Manual of histological techniques

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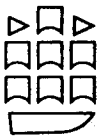

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1984

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by Churchill Livingstone Inc., 1560 Broadway, New York, N.Y. 10036, and by associated companies, branches and representatives throughout the world.

First published 1984

ISBN 0 443 02870 2

British Library Cataloguing in Publication Data
Bancroft, John D.
Manual of histological techniques.
1. Histology, Pathological — Technique
I. Title II. Cook, Harry C.
616.07'583 RB43

Library of Congress Cataloging in Publication Data
Bancroft, John D.
Manual of histological techniques,

Includes indexes.
1. Histology — Technique. 2. Histology — Laboratory manuals. I. Cook, Harry C. II. Title. [DNLM: 1. Histology — Laboratory manuals. QS 525 B213m]
QM 556.B28 1984 616.07'583 83-26173

Printed in Great Britain by the Pitman Press, Bath

Foreword

Modern histopathology is making increasing demands on laboratory scientific staff both in the quality and quantity of specimens with which they have to deal. Histologists have always enjoyed the use of a wide range of dyes to produce both attractive and informative preparations. However, in more recent times, a more precise scientific logic has been applied particularly with the introduction of enzyme histochemical techniques and immunocytochemistry. The histopathologist now frequently sets out to identify more precisely a particular substance within a cell or tissue which has been suggested by an appearance within a haematoxylin and eosin preparation. Fortunately the trend has been for greater co-operation between the medical and technical staff in the investigation of such problems and indeed both can learn from each other. All the more reason, therefore, why workers in this field need to be able to use their knowledge of staining techniques to solve problems posed by the histopathologist. This book, which has been prepared by two experienced senior members of their profession, is arranged in such a way that each chapter attempts to provide the basic answers to a particular line of enquiry. It is intended to be, and certainly should be, used as a bench book. The fact that one of the authors is the Chief Examiner for the Institute of Medical Laboratory Sciences in Cellular Pathology, and that both are active teachers in their profession, should reassure those readers who are concerned with the acquisition of knowledge for examination purposes.

Nottingham, 1984 David R. Turner

Preface

In writing this book we have tried to meet three objectives. Firstly, to integrate two existing texts; Cook's *Manual of Histological Demonstration Techniques* and Bancroft's *Histochemical Techniques*. Within this integration one of our aims has been to produce a textbook suitable for courses in cellular pathology in the United Kingdom and similar histological courses elsewhere. The second objective was to produce a book that is an introduction to, and a practical companion of, Bancroft and Stevens: *Theory and Practice of Histological Techniques* (Churchill Livingstone 1982), which is designed as a comprehensive reference work. Our final and major aim has been to produce a laboratory manual containing standard and occasionally non-standard methods. In attempting to meet these objectives we have tried to appeal to qualified and non-qualified personnel alike and to anticipate the staining techniques required in a busy histological laboratory.

This manual is designed to cover demonstration techniques only; the other specialised techniques are to be found in *Theory and Practice*.

Whenever possible, the theoretical aspects of the topic have been discussed, and where relevant, the associated pathology has been included.

Nottingham and Isleworth, 1984 J.D.B.
H.C.C.

Acknowledgements

The authors wish to acknowledge the assistance of the contributors to Bancroft and Stevens: *Theory and Practice of Histological Techniques* and other professional colleagues: in particular, Pat Chapman for helpful suggestions on clinical cytology, Dr Paul Forster and Dr James Lowe for comments on histology and pathology. Helpful advice resulted from discussions during the preparation of the text with Brian Chalk and Stanley Terras.

Secretarial assistance was provided by Audrey Cook, Flora Ottawa and Jane Burgass; we are grateful for their untiring efforts. Our thanks go to Dr Carol Tinkler for her patient proof reading and considerable help with the index.

We wish to thank colleagues in our departments for helpful advice and comment; additionally, thanks are due to Professor D. R. Turner and Dr A. Knudsen for their tolerance during the writing of the manuscript.

Contents

1

Fundamentals of normal histology and histopathology

A thorough preparation in normal histology is a prerequisite for the successful practice and study of histological technique. To achieve this it is necessary to have recourse to the appropriate textbooks and more importantly, stained sections. The following pages are intended as a reminder of the basic elements that form the various organs and structures of the body and are intended to complement the demonstration techniques to be covered. Equally important, is an elementary knowledge of pathological terms and lesions so that the employment of the various demonstration techniques is firmly related to the diagnosis (see Table 1.2).

Because the techniques later described are those at light microscope level the ultrastructure of normal and pathological tissues will not be dealt with.

NORMAL HISTOLOGY

TYPES OF TISSUE

The following descriptions apply to formalin fixed paraffin sections stained by haematoxylin and eosin.

EPITHELIUM

This type of tissue forms glands, and lines surfaces (Table 1.1). There are three main subdivisions:

1. *Simple (non-stratified)*

Squamous (syn. pavement). There is an abundant flattened cytoplasm and in profile an elongated, central, darkly staining nucleus in an extended cytoplasm found, e.g. in loop of Henle in the kidney.

Two other cells have a similar histological appearance but are mesenchymal in origin (as opposed to endodermal or ectodermal as is usually the case); these are the endothelial and mesothelial cells found lining blood vessels and serous membranes respectively.

Cuboidal. These are small round cells with a central nucleus found, e.g. forming the walls of small, glandular intralobular ducts, also renal tubules.

Columnar. This can be found as either 'low' or 'high' columnar and is an elongated cell having a basal nucleus. It usually contains mucin and is found, e.g. as the lining epithelium for much of the alimentary tract (stomach onwards). In the

Table 1.1 Epithelium: summary of types and location

Type	Location
Simple	
Squamous	Lung, kidney
Cuboidal	Kidney, glandular ducts
Columnar	Stomach, large and small intestine
Ciliated	
Pseudostratified columnar	Bronchus, trachea
Stratified	
Squamous keratinized	Epidermis of skin
Squamous nonkeratinized	Oesophagus, cervix
Transitional	Urinary bladder, ureters
Columnar	Large ducts, urethra
Mixed columnar-cuboidal	Epididymis
Glandular	
Tubular	Stomach, uterus
Acinar	Pancreas, salivary glands
Tubulo-acinar	Prostate

small intestine the free border of the columnar cells has a so-called 'brush border', which is a layer formed of microvilli, which appears as a darker staining line at the free surface.

Ciliated. Both cuboidal and columnar cells may carry on their free border fine hair-like processes termed *cilia*, and may be found lining organs such as the Fallopian tubes and uterus.

Pseudo-stratified ciliated columnar. This type of epithelium is synonymous with respiratory epithelium, and consists of somewhat misshapen ciliated columnar cells which, although they at first sight appear to be in layers, in reality extend from the basement membrane (basal lamina) to the free surface.

Goblet cells. A somewhat ubiquitous cell it is abundant wherever there is copious mucus production, e.g. mucous membranes of the respiratory tract and the small and large intestine. It is a large bulbous cell having a dilated cytoplasm filled with mucin and possesses a basal nucleus.

2. *Stratified (multilayered)*

Squamous keratinized. This forms the epidermis of skin and consists of a varying number of layers depending on the site, e.g. 'thin' skin as obtained from the inner aspect of the thigh, whilst 'thick' skin is exemplified by that from the solar aspect of the foot.

The deepest layer is columnar in type compared to the middle layers (the so-called 'prickle cell' layers) which are polyhedral in shape and bear desmosomal spines, a feature which is artefactually exaggerated in conditions of epidermal oedema. The superficial layers are more classically squamous in shape with an uppermost granular layer, the cells of which contain haematoxylophilic keratohyalin granules. Finally, there is a non-cellular keratinous layer, the stratum corneum. (There is also allegedly a 'stratum lucidum'; we have yet to see a convincing example in routinely prepared skin.) Also to be found in epidermis are melanoblasts, melanocytes and melanophores, together with Langerhans cells — a type of histiocyte.

Squamous non-keratinized. This is similar in structure to the foregoing but it lacks a keratinized layer and granular layer. It is found in a number of sites, e.g. ectocervix and oesophagus.

Transitional is composed of cuboidal-like cells. It resembles on casual inspection the stratified squamous epithelia, but more careful examination reveals more uniform cells, i.e. the basal cells are only slightly more columnar than the most superficial cells, in addition, 'prickle', granular and keratinized layers are lacking.

Columnar. True stratified columnar epithelium is only found in a few situations; these include the urethra and the major ducts of the salivary glands.

Mixed stratified. Occasionally a type of epithelium is seen which does not easily fit into one of the above categories. Such an epithelium occurs in the epididymis, where the tubules are lined by tall columnar cells, bearing stereocilia, with an underlying uniform layer of cuboidal-type cells.

3. *Glandular*

Tubular. These glands may be coiled or straight and have wide lumina lined by large columnar-type cells, with basal nuclei. This type of gland is found in the stomach and endometrium.

Acinar (syn. alveolar). The component cells have a broad base and narrow apex with the nucleus occupying a basal position. Each group of cells or acinus has an ill-defined central ductule which communicates with intra and extralobular ducts. The pancreas and the salivary glands are good examples.

Mixed tubulo-acinar. As the name indicates, these are formed of mixtures of both of the above gland elements. The prostate shows this type of feature well.

MUSCLE

Skeletal muscle (syn. striated, voluntary)

This is the main motor tissue of the body and is under the direct control of the central nervous system. Examination of H and E stained sections with the light microscope gives only limited information as to structure and composition, enzyme histochemistry and electron microscopy being necessary for systematic study. Skeletal muscle consists essentially of adjacent fibres varying in length from 1–40 mm, and in diameter from 10–100 μm. Each fibre is surrounded by a thin membrane, the sarcolemma, which encloses the

cytoplasm or sarcoplasm. Nuclei are multiple and peripherally situated, a feature which is most easily seen when the fibres are in cross section.

Within the sarcoplasm are longitudinal myofibrils and transverse striations. The latter cross striations consist of alternating light I discs with a narrow central darker Z band, and darker A discs having a lighter central H band.

Skeletal muscle is rich in mitochondria and glycogen, and in H and E preparations usually stains a brighter red colour, compared to the other types of muscle.

Cardiac muscle

Under this heading may be considered two different muscle fibres, the contractile heart muscle per se and the specialized conductor muscle which initiates and propagates heart contraction. The former type of muscle is similar to skeletal in having longitudinal myofibrils and cross striations (although these are less evident in H and E type preparations), but differ in that they branch and anastomose and are somewhat shorter (100–150 μm). Present in the sarcoplasm in the Z band region are intercalated discs which mark the cell boundaries. The nuclei are single and centrally placed. Mitochondria and glycogen are again abundant, and granules of lipofuscin are usually present in older age groups. The conducting system of the heart comprises the SA node, AV node and bundles of His, also the interventricular Purkinje fibres. These are not easily seen in human heart and, indeed, careful dissection is needed in order to locate the various constituents (Hudson, 1963).

Broadly speaking the muscle cells of the nodes are somewhat smaller and more compact than the main cardiac muscle cells, whilst the Purkinje fibres are composed of larger somewhat ovoid muscle cells, which have a perinuclear centre rich in glycogen. But it must be stressed again, that experience (and patience) are needed to successfully visualize the conductor system of the heart.

Smooth muscle (syn. involuntary, nonstriated)

Muscle controlled by the autonomic nervous system has a wide distribution, being found in the walls of the alimentary, respiratory and genito-urinary tracts. As the name implies, there are no cross striations in the sarcoplasm of these cells, only longitudinal myofibrils. Typically smooth muscle cells are 'cigar' shaped in that the poles are somewhat tapered. The nuclei are single and centrally placed, and the sarcoplasm contains fewer mitochondria and glycogen granules compared to the other muscles. The overall size, too, is less being in the order of 20–100 μm in length.

NERVOUS TISSUE

The microscopic study of both the central (CNS) and peripheral (PNS) nervous systems is best carried out with the aid of special techniques — usually silver impregnation on frozen or celloidin embedded material. However, a certain amount of detail can be visualized in H and E stained paraffin sections and this will be described.

CNS

This comprises the brain and spinal cord.

Neurones. The larger neurones such as the Betz cells of the cerebrum, Purkinje cells of the cerebellum and the anterior horn cells of the spinal cord have well-marked characteristics. There is a large cell body with discrete granules of ribonucleic acid termed Nissl substance and, as with cardiac muscle fibres, granules of lipofuscin pigment may be present in tissue from the older age-groups. The nuclear membrane and chromatin are ill-defined but there is a prominent nucleolus. (Myelinated nerve fibres stain pink in the H and E unlike the non-myelinated which may hardly stain at all.)

Small or medium size neurones have comparatively indistinct cell bodies and are often shown solely by their nuclei which, compared to the large neurones, tend to have a more distinct chromatin pattern.

Neuroglia. There are several different types of glial cell having a variety of functions, varying from supportive (the astrocytes), ventricular lining cells (ependymal) to myelin formation (oligodendrocytes). Another cell, the microglial cell is, embryologically speaking, not a true neuroglial element being derived from mesenchyme and is usually included because of its possession of

processes; it is a reticulo-endothelial (RE) cell and thus has a marked phagocytic function.

Astrocytes. The two members of this group — fibrous and protoplasmic — are indistinguishable in a routine H and E, and indeed, show virtually no cytoplasmic detail or evidence of processes. Their position is solely indicated by their round to ovoid moderately-sized nuclei, having a well marked chromatin structure. Special stains reveal abundant, substantial processes.

Ependyma. These cells are largely found lining the ventricles of the brain and appear as ciliated cuboidal-like cells. Unlike the other neuroglial cells, special methods do not materially assist in their identification.

Oligodendrocytes. These are the smallest cells of the neuroglial system and appear not unlike lymphocytes in an H and E stained section having a scanty cytoplasm and a small round pyknotic nucleus. Special methods reveal these cells to have scanty, relatively small processes.

Microglia. As is the case with the astrocytes, H and E staining shows only the nuclei to advantage which are intermediate in size between astrocytes and oligodendrocytes. Unlike those cells the microglial nucleus is more irregular in shape and usually more elongated. Special stains reveal a somewhat elongated cell body with short processes extending from each pole.

Axons. These are the nerve fibres which comprise the main substance of both grey and white matter. They vary considerably in both length and diameter; fine fibrils, the *neurofibrils* can often be demonstrated within the nerve fibre.

Axons of white matter are surrounded by a dense fatty sheath termed *myelin*. By contrast, the fibres in the grey matter are largely nonmyelinated.

PNS

The nerve fibre appearance is very much that found in the CNS excepting that they may be formed into discrete nerve bundles with accompanying specialized connective tissue. The nerve cell bodies or ganglion cells as they are termed in the PNS tend to be modelled on the larger type of CNS neurone, although the Nissl substance is less pronounced. Lipofuscin is often a prominent feature. Posterior root ganglia of the spinal cord are formed of sensory neurones and their processes the nerve fibres, but unlike the CNS there is an abundant supporting framework of collagen fibres.

Nerve endings, either sensory or motor, are not usually distinguishable in H and E preparations. Exceptions to this rule are the Pacinian corpuscles, which are sensory end organs found prominently in the deeper layers of skin, and neuromuscular spindles, another form of sensory nerve ending found in the skeletal muscle.

The former nerve ending is often quite large (up to 3 mm in diameter) and fairly readily identified in that it is not unlike an onion in appearance, consisting as it does of concentric pale-staining layers of connective tissue surrounding a central nerve fibre. By contrast, neuromuscular spindles are much less easily found; they appear as a pale-staining area in muscle, which on closer inspection is made up of thin muscle cells and nerve cells, invested and surrounded by fine connective tissue.

CONNECTIVE TISSUE

The role of connective tissue is largely that of support, and because this supporting function is a variable one depending on the structure involved, so does the form vary in appearance, elasticity and strength. In addition to this supporting function there are certain types of connective tissue which have a distinct metabolic role, e.g. adipose tissue.

Associated with the tissue entities which comprise connective tissue, there are several different types of cell which are in environmental association. These cells are as follows:

Plasma cells are found in lymphoid tissue, spleen, bone marrow. The plasma cells have an ovoid cytoplasm and eccentric nucleus with prominent radial chromatin. They are derived from B lymphocytes and secrete immunoglobulins.

Mast cells. Occurring in loose connective tissue such as that of the wall of the large and small intestines, these cells are small with a large central nucleus and prominent cytoplasmic basophilic granules (note that human mast cell granules are not easily seen in H and E stained sections).

Fibroblasts. These cells occur wherever there are collagen fibres, e.g. dermis of skin and submucosa

of the alimentary tract, and are relatively large elongated cells having an indistinct cytoplasm and a large elongated pale-staining nucleus. Fibroblasts produce the protein tropocollagen which is the precursor substance for collagen.

Histiocytes are found in similar situations to fibroblasts. These are round to oval cells intermediate in size between a fibroblast and a mast cell. The cytoplasm stains weakly as does the nucleus which in shape is typically oval or indented (kidney shaped). They have a phagocytic role.

Blood cells. In non-haemopoietic tissue these comprise neutrophils, lymphocytes and eosinophils and have the classical normal features of the circulating blood cells. They may be seen to greatest advantage in loose connective tissue such as the lamina propria of the alimentary tract.

TYPES OF CONNECTIVE TISSUE

Collagen and reticulin

Using special biochemical techniques it is current practice to classify collagen into subgroups I to V. So for example, conventional collagen found in dermis or colonic wall is type I, whilst reticulin is type III; this will be referred to again in subsequent chapters. However, purely from a standard morphological point of view, collagen presents as a non-branching coarse fibre (maximum diameter 100 μm) made up of fibrils which stain bright pink with eosin, whilst reticulin fibres are much finer (up to 1.5 μm) and branch freely, needing special silver staining to be clearly delineated.

The latter fibres whilst often present in association with collagen may be found as a separate entity lining the liver sinusoids or investing lymphoid tissue.

Elastic tissue

This connective tissue is formed of branching fibres of varying diameter (1–10 μm). They possess the ability to expand or contract, and are thus to be found wherever this property is required, e.g. blood vessels, skin and lung. They stain intensely pink with eosin.

It is interesting that whilst the formative cells for most connective tissues are known, that for elastic fibres has yet to be firmly established, although the weight of opinion seems to favour a modified fibroblast.

Loose connective tissue (syn. areolar tissue)

Found as a 'filling' between muscles and fascia also as the lamina propria of alimentary tract, this is a composite tissue formed of fine collagen, elastic and reticulin fibres. Also present are the connective tissue cells described earlier and to a variable degree, a mucoprotein ground substance.

Cartilage

Essentially this is formed of polygonal chondrocytes which lie in varying sized groups in the lacunae (spaces) of a dense mucoprotein matrix. There is a varying fibre content. Three forms are recognised:

Hyaline. The matrix is low in fibre content which is principally collagenous. The articular cartilage of bone is comprised of this tissue.

Fibrous. The collagen fibre content of the matrix is high giving greater strength. Fibrocartilage forms the intervertebral discs.

Elastic. The main fibres present are elastic in this less common cartilage which forms the epiglottis and pinna of the ear.

Bone

Structurally, bone presents either as dense compact material such as is found forming the cortex of long bones, or as the cancellous (spongy) tissue of the medulla of the long bones. The latter encloses the cells of the bone marrow. In either case, bone is essentially collagen and ground substance termed osteoid impregnated by various calcium salts.

The mature bone cells are termed osteocytes, and have small dense round to elongated nuclei with an ill defined cytoplasm. These cells lie in bone spaces called lacunae, which interconnect by means of fine channels — the canaliculi.

Active or growing bone is evidenced by the presence of bone-forming cells — the osteoblasts — and multinucleated bone remodelling cells — the

osteoclasts. Both cells are found principally at the interface of bone trabeculae with the bone marrow.

Adipose tissue

Depot fat of the body contains the major proportion of adipose tissue such as in the mesentery and omentum of the abdominal cavity, and the subcutaneous layer of the skin.

The individual fat cells have a large vacuolated (in paraffin sections) cytoplasm with a thin nucleus compressed to one side. Fine collagen or reticulin fibres surround the cells which are formed into lobules. Small blood vessels run in the somewhat denser collagen fibres of the interlobular tissue.

Lymphoid tissue

It is convenient to describe lymphoid tissue in this context, as while its function is far removed from that of being merely supportive many of the cellular elements of lymphoid tissue and the supportive connective tissue have a common origin. It is found to greatest extent in lymph nodes, spleen and large and small intestine.

The following necessarily oversimplified description of this complex and controversial tissue is merely an attempt to highlight the more important constituents. In addition it must be appreciated that many of these constituents are not readily identified if only conventional paraffin H and E preparations are available.

A typical lymph node exhibits the following structure:

Cortex. An inactive gland shows follicles containing small lymphocytes (B type) tingible body macrophages and dendritic reticulum cells; the former cells are round with pyknotic nuclei and the latter two cells are much greater in size with large irregular pale-staining nuclei.

An active gland will show in addition germinal centres. These are paler-staining zones compared to the primary follicles and contain histiocytic cells and different forms of B lymphocytes such as the cleaved (centrocyte) and noncleaved forms (centroblast) — the description referring to the nuclear presentation. Immunoblasts and plasma cells are also present, plus scanty T lymphocytes.

Paracortex. This occupies a position internal to the cortex, but external to the medullary cords, and is the domain of the T lymphocyte series.

Both cortex and paracortex are drained by a system of cortical sinuses having a reticulin fibre framework which lead into the medullary cords.

Medullary cords. The cortical sinuses drain into a system of medullary sinuses. These run between medullary cords which are formed of various lymphocytic cells, plasma cells and histiocytes.

Gross structure. The efferent lymph flow is from the medullary sinuses and the afferent lymphatics via channels in the fibroreticular membrane that covers the lymph node, and thence into subcapsular sinuses.

It should be noted that a dense reticulin pattern can be demonstrated throughout the lymph node, with the exception of the germinal centres where it is perifollicular in distribution.

GENERAL NOTES

Having set out the various tissue types and subtypes, it may be helpful to note the following points.

1. The structure of a tissue often mirrors its function or physiological role. For example epithelial cells lining an intralobular duct in an acinar gland are usually cuboidal in type, whereas those cells lining the extralobular, i.e. larger ducts will tend to be more columnar. The main duct for the gland may well be lined by stratified (layered) columnar cells. Similarly, stratified squamous epithelium forming external skin surfaces are keratinized to varying degrees, whereas the internal 'skin' surfaces such as the oesophagus lack a protective layer of keratin. Both of these examples exemplify the arrangement of cells according to their role; in the case of the ducts, the degree of secretion required and resistance to the flow-stress of the duct contents, and in the case of the skin layers the degree of surface protection required against trauma.

2. When comparing sections of tissues with descriptions or pictures in textbooks, remember that 'normal' is a relative word and that, of necessity, textbook illustrations show tissue under ideal conditions of fixation, preservation and demonstration. It is important to expect minor variations

in presentation due to these modifying factors.

3. In the authors' experience the important microscopy objectives for studying the normal histology of stained slides are a low-power scanning lens, e.g. × 2.5 and a higher power lens such as × 40.

The low-power lens should be initially employed so as to afford a general idea of the cellular arrangement followed by examination with the higher-power lens to confirm or resolve important fine structural details. A lens of intermediate magnification such as the × 10 is normally less useful than the objectives mentioned, whilst the × 100 oil-immersion objective is rarely called for.

BASIC HISTOPATHOLOGY

The type of pathological change to be discussed here is that which is most likely to be encountered in the routine diagnostic laboratory. It will be described in relation to the type of material most likely to be handled by such a laboratory.

NON-TUMOUR PATHOLOGY

Under this umbrella is to be found a wide spectrum of disorders involving tissue. Some degree of inflammation is usually involved either as the primary lesion, or as a secondary reaction to other disease processes.

The following explanation of some common terms may be found useful:

Aneurysm is a localized dilatation of an artery which may be a large vessel, e.g. aorta, or quite small, as found at the base of the brain. The media of the affected area is defective and there is associated degeneration of elastic fibres. There are various types, such as atheromatous, congenital, syphilitic and dissecting.

Embolism. There are various forms of embolus which usually consist of detached material which is transported from one part of the circulation to another. Examples of emboli are pieces of blood clot, fat or tumour.

Diverticulum. The large intestine is most commonly involved in diverticula formation which is the presence of a pouch-like dilatation of the bowel wall. The diverticular wall is usually thin with a scanty muscle coat. The condition is known as diverticulosis, and if inflammation is present, is termed diverticulitis.

Endometriosis. When normal tissue is found in an abnormal situation it is termed 'ectopic', and ectopic endometrium gives rise to the condition of endometriosis where nodular lesions occur in a number of sites including the ovary, umbilicus and gut. The wall of the uterus commonly shows this condition where it is known as 'adenomyosis'.

Granuloma. Inflammatory reactions may take many forms and a granulomatous reaction is one where there is a marked proliferation of abnormal histiocytes called 'epithelioid' cells plus their conglomerates the giant cells. Examples of this type of reaction are tuberculosis and sarcoidosis.

Infarct. Infarction is a localized area of tissue necrosis caused by an interruption to the blood supply (resulting in haemorrhage to the area). Whilst this can occur to almost any situation, it is seen to greatest advantage in placenta, heart (myocardial infarction), brain, lung, kidney and spleen.

Inflammation. In purely histological terms an acute inflammatory reaction involves to varying degrees, hyperaemia, oedema and neutrophil proliferation. This reaction gradually lessens until the chronic stage is reached when lymphocytes, and to a lesser degree, histiocytes and fibroblasts are predominant. The final stage is one of collagen overgrowth (fibrosis).

Ischaemia. Sometimes the blood supply to a given tissue is decreased because of partial obstruction or narrowing of the relevant blood vessel. This partial occlusion commonly occurs in the heart muscle due to coronary artery deficiency, and results over a period of time in fibrotic diseases in the myocardium. A similar diminution of blood supply can occur in the large intestine — a condition termed 'ischaemic colitis'.

Metaplasia denotes a change in differentiation of tissue from one mature type to another and is thought to be due to the proliferation and differentiation of totipotential cells. Metaplastic change can be nonspecific or as a result of inflammation. Examples are squamous metaplasia of bronchial mucosa in chronic bronchitis, the appearance

of bony areas in scar tissue, and intestinal metaplasia of the stomach. In certain tumours squamous metaplasia is often present, particularly adenocarcinoma of the uterus and transitional cell carcinoma of the bladder.

Necrosis. Implied in this term is death of cells or tissues and, as would be expected, a loss of normal cellular detail and architecture. Nuclear changes are evident including pyknosis (condensation of chromatin), and karyorrhexis (nuclear breakdown). Calcification is often present so that this type of tissue change is less than popular with the microtomist!

There are different types of necrotic change; infarction (see above) is one such, as is the caseation necrosis associated with tuberculous lesions.

Disturbances of growth

Anaplasia. The term anaplastic implies immaturity or lack of differentiation and cells showing anaplasia often present as highly malignant tumours.

Atrophy. Atrophic change is applied to the macroscopic appearance of shrinkage of a structure and can be due to a decrease in number or size of the component cells. Examples are disuse atrophy of muscle, and renal atrophy due to ureteric blockage.

Dysplasia denotes an abnormal growth pattern.

Hyperplasia/hypoplasia. Denotes an increase or decrease, respectively in the number of component cells. Endocrine glands such as the thyroid or pituitary may exhibit these features; also haemopoietic bore marrow in a variety of diseases, and tumour cells generally.

Hypertrophy. This is the converse of atrophy and denotes an increase in cell volume. An example is ventricular hypertrophy of heart muscle in hypertension.

TUMOUR PATHOLOGY

A benign tumour differs in a number of significant respects when compared to a malignant tumour. This is true not only clinically but histologically too, and the following few guidelines may be found useful. It is important to bear in mind that there is not infrequently overlap as regards the histological parameters of benign and malignant tumours, and that type of situation calls for long experience and/or high interpretative skills.

Benign tumours. These may be small or large, but the component cells are distinguished by a relatively uniform appearance with very little variation in size and shape of both nucleus and cytoplasm. Important is the lack of invasion into deeper structure. Examples are squamous cell papillomas of the skin and uterine fibromyomata ('fibroids').

Malignant tumours. Most malignant tumours arise from epithelium and are termed 'carcinoma' and thus commonly arise in glands and lining surfaces.

Microscopically the cells exhibit some or all of the following features.

1. Pleomorphism (variation in size and shape of nuclei and cytoplasm)
2. Invasion of deeper structures
3. Nuclear immaturity (abnormal chromatin clumping, prominent nucleoli)
4. Mitotic activity.

The other, less common, type of malignant tumour is the sarcoma and consists of tissue elements of mesenchymal origin. This is a complex group and tends to present the pathologist with greater diagnostic problems. The names of the individual sarcomata reflect the type of tissue from which they spring, e.g. fibrosarcoma, myosarcoma, osteosarcoma, liposarcoma, etc. The sarcomas affect all age groups, unlike the carcinomas which have a predeliction for the older age groups.

The following explanations of some commonly employed terms may be found useful:

Adenoma is a benign tumour of glandular or secretory tissue. Common examples are to be found in rectum, skin appendages and thyroid. The malignant variant is known as an adenocarcinoma and is the commonest type of tumour in the gastro-intestinal tract.

Neoplasm refers to an abnormal growth or tumour which can be benign or malignant.

Papilloma. A papillomatous growth is one where there are finger-like projections of tissue. It is most commonly a benign tumour and examples are

squamous papillomas of the skin and villous adenomas of the rectum. In each case there is an epithelial lining overlying a connective tissue core of the papillary projections.

Polyp. 'Polyps' (or more correctly 'polypi') are round, usually benign growths which often have a connecting stalk and are 'pedunculated'; a non-pedunculated polyp is termed 'sessile'. Occasionally malignant change takes place, more particularly in those arising in the gastro-intestinal tract. The cervix uteri and rectum are common site for polyps.

Tumour differentiation. When one refers to a given malignant tumour as being histologically *well* — or *poorly* — differentiated, it denotes the degree to which the malignant tissue resembles the normal parent tissue. Thus a 'well differentiated' adenocarcinoma of the colon will show a well marked glandular structure often with mucin secretion. A 'poorly differentiated' adenocarcinoma, on the other hand, will consist of cell masses with little or no glandular structure or mucin secretion, and the cells themselves will possess the stigmata of immaturity, particularly as regards the nuclear presentation.

Analogous to the mucin production of the well differentiated adenocarcinoma is the keratin formation of well differentiated squamous cell carcinomas. In a similar way, a fibrosarcoma if well differentiated will show a greater *fibrillar* content and a lessened *cellularity* compared to a poorly differentiated sarcoma, which will be more cellular and less fibrillar.

The more important organs and structures in the body are shown in Table 1.2 which relates to the more important histopathological conditions. It is not an exhaustive list, merely an attempt to highlight for the inexperienced worker the location of the more common lesions, i.e. those that are likely to be presented to the average histology laboratory.

Table 1.2 Some commonly received specimens in the surgical laboratory and their more common pathologies.

Tissue	*Non-tumour pathology*	*Tumour pathology*
Appendix	Appendicitis Mucocele	Carcinoid tumour Carcinoma, primary (rare) Carcinoma, secondary (uncommon)
Artery	Arteritis Atheroma Aneurysm	Chemodectoma (uncommon)
Bladder (biopsy or cystectomy)	Cystitis Diverticulae Fistulae Tuberculosis Shistosomiasis (uncommon)	Transitional cell carcinoma Squamous cell carcinoma Adenocarcinoma (rare)
Bone and bone marrow	Osteoporosis Osteomalacia Osteomyelitis Paget's disease	Osteochondroma Myeloma Metastatic tumour (e.g. breast, bronchus, thyroid, prostate, kidney) Leukaemia Osteosarcoma
Breast (biopsy or mastectomy)	Cysts Fibrocystic disease Abscess Fat necrosis	Adenoma Fibroadenoma Adenocarcinoma Paget's disease of nipple
Bronchial biopsy	Inflammation Squamous metaplasia	Squamous cell carcinoma Oat cell carcinoma
Cervix (cone biopsy or punch biopsy)	Inflammation Dysplasia (CIN)	Squamous carcinoma (in-situ (CIN) or invasive)
Colon and rectum (biopsy or colectomy)	Ulcerative colitis Crohn's disease Amyloidosis Fistulae Amoebiasis (uncommon) Diverticular disease	Adenomatous polyps Adenocarcinoma Lymphoma (uncommon) Polyps

Tissue	*Non-tumour pathology*	*Tumour pathology*
Endometrium (curettings)	Endometritis Abnormalities of cycle Hyperplasia	Adenocarcinoma Sarcomas (uncommon) Polyps
Epididymis	Cysts Inflammation Tuberculosis	Adenocarcinoma (rare)
Fallopian tubes	Salpingitis Ectopic pregnancy Endometriosis	
Gall bladder	Cholecystitis Calculi	Adenocarcinoma (uncommon)
Joints/tendons	Arthritis Crystal synovitis (e.g. gout)	Sarcoma (rare)
Kidney (biopsy or nephrectomy)	Amyloidosis Glomerulonephritis Pyelonephritis Cysts/calculi Tuberculosis	Adenocarcinoma Transitional cell carcinoma of pelvis
Larynx and vocal cords	Laryngeal nodules Inflammation Polyps	Squamous cell carcinoma
Liver (biopsy)	Hepatitis Cirrhosis Obstructive jaundice Sarcoidosis Amyloidosis Storage disorders	Hepatocellular carcinoma Secondary tumour, e.g. colon stomach, breast and pancreas Lymphoma
Lung (biopsy)	Pneumonia Alveolar fibrosis Pneumocystic carinii	Squamous carcinoma Oat cell carcinoma Adenocarcinoma Secondary tumours
Lymph node	Reaction to inflammation Tuberculosis Sarcoidosis	Hodgkin's and non-Hodgkin's lymphoma Secondary tumours, e.g. lung, breast, colon, testis
Muscle, voluntary (biopsy)	Myopathies Neuropathic atrophy	Rhabdomyosarcoma (rare)
Nasal mucosa	Polyps Inflammation	Muco-epidermoid carcinoma Squamous cell carcinoma
Oral cavity	Cysts (dental) Inflammation Polyps	Squamous cell carcinoma Salivary gland tumours
Oesophagus (biopsy)	Oesophagitis Strictures Ulceration (peptic)	Squamous cell carcinoma
Ovary	Cysts Endometriosis	Both benign and malignant tumours of: Epithelium, e.g. mucinous cystadenoma Stroma, e.g. thecoma Germ cells, e.g. dysgerminoma Metastatic tumours, e.g. stomach
Pancreas (biopsy)	Cysts Pancreatitis	Adenocarcinoma (exocrine elements) Apudomas (rare)
Parathyroid gland	Hyperplasia	Adenomas
Placenta and umbilical cord	Malformations Infarction	Hydatidiform mole Choriocarcinoma