from patients with lichen planus, pityriasis lichenoides and mycosis fungoides were processed for electron microscopy. The lymphoma group comprised 11 patients with lesions of mycosis fungoides, Stages I-V. Using a stratified random sampling strategy, micrographs of lymphoid cells were obtained and with a digitising tablet and a MOP AM03 image analysis system, cellular and nuclear areas were measured. An estimate of maximum diameter was generated automatically. Nuclear cytoplasmic ratio and nuclear volume density were calculated from the area measurements. Generally, both parameters were reduced substantially in MF when compared with normal skin; lichen planus and pityriasis lichenoides lesions possessed intermediate values. Maximum nuclear diameters were consistently elevated in all MF lesions when compared with normal skin. For all parameters some overlap was detected between early MF lesions and the control skin lesions. Reductions in the nuclear cytoplasmic ratio and the nuclear volume density together with increases in nuclear diameter suggest that both nuclear and cytoplasmic enlargement is occurring during the progression of MF. The evaluation of absolute cellular and nuclear volumes may provide a further source of quantitative information which might be helpful diagnostically.

QUANTIFICATION OF INTERMEDIATE FILAMENTS IN NORMAL, HYPERPLASTIC AND PRENEOPLASTIC STRATIFIED SQUAMOUS EPITHELIUM

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80 nm intermediate filaments aggregate into bundles and form extensive cytoplasmic networks in basal, spinous and granular cell layers of stratified squamous epithelium. These components are differentiation products and we hypothesise that they are reduced in transforming epithelial cells since neoplastic transformation can be considered as a failure of cellular differentiation. In this report, we have used stereological intersection counting to quantify volume densities of aggregated intermediate filaments in differentiating strata of normal and carcinogen-treated hamster cheek pouch epithelium. In conjunction with morphometric estimates of cellular volume, we have calculated absolute volumes of filaments on an 'average cell' basis in normal (N), hyperplastic (H) and preneoplastic or dysplastic (D) epithelium. Volume density estimates of filaments were generally lower in hyperplastic and dysplastic epithelium when compared with normal epithelium in each cellular stratum examined. However when related to the 'average cell', the following results were obtained. Basal N=38.8 \( \mu m^2 \); H=51.2 \( \mu m^2 \); D=61.4 \( \mu m^2 \); Spinous N=91.1 \( \mu m^2 \), H=149.7 \( \mu m^2 \); D=148.8 \( \mu m^2 \); Granular N=144.2 \( \mu m^2 \), H=275.1 \( \mu m^2 \), D=435.5 \( \mu m^2 \). We conclude that following carcinogen treatment, epithelial cells increase in size producing a reduction in the cytoplasmic density of intermediate filaments. However, careful calculation of 'average cell' data reveals an increased synthesis of cytoplasmic structural proteins during carcinogenesis. This alteration in phenotypic expression is probably related to the presence of abnormal keratinisation or dyskeratosis which is commonly observed in preneoplastic epithelia.
UNUSUAL GAP JUNCTION VARIANTS IN DMBA-INDUCED EPITHELIAL HYPERPLASIA AND DYSPLASIA

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Gap junctions are cell membrane specialisation in which adjacent cell plasma membranes differentiate to produce protein channels, the connexons, through which direct cytoplasmic communication may be effected. These junctions are found in basal, spinous and granular layers of stratified epithelia in two forms. The peripheral junction is found at the cellular periphery as a straight or slightly curved specialisation whereas the annular junction is found deep within the cytoplasm and is ovoid or circular in profile. During our investigations into the ultrastructural alterations induced in stratified epithelium by the chemical carcinogen DMBA, we observed an increased incidence of annular gap junctions in both hyperplastic epithelium which demonstrated minimal atypical histological features and in dysplasia, in which epithelial atypia was prominent. Annular junctions were detected at all levels within the epithelium and in relation to an individual cell. were apparently randomly distributed throughout the cytoplasm, many lying close to the nucleus. The cytoplasm delineated by the ellipsoidal or circular annular junctions frequently contained organelles which included mitochondria, ribosomal particles, rough endoplasmic reticulum, membrane-coating granules, lipid droplets, small vesicles and often other annular junctions. The presence of the annular variant within the annular junction suggests that there is an unusual structural modification of the epithelial cellular membranes during carcinogen-induced transformation which results in multiple differentiated areas of invagination of one cell into its neighbour. This would produce extensive areas through which intercellular communication could take place although from our morphological study, we cannot be certain that such junctions are functioning normally, i.e. whether the connexons are patent.

THREE-DIMENSIONAL RECONSTRUCTION AND SIMULATED RESECTIONING OF CRESCENTIC RENAL CORPUSCLES FROM SERIAL SECTIONS USING COMPUTER GRAPHICS

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In section, structures of the renal corpuscle, such as the glomerular tuft and the vascular and tubular poles, vary in appearance according to orientation. Three-dimensional structure can be appreciated through conventional reconstruction from serial sections. Such methods are time-consuming and do not allow viewing of cross-sections other than those originally cut. Quantitative study is therefore difficult. A program ‘GLOM’ has been developed which allows the main structures in up to 70 serial sections of the renal corpuscle to be recorded by tracing micrographs on a bit-pad. Structures are colour-coded and successive layers can be aligned manually or automatically. The reconstruction can be viewed from any angle, and re-sectioned in any plane. Quantification of the volume, area and perimeter of the structures is also available. The program is written in Fortran 77 using the GKS graphics subroutines and runs on a VAX/780 mini-computer. One normal and five crescentic glomeruli from a patient with polyarteritis nodosa have been studied. Thinning and rupture of Bowman’s capsule is seen, and the spatial relation of this lesion to glomerular structures can be investigated. The extent of compression of the glomerular tuft by crescentic proliferation can be quantified.

EPITHELIAL MONOCLONAL ANTIBODY IMMUNOSTAINING IN CARCINOID TUMOURS OF APPENDIX

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The origin of gastrointestinal carcinoid tumours has been a matter for debate for a considerable period. Recently the finding of cytokeratin immunostaining in these
tumours has established their epithelial nature and suggested an epithelial origin for these tumours. Therefore we decided to investigate the pattern of immunostaining in carcinoid tumours using five epithelial monoclonal antibodies. Ten carcinoid tumours of the appendix were fixed in formalin, embedded in paraffin wax and 5 μm sections cut and immunostained with antibodies to AuA1 (colonic carcinoma cell line) 5.2 (cytokeratin) HMFG1, HMFG2 (human milk fat globulin) and LP34 (cytokeratin) using the indirect peroxidase technique. Polyclonal antibodies to neuron specific enolase (enzymatic neuroendocrine marker) were also used with the PAP technique for comparative purposes. Results show that all 10 carcinoid stained strongly with antibodies to AuA1, 5.2, HMFG1, while HMFG2 and LP34 gave uniformly negative results. All 10 carcinoids also stained strongly with NSE antibodies. These results confirm that carcinoids of the appendix share epithelial antigens as well as neuroendocrine antigens and the preservation of the antigens in routine fixatives makes possible retrospective studies on gastrointestinal carcinoids.

INTERMEDIATE FILAMENTS IN MERKEL CELL TUMOURS

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The presence of cytoskeletal proteins was studied in a series of 10 Merkel cell tumours (MCT) with a polyclonal antiserum directed against cytokeratin and monoclonal antibodies against cytokeratin, neurofilament and vimentin using the immunoperoxidase procedure. Cytokeratin was demonstrated in 9 out of 10 MCT. Neurofilament was present in the 2 snap frozen tissues tested and could be demonstrated in 3 out of 8 formalin-fixed and paraffin embedded tissues. No reactivity for vimentin was found. By electron microscopy desmosomes were demonstrated in all 7 cases studied, while tonofilaments were only found in 2 cases. Neurosecretory granules were seen in all tumours but were mostly found in low numbers. This study indicates that the MCT represents a poorly differentiated small-cell carcinoma which has the ability to express some neuroendocrine features.

SPECIFIC ENZYME TREATMENT IS REQUIRED FOR INDIVIDUAL MONOCLONAL ANTIBODIES IN IMMUNOHISTOCHEMISTRY WITH FORMALIN FIXED SECTIONS

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Trypsin, pronase, pepsin and chymotrypsin were tested as means of restoring antigenicity in formalin fixed paraffin embedded sections for immunoperoxidase staining with monoclonal antibodies (MC). For example, common leucocyte antigen MC PD7 gave positive results with trypsin, but poorer results with pronase; while C3b receptor MC T05 required pronase treatment and trypsin was ineffective. Individual MCs require testing with a battery of enzymes to determine the best for use in formalin fixed sections.

MONOCLONAL ANTIBODIES SELECTED TO DISCRIMINATE BETWEEN MALIGNANT MELANOMAS AND NEVOCELULAR NEVI

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Two monoclonal antibodies, PAL-M1 and PAL-M2, are described that were selected to discriminate between melanomas and nevocellular nevi (NN) in frozen sections. MoAb PAL-M1 reacted with all 15 melanoma metastases (MM), with 14 out of 19 primary cutaneous melanomas (PCM), 9 out of 35 dysplastic nevi (DN) and 2 out of 26 NN. The two NN stained were removed from patients with the dysplastic nevus syndrome. MoAb PAL-M1 reacted with 9 out of 15 MM, 5 out of 19 PCM, 3 out of 35 DN and did not react with 26 NN after usual staining conditions. The proportion of melanocytic cells stained was low in DN and much higher in PCM and especially in MM. Staining in DN was restricted to intraepidermal or subepidermal nests of atypical melanocytes. In PCM
staining with PAL-M₂ was only observed in tumours with a Breslow thickness of 0.76 mm or higher. PAL-M₁ and PAL-M₂ may be immunohistochemical markers for tumour progression in melanocytic proliferations.

MONOCLONAL ANTIBODY PAL-E SPECIFIC FOR ENDOTHELIUM

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A monoclonal antibody, PAL-E, is described that is specific for endothelial cells. The monoclonal antibody, an IgG₂a, markedly stains endothelium of capillaries, medium-sized and small veins and venules in frozen sections of human and some animal tissues tested. It reacts not or only weakly with endothelium of large medium-sized and small arteries, arterioles and large veins and does not stain the endothelial lining of lymphatic vessels and sinus histiocytes. The cellular staining pattern and tissue staining were different from those obtained with anti-Factor VIII R:Ag antiserum and Ulex Euopeaeus I lectin. Blocking experiments indicated that these three reagents recognize different endothelial binding sites. PAL-E therefore is a new staining reagent for endothelium in frozen sections. Based on immunoelectronmicroscopical observations the antigenic determinant recognized by PAL-E is associated with endothelial vesicles.

ANIMAL MODEL OF WILMS' TUMOUR

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A single injection of 1,2 dimethylhydrazine hydrochloride (DMH) (200 mg/kg s.c.) administered to 40 weanling rats resulted in the development of a high incidence of renal tumours within one year. Most of these closely resembled human nephroblastoma in their bilaterality, size, variegated macroscopic appearance and triphasic histological appearance. Of 36 rats which survived (4 died of acute DMH toxicity), 25 rats developed tumours ranging from small cortical nodules to massive tumours up to 9 cm in diameter and weighing up to 128 g. Bilateral tumours were present in 9 rats (36%) and one rat had metastasis to the lung. Histological evaluation, using the human morphological criteria of Bennington and Beckwith for human nephroblastoma, showed that most of these tumours (73%) exhibited the classical triphasic morphological features of nephroblastoma. Varying proportions of epithelial (glomeruloid and tubular structures), stromal (fibrous tissue and smooth muscle) and blastematous elements were present. The pathological resemblance of these tumours to human nephroblastoma suggests the possibility of using DMH-induced rat renal tumours as a model of Wilms' tumour.

METASTASISING MALIGNANT PHAEOCHROMOCYTOMA IN RAT

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Malignant phaeochromocytoma occurring in the adrenal of two aged male rats are reported. The primary tumours were unilateral and revealed metastasis into the second adrenal, pancreas, liver, spleen, lung, kidney and bone marrow. Metastatic deposits were also seen in a pituitary tumour and in an islet cell tumour of the pancreas. The cells, although resembling normal medullary cells, were smaller with less cytoplasm. The nuclei were generally hyperchromatic. The cells were arranged in nests or sometimes in trabecular form. In general, phaeochromocytomas of rats are not prone to metastasize, therefore the presence of malignant phaeochromocytoma with widespread metastasis was thought to be of interest. In addition, invasion into a pancreatic islet cell tumour and into a pituitary tumour appears to be the first recorded case.
FINE NEEDLE ASPIRATION CYTOLOGY OF HEPATOMA IN THE GAMBIA

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Cytological diagnosis of fine needle aspiration of the liver was used to confirm or exclude hepatoma in 151 Gambian patients. Of 131 with hepatic tumours a correct positive diagnosis was made in 116 (87.2%). Confirmation of the diagnosis was obtained from a combination of isotope scan (44 patients), serum alphafetoprotein, histological biopsy (10 patients) and clinical follow up. Serum alphafetoprotein estimations, which were made by radioimmunoassay were raised (>1,000 μg/l) in 76 (60.8%) of the 125 patients with hepatocellular carcinoma. Tumour cells were present in ascitic fluid from only two patients out of 49 with hepatoma who had paracentesis. The cytological appearances are illustrated. Most of the specimens could be easily recognized as benign or malignant but diagnostic difficulty in distinguishing between well differentiated hepatoma and benign abnormalities resulted in one false positive result and two inconclusive reports. There were no complications due to the procedure which was well tolerated and simple to perform. The technique is particularly suitable for use in countries where HCC is prevalent and limited medical resources exclude histological biopsy as a routine procedure.

DRINK AND THE KUPFFER CELL

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Patients with alcoholic liver disease have impaired clearance of injected radiocolloid as well as endotoxins and microorganisms. Lysozyme (muramidase) positive Kupffer cells were studied by immunoperoxidase in 54 liver biopsies from 41 patients with different forms of alcoholic liver disease, including fatty liver, fibrosis, alcoholic hepatitis and cirrhosis. Fifteen histologically normal livers from non-alcoholic patients provided control material. Positive cells were counted by means of an eyepiece graticule, in 5 high-count and 5 low-count areas of each biopsy. There were fewer positive Kupffer cells in alcoholic patients than in normal controls, in patients with alcoholic hepatitis compared with those without hepatitis, and in patients with cirrhosis compared with those without cirrhosis. All these differences were statistically significant (p<0.01). The findings may reflect a change in the number of a sub-population of hepatic sinusoidal macrophages or in their activity, and support the clinical observation of impaired phagocytic activity. Loss of functioning Kupffer cells may contribute to the pathogenesis of alcoholic liver disease.

CASE OF HUMAN CHLAMYDIAL PLACENTITIS CAUSING ABORTION

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Chlamydia psittaci is well known to be a major cause of abortion amongst sheep. It has been suspected of causing abortion in man, but this has not been conclusively proven in the few cases reported. In our case, a pregnant sheep farmer's wife who, sometime after helping with lambing, spontaneously aborted at 28 wk gestation. Chlamydia psittaci was cultured from fetal blood and tissues, and post partum the mother had rising titres of IgM and IgG antibody to C. psittaci. Sheep on her farm were found to be carriers of C. psittaci. The severe placentitis present was similar to that found in infected aborted lambs. Numerous chlamydial inclusions were visualised in the trophoblast using H & E, methylene blue and Giemsa stains. Immunoperoxidase labelled antibody to ovine C. psittaci also indentified the inclusions. Electron microscopy showed the ultrastructure of the inclusions to be that of C. psittaci. This is an important case because it demonstrates conclusively that ovine C. psittaci, common in sheep in the UK, can cause abortion in
man. Pathologists should be aware of this possibility and of the relatively simple methods used to identify the organism in formalin fixed material.

ANATOMICAL RELATIONSHIP BETWEEN PEPTIDE-CONTAINING AND CLASSICAL NEURAL COMPONENTS IN THE PARACERVICAL GANGLION OF RAT STUDIED BY IMMUNOCYTOCHEMISTRY

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At the transition of rat uterine cervix to vagina, the paired paracervical ganglia (Frankenhaus) are embedded in parametrical connective tissue, attached bilaterally to the utero-vaginal muscle coat. The ganglia receive a lumbar sympathetic supply and a sacral parasympathetic supply and are known to contain both adrenergic and cholinergic neurons. In addition to these classical ganglion cells, the paracervical ganglion also includes peptide-containing neurons. Little work has been carried out to establish the relationship between the different types of neurons within the ganglion. We have studied some of these relationships and using immunocytochemistry we demonstrated vasoactive intestinal polypeptide (VIP), neuropeptide tyrosine (NPY), calcitonin gene-related peptide (CGRP), dopamine-β-hydroxylase (DBH) and neurofilaments protein triplet (NF) immunoreactivities in this region. The VIP, NPY, DBH and NF immunoreactivities were located in ganglion cells and nerve fibres whilst CGRP immunoreactivity was localized only in nerve fibres found in certain regions of the ganglia. Consecutive sections stained with VIP, DPH and NPY revealed that many cell bodies immunoreactive with DPH antiserum were also immunoreactive with NPY antiserum whilst a small number of cells immunoreactive with VIP antiserum were also immunoreactive with NPY antiserum.

VISUALISATION OF INNERVATION OF PERIPHERAL ORGANS BY USE OF ANTIBODIES TO NEUROFILAMENT PROTEINS, NEURON SPECIFIC ENOLASE, GLIAL FIBRILLARY ACIDIC PROTEIN AND 2-100

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Until recently, it was very difficult to visualise all components of the innervation of peripheral organs in single tissue sections. In a comparative study, we have used monoclonal and polyclonal antibodies to neurofilament (NF) proteins, neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP) and S-100 to demonstrate nerves, ganglion cells and the supportive glial system in various organ systems. These included the female genitalia, the urinary system, the respiratory tract, the pancreas, the heart and the skin of several mammalian species including man. Immunocytochemistry was carried out by use of indirect immunofluorescence, peroxidase anti-peroxidase and immunogold-silver staining techniques on serial cryostat or wax sections fixed in benzoquinone solution or Bouin's fluid. In tissues of the different species examined, no differences in the general staining characteristics were found, and the main distribution of immunoreactive cells and fibres was very similar. Antibodies to NF proteins were found to reveal the innervation specifically, whereas antibodies to NSE allowed the demonstration of both neural and endocrine components. Antibodies to S-100 seemed to be a good marker for the detection of Schwann- and other glial cells, whereas antibodies to GFAP demonstrated a sub-population of glial cells.
CGRP-IMMUNOREACTIVE NERVES IN TONGUE OF RAT

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The tongue presents a rich peptidergic innervation subserving possible motor and sensory functions. Recently, calcitonin gene-related peptide (CGRP) was found widely distributed in sensory and motor nerves of the upper part of the digestive tract, such as in palate, pharynx and oesophagus. In this study, we investigated the distribution, nature and possible origin of immunoreactive CGRP in the tongue of rat, both by immunocytochemistry and radioimmunoassay. Numerous CGRP-immunoreactive fibres formed a dense plexus in the lamina propria; some of these fibres entered the papillae (foliate, fungiform and circumvallate), innervating the epithelium and taste buds. CGRP-immunoreactive nerves were also observed in the muscle layer and around the blood vessels of the tongue. After selective denervation of the tongue (trigeminal, glossopharyngeal and hypoglossal nerve section) there was a decrease of CGRP immunoreactive fibres in the mucosal layer. Following systemic capsaicin treatment, a substance toxic for sensory neurons, CGRP-immunoreactivity depletion was observed, particularly in the epithelium and around taste buds. These data indicate that part of the CGRP-immunoreactive nerves might have a role in the sensory function of the tongue. However, further studies will be needed to clarify the possible motor component of these nerves.

CORRELATION BETWEEN TESTICULAR EFFECTS PRODUCED IN VIVO AND IN VITRO BY ETHYLENE GLYCOL MONOMETHYL ETHER (EGME) AND 2-METHOXYACETIC ACID (MAA) IN RAT

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Ethylene glycol monomethyl ether (2-methoxy ethanol; methyl cellosolve: EGME) is a water soluble organic solvent used extensively in industry. Recent work has shown that this compound can exhibit teratogenic, haematological and testicular effects in a number of species by a variety of routes of administration. The purpose of the present study was to compare testicular toxicity produce by EGME and its major metabolite, 2-methoxyacetic acid (MAA) after a single oral dose in vivo and on Sertoli-germ cell cultures in vitro. Mature male rats were given EGME (500mg/kg) or an equimolar dose of MAA by gavage. Control animals received an equivalent volume of the water vehicle (5ml/kg). Groups were killed 3, 6, 12 or 24 hr after treatment. Testicular effects with both EGME and MAA were first observed after 6 hr when early degenerative changes were evident in late pachytene spermatocytes in tubules staged at IX, X and XI. By 12 hr early pachytene spermatocytes were affected and at 24 hr most of the pachytene and secondary spermatocyte populations exhibited degeneration or cell loss. In vitro, only MAA produced preferential effects on pachytene spermatocytes at doses approximating to steady state plasma concentrations (5mM) after a testicular toxic dose of EGME (500mg/kg). Thus the metabolite (MAA) would appear to be responsible for the testicular damage produced by EGME with a good correlation between in vivo and in vitro response.

EXAMINATION OF ORGAN OF CORTI

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To prevent autolysis of the hair cells of the organ of Corti it is necessary to perfuse the perilymph space with fixative. This may be carried out even up to 36 hr after death with useful results. Perilymph perfusion is performed by opening the tympanic membrane, removing the stapes and footplate from the oval window, incising the round window membrane and injecting 2% glutaraldehyde solution into the vestibule. This procedure may be performed directly on the cadaver, or after the temporal bone has been
removed. The temporal bone is microsliced at 2 mm, the tectorial membrane is extracted and pieces of basilar membrane from all three coils carefully removed and examined after exposure to osmic acid solution or staining by toluidine blue. In bones perfused within three hours after death scanning and transmission electron microscopy can be carried out on the organ of Corti.

CELLULAR INFILTRATION, FIBRIN DEPOSITION AND FIBROSIS IN MESENCHYMAL INFLAMMATION

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Most chronic inflammatory disorders like rheumatoid arthritis, where inflammation persists within a mesenchymal space, are characterised by cellular infiltration, fibrin deposition and fibrosis. Are these the result of a single disease mechanism or a combination of related processes? We examined this question using the rat air pouch model of inflammation. We studied 3 groups of rats with non-inflamed pouches and inflammatory pouches induced by pertussis vaccine and carrageenan. There were 58 animals studied over a 30 day period. The control pouches showed the development of a mononuclear cell lining with a mild fibrotic reaction. The inflammatory models showed a polymorph infiltrate and later mononuclear cell infiltrations. Only the pertussis model showed fibrin deposition and this occurred early in the course of the reaction. There were distinct differences in the fibrotic reaction induced by pertussis vaccine and carrageenan. The latter model showed marked collagen changes with both considerable increases in the amount of collagen and also a change in the staining properties of some areas of collagen and the appearances of fibrinoid like changes. We conclude that different inflammatory stimuli produced grossly different reaction in the simple rat air pouch. There is also evidence of its association between inflammatory cell infiltration, fibrin deposition and fibrosis.

SERUM LEVELS OF PREGNANCY-ASSOCIATED \( \alpha_1 \)-GLYCOPROTEIN (\( \alpha_1 \)-PAG) DURING PREGNANCY IN AUTOIMMUNE THYROID DISEASE: RELATIONSHIP TO DISEASE ACTIVITY

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Serum concentrations of \( \alpha_1 \)-PAG were measured by an enzyme-linked immunosorbent assay serially during 24 pregnancies in 18 patients with Graves' disease and 4 with Hashimoto's thyroiditis. During each trimester, \( \alpha_1 \)-PAG levels were significantly higher than in normal pregnant controls, matched for week of gestation. Remission of disease activity was associated throughout pregnancy with progressively higher \( \alpha_1 \)-PAG levels than those in patients with active disease. The data suggest that \( \alpha_1 \)-PAG may play an important role in inducing and maintaining the clinical remissions observed in some women with autoimmune thyroid disease during pregnancy.

INHIBITION OF CONTACT SENSITIVITY REACTIONS TO DNFB BY TOPICAL CYCLOSPORIN APPLICATION IN THE GUINEA PIG

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Contact sensitivity skin reactions to dinitrofluorobenzene (DNFB) in the guinea pig were inhibited by twice daily topical application of cyclosporin (CsA; 2% w/v) commencing at the time of skin testing. Suppression of the characteristic mononuclear
inflammatory cell response was observed both in normal guinea pigs and in animals with enhanced skin reactions following pretreatment with cyclophosphamide. In contrast to oral administration of CsA (25 mg/kg) for four days, topical application of the drug over the same period did not result in systemic absorption, as measured by radioimmunoassay, or in any evidence of nephrotoxicity.

CYCLOSPORIN-INDUCED FETOTOXICITY IN RAT
P. A. J. Brown, Elizabeth S. Gray, J. G. Simpson and A. W. Thomson

We have found that cyclosporin A (CsA; 25 mg/kg/day) administered orally to either syngeneically or allogeneically-mated rats throughout pregnancy leads to a high incidence of fetal death. This phenomenon has been examined further in non-inbred Sprague-Dawley rats given the same dose of CsA during different phases of gestation, the effects on the outcome of pregnancy being ascertained on day 18. CsA given from days 1 to 7 caused a small but significant reduction in litter size, with no significant increase in the number of resorptions. When the drug was administered from day 8 to 14 there was no significant change in litter size but a very striking increase in the incidence of resorptions. This fetotoxic effect was also evident but less marked when the drug was withheld until day 15. Reduction in fetal weight was only present in the group given CsA from days 8-14. In surviving fetuses the presence of focal decidual necrosis was more frequent in mothers receiving CsA, suggesting a possible mechanism whereby CsA may mediate its fetotoxic effects.

LYMPHOCYTE SUBSETS AND LANGERHANS CELLS IN EPICUTANEOUS PATCH TESTS SHOWING ALLERGIC AND IRRITANT REACTIONS: HISTOMETRIC STUDIES
J. Ferguson, J. H. Gibbs and J. Swanson Beck

The study has attempted to distinguish between 'allergic' and irritant reactions to patch tests by quantitative histological methods. The extent of perivascular chronic inflammatory infiltrate at 72 h in 'irritant' patch test reactions to sodium lauryl sulphate was shown to be small and very consistent, whereas in 'allergic' reactions to nickel sulphate it was generally larger and more variable in size (p<0.02). The two major lymphocyte subsets (T4 and T8) were randomly intermixed in both types of reaction and formed the major component of both the perivascular and diffuse dermal infiltrate, without any evidence of selective migration. The T4:T8 ratios were similar in focal and diffuse infiltrates. The number of T6 dendritic (putative Langerhans) cells in the epidermis was usually greatly reduced in 'irritant' reactions, but remained within normal limits in 'allergic' reactions (p<0.001). Comparable results were seen with other irritants (mercuric chloride and benzalkonium chloride) and other allergens (neomycin sulphate, ethylene diamine and potassium dichromate). In additional experiments pairs of biopsies were taken from the reaction and from adjacent unaffected skin. The T6 cell density in the epidermis was slightly greater in 'allergic' reactions than in control skin (not significant). By contrast the 'irritant' reactions had fewer T6 cells than the control skin (p<0.001).