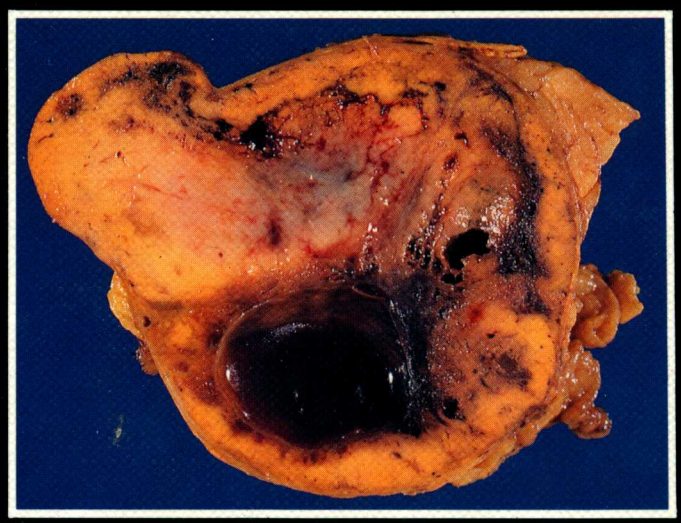
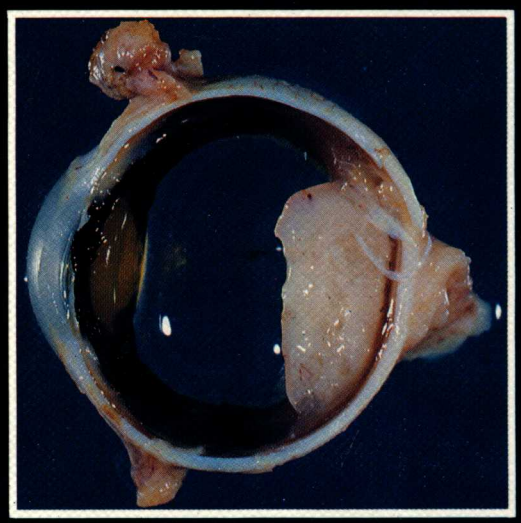
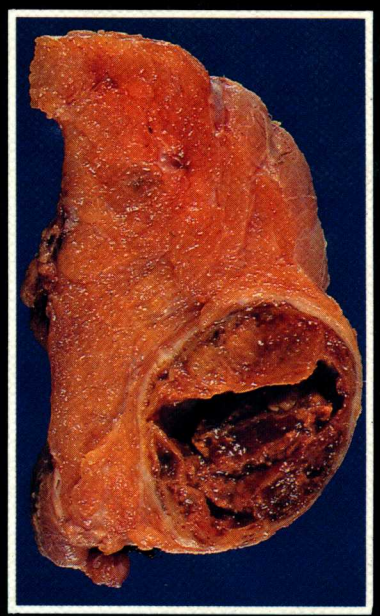
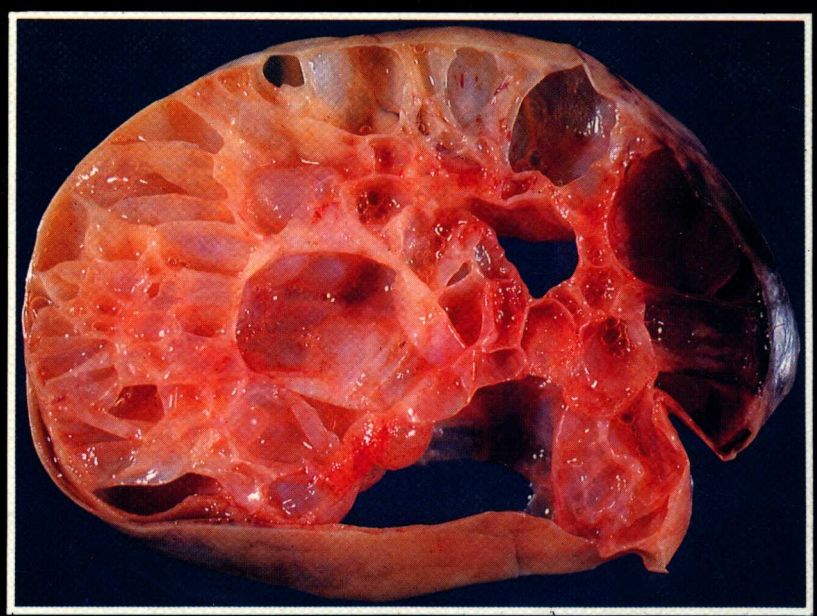
SURGICAL PATHOLOGY TECHNIQUES

D.G.Lowe • I.M.Jeffrey









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D.G. Lowe
MD MIBiol MRCPath
Senior Lecturer/Honorary Consultant
Department of Histopathology
St Bartholomew's Hospital, London

I.J.M. Jeffrey
BSc MB ChB
Senior Registrar
Department of Histopathology
St Bartholomew's Hospital, London

B.C. Decker Inc.
Philadelphia • Toronto

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PREFACE

Diagnostic histopathology is largely an apprenticeship, learned through experience and by demonstration and example. The laboratory process begins with the macroscopical description and dissection of specimens received from theatre (the 'cut-up'), and it is at this stage that blocks of tissue for macroscopical examination are selected and taken. These techniques are usually learnt at an early stage in the histopathologist's career, by observing experienced members of staff and by practising under supervision.

This illustrated book is intended to help this learning process and is aimed primarily at the trainee histopathologist. It should continue to be of use to the pathologist once the basic techniques have been mastered, as even after practising for some time he or she is likely to receive specimens that have not been encountered before. Surgeons, gynaecologists and other clinicians would also greatly benefit from some knowledge of what happens to a specimen once it has left their hands.

These pages provide guidance on how to approach the preparation and dissection of a surgical specimen to demonstrate the pathology present. It also gives advice on the structuring and contents of macroscopical reports, and examples of these are paired with colour illustrations of the relevant specimens. Much of the descriptive and procedural data has been tabulated and highlighted to make the book easier to use at the dissection bench. Although the number and types of blocks taken in different laboratories may vary, in general the same principles of sampling can be applied; line drawings of the photographs are provided to indicate where blocks of tissue should be taken for microscopical examination.

We would like to acknowledge the help of Mr J.L. Hopwood, Museum Conservator at the Pathology Museum, St Bartholomew's Hospital, London, who took almost all of the colour photographs in this book. Figures 34-36 are reproduced by kind permission of Professor J. Jass of the University of Auckland (formerly of St Mark's Hospital, London). Figure 78 is reproduced by kind permission of Professor C.L. Berry of the London Hospital, the editor of the *British Medical Journal*, and the editor of the *Journal of Clinical Pathology*.

INTRODUCTION

The work of the surgical pathologist starts in the cut-up room, where specimens are identified, logged in, examined, described, dissected and prepared for histological processing. The purpose of the gross examination is to establish what the specimen consists of, what it looks like (and feels like, in many cases), and how it differs from normal. The components of the macroscopical report are therefore the specimen type (e.g. mucosal biopsy, hysterectomy, laryngectomy) or the tissues present (e.g. pericardium, small bowel diverticulum); measurements of the specimen (e.g. size, number and weight); a description of the pathology and of other tissues present; and, from large specimens, a description of the blocks of tissue taken for histological examination, with a record of the sites from which they were obtained and their numbers.

Specimen identification

Specimen identification is clearly a crucial part of the initial examination of a pathological specimen. The first sign of a mix-up of specimens will emerge at this stage. The clinical details on the request form should indicate the types and numbers of specimens, and these should be appropriate for the specimen received.

The pathological report identifies the surgical procedure that was performed or the tissues that were removed, and may be the only record of the operation if the case notes are lost. As the size and weight of the organ or tissue removed are recorded, it also provides a record of the extensiveness of the surgical procedure.

Photography has proved to be an excellent way of identifying a resection specimen and recording its macroscopical features. Colour photography should be used if possible, but good results can be obtained with monochrome Polaroid instant prints or black-and-white reel film. In complicated dissections (such as a laryngectomy or a Whipple's operation specimen), a second print can be used to mark the positions of the blocks of tissue taken and can greatly facilitate the macroscopical and microscopical reporting. Photography of fresh tissue has the particular advantage that the colours are preserved but there are several practical constraints, particularly the hazards to staff from infection and the need for staff and equipment to be available throughout the day, which may well limit feasibility. No such limitations affect the use of fixed specimens, so these are more likely to be used for photographic records, especially in a busy department.

Fixation

Most specimens are received fixed in 10 per cent formalin solution, though some tissues may be sent to the histopathology department fresh. Fresh material is needed for the following purposes:

- frozen section
- immunocytochemistry
- hormone receptor estimation and other biochemical studies
- cytological examination
- microbiological sampling before histopathology
- chromosome analysis
- research
- museum display.

Occasionally, specimens will be sent unfixed because they will not fit into a pot (e.g. a lower limb) or, in complicated cases, because a fresh specimen is necessary for satisfactory orientation and dissection.

When the above points have been dealt with, the tissue should be fixed before dissection. If suitable, the entire specimen should be fixed in as intact a state as possible, so that the relations between its parts are preserved. Some specimens, such as hollow organs, cysts, and masses of adipose tissue, will not fix well because the diffusion of fixative into the centre of the specimen will be delayed, but where cuts must be made to aid fixation, distortion of the specimen should be

kept to a minimum. These cases will be discussed in the appropriate chapters.

Macroscopical description

The macroscopical report of a specimen records the gross features of the tissue received in the pathology department, and serves several important functions. With all specimens, but especially with biopsies, the report provides a check that the histological slides which are eventually prepared are representative of the tissue supplied, in both the number and the area of fragments. With a large resection specimen, the macroscopical description is usually the only recorded evidence of its appearance before it was cut, separated, dissolved or otherwise distorted for examination purposes. A good description of a specimen is therefore essential.

Some diagnoses are made principally from the macroscopical features; for example, cholelithiasis and sialolithiasis can be macroscopical diagnoses. The gross appearance of the specimen may also influence the final diagnosis, as with the distribution of lesions in the differential diagnosis of Crohn's disease and ulcerative colitis. If by mishap the blocks are embedded incorrectly or if the sections prove difficult to interpret, the macroscopical report may be the only means of diagnosing whether a tumour extends to an excision line or involves a serosal surface.

The report recording the appearance and other physical characteristics of a pathological specimen may also be of interest to clinicians, especially when the lesion has been seen, for example, at laparotomy or through an endoscope or laparoscope. Gynaecologists will probably be more interested to learn that a fibroid weighed 5 kg than to have it confirmed that it was indeed histologically diagnosable as a leiomyoma. The site of occurrence of a particular disease can be important for epidemiological studies and is usually defined, at least in part, in the macroscopical description. For forensic purposes, a record that an organ or part of one has been excised can occasionally be of value, for example, in helping to identify a cadaver.

Macroscopical descriptions should be short but they must contain all the salient details of a case. The active voice 'The kidney shows . . .' is generally preferable to the passive '. . . is found in the kidney', and in most cases the present tense is most suitable.

Estimation of the size of a lesion by comparison with familiar objects should be avoided. Oranges, eggs and peas, for example, come in a range of sizes, and if a report has to be sent overseas the original inexactitude may well be exaggerated. Measurements are usually given in centimetres (i.e. 3.7 cm rather than 37 mm) unless the specimen is very small, in which case millimetres can be used.

Weight is often an important descriptive parameter, especially when dealing with endocrine organs. When the average weight of the normal organ is known (or can be found in reference books), the weight of the abnormal organ may be significant, and may give some indication of the size or extent of the abnormality. Even when the normal weight is very variable, as for the prostate, the specimen weight can indicate whether a resection was modest or extensive. The significance of the weights of some tissues, such as mastectomy specimens or hysterectomy specimens, is more doubtful, though they may give a general indication of size.

When a tissue such as the endometrium or prostate is biopsied randomly or diffusely, the number of pieces tends to be unimportant, and the volume of the aggregated pieces gives a better impression of sampling. Directed biopsies such as endoscopic or colposcopic biopsies should be counted, to confirm the number of specimens received and to indicate the number of fragments that should appear on the histological sections. When biopsies are taken from different sites in an organ they must be labelled separately.

After fixation in formalin most tissues lose their natural colours and become grey or brown. Nevertheless, some idea of the colour is useful, especially when different parts of a tumour are differently coloured, since this may indicate that more than one tissue, host response, growth pattern, etc., is present. The same is true of differences in the consistency of the tissue and the texture of the surface.

Descriptive terms should not be too obscure. Clinicians are unlikely to reach for their dictionaries when faced with *grumous* or *pultaceous*, and the meaning of the report

Table

	DESCRIPTIVE TERMS	INTERPRETIVE TERMS
Colour	Red, clear, grey	Haemorrhagic, mucoid, necrotic
Contour	Round, irregular, ill-defined	Encapsulated, invasive, infiltrative
Consistency	Hard, soft, rubbery, gelatinous	Bony, calcified, necrotic, mucoid, fibrosed
Texture	Granular, fibrillary, smooth	Ulcerated, fibrinous
Size	Usually in centimetres; in millimetres for tiny objects	Enlarged, atrophic

may be obscured by such words. Pathologists' terms can be divided broadly into two groups: those which qualify the physical appearance of the specimen alone (descriptive terms); and those which draw from the physical appearances some conclusion about their nature (interpretive terms) (**Table**).

Morphological features can usually be described with impunity, since they can be appreciated by most people. For example, a tumour may be described as pink, well circumscribed, and hard, and unless the prosector is colour-blind or careless, the description is unlikely to be mistaken. Interpretive terms, on the other hand, may cause problems. The same tumour could be described by one prosector as an encapsulated, bony mass with areas of fibrosis, and by another as calcified tumour tissue which compresses the adjacent tissues. Interpretive terms may be admissible, however, when there is no doubt that haemorrhage, necrosis etc. has occurred.

Some terms have been used in specific contexts for many years and cannot now be used generally because of their associations. For example, if a soft grey or yellow area of tissue is described as *caseous*, clinicians may assume that tuberculosis is present; *waxy* is very rarely used, except to describe amyloidosis.

It is helpful to both pathologist and clinician if a report follows a logical sequence or progression. For the former, this provides a framework or check-list so that dissection progresses more quickly. The latter will be able to find points of interest more easily. Examples of sequences in common use (the direction of the sequence is not important) are as follows:

Vagina/cervix/endometrium/
myometrium/fallopian tubes/ovaries;
Oesophagus/fundus/body/antrum/
pylorus/duodenum.

For other specimens, the different aspects may provide a framework:

Mucosa/cut surface of wall/serosa/
attached tissues;
External surface/cut surface/contents.

If the specimen has an obvious lesion such as a carcinoma, however, it would be irritating if its description was to be submerged by an academic sequence. In such a case, the carcinoma and the tissue where it arises should be described first, and the sequence used in a subsequent paragraph to remark on the other tissues present.

Principles of dissection

Dissection will depend on the tissues present in the specimen and on the type of organ or tissue received; whether it is intact or opened in theatre; whether it is fresh or fixed; the nature of the pathology; and whether this can be identified with certainty at an early stage in the procedure. The general principles of dissection are that all of the pathology should be assessed, that the features of prognostic importance should be noted, and that there should be as little distortion and destruction of the specimen as possible. This last principle is important if it becomes necessary to demonstrate the case to a clinician or to another pathologist, or to return to a particular area of the specimen in order to take more blocks.

On the whole, the procedures used to dissect the specimen are not detailed on the

macroscopical report. It is not usually of interest to clinicians that, for example, 'The mesentery was then incised at 1 cm intervals . . .' or 'The stomach was opened along the greater curve . . .'. On the other hand, one might record that 'Despite a prolonged search for lymph nodes in this specimen, only two were found'.

Blocks

In the scope of this book it is possible only to give a general suggestion of the number of blocks which might be taken in any particular case. Obviously the exact number will depend on many differing factors, such as the size and complexity of the specimen, the research interests of the histopathology department, the forensic or legal implications of the case, and the individual practice and experience of the pathologist who is responsible for the surgical reporting. In the USA, for example, it is customary to take more blocks from certain specimens than is usual in the UK. If there is any doubt the advice of a senior staff member must be sought.

The size of the blocks of tissue taken from large specimens will be limited by the size of the processing cassettes in which they will be placed. In general, they should not be more than 4 mm thick and should not fit tightly into the cassette, so that diffusion of processing fluids into the tissues is not impeded.

The gross assessment of a case will determine which blocks are taken for preservation in the department's files. When a case is reviewed some time after initial reporting, the macroscopical description may be the only indication of the sites from which the blocks were taken. For example, if a resection specimen was found to have a focal lesion, the original description could serve to explain why no blocks were taken from some areas, and why numerous blocks were taken from a particular area.

In many cases, microscopical diagnoses cannot be made if the tissue submitted for processing is wrongly selected. For example, tissue from the periphery of a large bowel neoplastic polyp which does not include submucosa may prevent the histologist confirming or refuting a diagnosis of carcinoma.

In recording the sites of blocks, some degree of orientation will usually be necessary. The site of origin of the tissue may be important, and the way that it is embedded and sectioned may need to be specified. The parts of the block, such as resection margins, should be clearly identifiable.

Depending on the pathology present, standard blocks in addition to the lesion itself might include lymph nodes, samples of the tissue away from the lesion, vascular pedicles and resection margins.

The site from which a block is taken is usually recorded by giving the block an identifying letter or number (the back of the request form could be used for the record):

A	cord of tissue attached to mucosa
B	small polyp
C	large polyp
D	carcinoma
E	lymph nodes adjacent to tumour
F	distant lymph nodes
G	lymph nodes at highest point
H	proximal excision end
J	distal excision end.

The orientation of the tissue in the wax block is of great importance. It will determine which aspect of the tissue is lost when the block is trimmed ready for sectioning, which aspect of a lesion is cut and mounted on a slide, and the relationship of tissue components such as epidermis and dermis on the sections. Incorrect embedding may make a case unreportable, though fortunately the orientation of most blocks will be obvious to the experienced staff responsible for the embedding. If the orientation of a tissue in the block is critical, suitable directions should be recorded. Phrases in common use include:

- **On end** for a cord of tissue such as an artery or vas deferens (block A, above);
- **On side** when a cord or strip of tissue is not to be embedded on end;
- **On edge** when a flat sheet of tissue is from a hollow viscus and the layers of the wall are to be displayed (blocks H and J, above);
- **Cut side down** when an irregular, spherical or cylindrical specimen is bisected (blocks B, C, E, F and G, above).

Flat blocks (e.g. block D, above) are placed

into cassettes so that the face which is to be trimmed and sectioned first is downwards, away from the lid. In blocks H and J above further orientation might be necessary to identify which aspect of the block represents tissue at the excision lines.

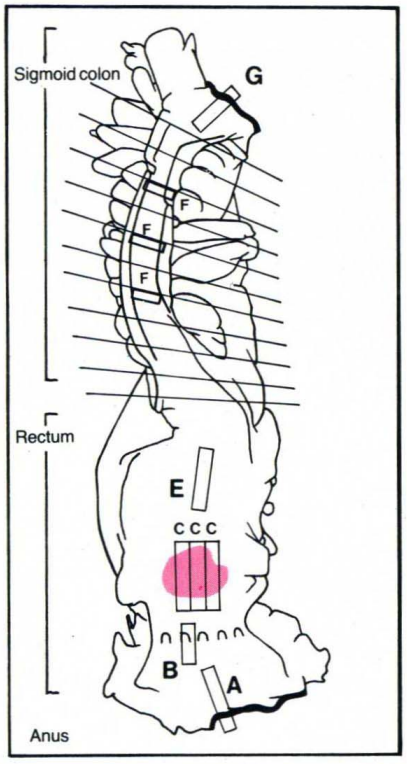
Marking of the specimen with insoluble pigment may be essential in some cases, especially when the tissue may be distorted or fragmented by processing. There are several possible markers, the most commonly used being carbon particles from India ink, which can be carefully painted onto the surfaces to be identified and blotted dry. There is a risk that when a tissue is cut carbon will be carried into it on the knife blade but in practice this is seldom a problem. Other pigments which have been successfully used include alizarin crimson and viridian. When fresh specimens are received, inking of the excision margins and other important structures should where possible be done before fixation so that any distortion from fixation or processing can be allowed for. Blocks may also be marked with a notch or incision on the border away from or opposite the excision margin.

Decalcification

Tissues may be very hard or have focal gritty areas. If foreign material can be excluded, this means that the tissue is almost certainly calcified and will need decalcifying before routine processing. Common specimens which contain foci of calcification or fragments of bone include:

- nasal polyps
- apical granulomas and other dental specimens
- bone marrow aspirates
- some ovarian tumours
- meningiomas
- craniopharyngiomas
- areas of fat necrosis
- breast lesions
- products of conception
- intervertebral discs.

The staff responsible for the embedding should be warned if there is the possibility that calcium or bone may be present because of the damage that these cause to microtome blades, and to the surrounding tissues when the sections are cut. Before a specimen is decalcified, any special stains that might be needed should be discussed with the staff; some, such as methyl green pyronin stain for RNA, will not work well on sections which have already been decalcified with formic acid. Decalcification with EDTA chelates iron salts and makes Perls' stain unreliable.



Key to illustrations

In the ensuing chapters line drawings of specimens, paired with photographs, are used to illustrate where blocks should be taken for microscopic examination. Figure 28b shown here is representative of the diagrammatic style.

Pathological lesion

Resection margin

Cuts into specimen at dissection

Blocks of tissue taken from specimen

CHAPTER 1
ALIMENTARY TRACT

Small specimens

Biopsies

Biopsies from the alimentary tract are among the commonest specimens received by general histopathology laboratories. In most cases, fragments of tissue from different parts of the tract are handled in the same way. When several biopsies are received from one patient, the site of each must be specified in the report. This is made easier if the clinician uses a schematic drawing of the oesophagus, stomach or bowel and marks the biopsy sites on it (**1**).

Endoscopic mucosal biopsies are taken from almost anywhere in the tract but especially from the oesophagus, stomach, duodenum, terminal ileum and large bowel to investigate ulcers, neoplasms, diffuse and focal inflammation, and malabsorption. The biopsies are usually small and difficult to orientate. Jejunal biopsies taken by Crosby capsule for the investigation of small bowel disease are somewhat larger, but can become contracted and distorted. Where possible, biopsies are embedded at right angles to the luminal surface so that the sections will show the full thickness of the mucosa and include any submucosa that is present in the biopsy (**2**).

In some hospitals the gastroenterologists and surgeons arrange the biopsies mucosa upwards on small sheets of card before fixation. Strips of ruled cellulose acetate are very useful (**3**), since the biopsies can be placed along a line and the tissue and acetate processed, embedded and sectioned together. Distortion of the tissue is minimized and the sites of the biopsies can easily be identified by their position along the strip.

Small mucosal biopsies rarely need dissection or a detailed macroscopical description. Biopsies from the small bowel should be examined under a dissecting microscope before processing, to assess the villous pattern and check the orientation of the specimen if it is received attached to card or acetate sheet (**4**). Mishaps can sometimes occur; the specimen may be placed with the mucosal side downwards on the card, and the flat surface of the upward-facing muscularis mucosae or submucosa may be mistaken under the dissecting microscope for total villous atrophy.

The clinical details supplied with a case may indicate that levels or special stains will be needed. These should be asked for at the time of macroscopical reporting to avoid delay. If there is any doubt about the orientation of a specimen, however, it is best to begin by examining a single section. Otherwise the entire specimen may be lost by cutting multiple sections in the wrong plane.

Biopsies: Macroscopical report

Site	Oesophagus, stomach, duodenum, etc.
Type of specimen	Mucosal biopsy, tissue fragment, friable material
Measurements	Number of fragments Size, range of sizes or greatest dimension if very small
Description	Colour Whether attached to card or acetate

Polyps: Macroscopical report	
Site	Rectum, sigmoid colon, etc.
Type of specimen	Sessile or pedunculated polyp(s)
Surgical additions	Suture markers, clips
Measurements	Diameter of head (pedunculated) or dimension of whole polyp (sessile) Length and diameter of pedicle if present Width of cuff of uninvolved tissue if present
Description	Colour Contour (villous, lobulated, smooth) Cut surface (cystic, papillary, solid)

Polyps

Polyps are circumscribed masses extending above the adjacent surface and may be pedunculated or sessile (**5**, **6**). They may be the result of a variety of pathological processes, such as inflammation, traction, hyperplasia, metaplasia or neoplasia. Histological examination is needed to establish the type of polyp and in neoplastic polyps to assess the extent of spread of neoplasia and the adequacy of the surgical excision.

Small polyps can be embedded whole, and if necessary multiple levels can be cut to display the stalk. Larger sessile polyps and pedunculated polyps with wide stalks can be bisected and embedded to show the cut surface (**5b**). If a polyp has a slender pedicle it should not be bisected since the very thin strand of tissue would tend to curl and distort. In this case, the head should be cut into three or more longitudinal blocks with a central block bearing the uncut pedicle (**6b**).

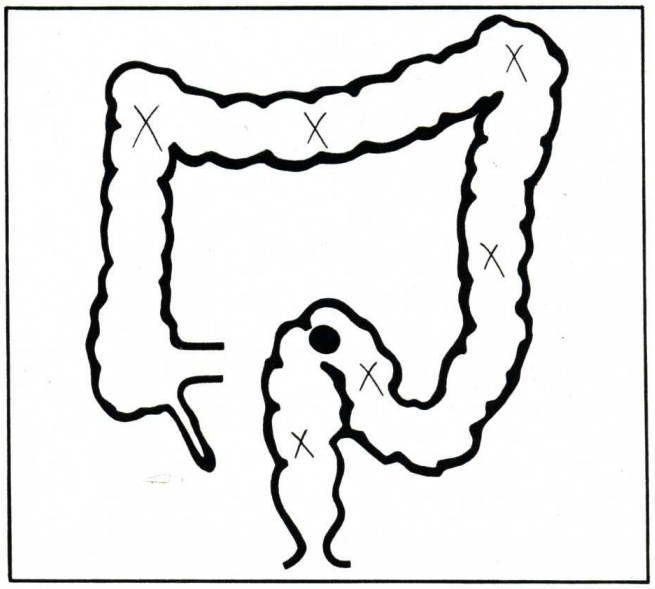
Small lengths of wall

Narrow rings or 'doughnuts' of wall can be taken from the excision margins and sent separately from a resection specimen (**7a**). Full thickness biopsies of bowel wall may also be taken for the investigation of Hirschsprung's disease. The dissection of an excised Meckel's diverticulum is described below (p 21).

The specimens should be measured and any abnormality of the mucosa, cut surface of wall or serosal surface recorded. If the doughnut is more than about 0.5 cm long, longitudinal blocks can be taken (**7b**). Thinner specimens should be divided into three or four pieces and embedded transversely.

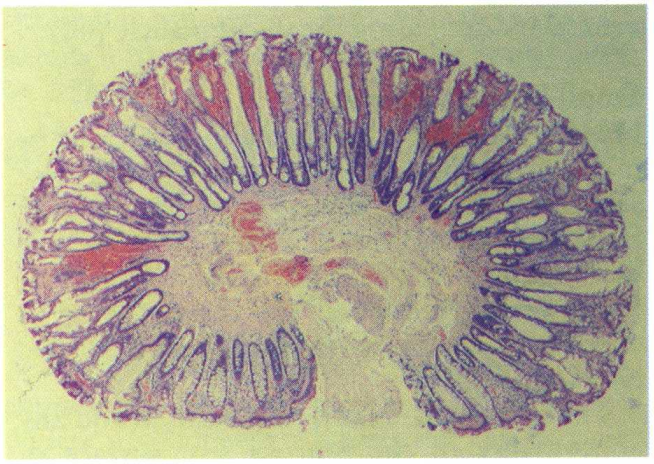
Small lengths of wall: Macroscopical report	
Site	Oesophagus, stomach, ileum, colon, etc.
Type of specimen	Ring (doughnut), sheet/strip of bowel wall
Measurements	Length and circumference, or area and thickness if a sheet of bowel is received
Surgical additions	Suture markers, clips, staples
Description	
Focal lesions	Ulcer, polyp, diverticulum
Rest of specimen	Mucosa, cut surface of wall and serosa: colour (red, purple, pale, etc.); consistency (friable, firm, fibrosed); contour (ulcerated, distorted, polypoid, fibrinous)

1



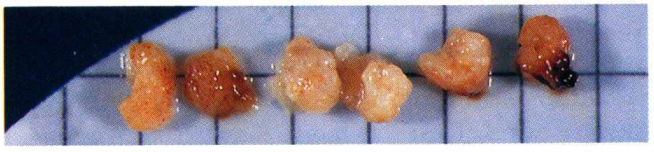
1 Diagram of the large bowel (made by a rubber stamp) on which the gastroenterologists have marked the sites from which a metaplastic polyp (●) and six biopsies (**X**) were taken. This method allows easy correlation of the pathological findings with the clinical features.

2



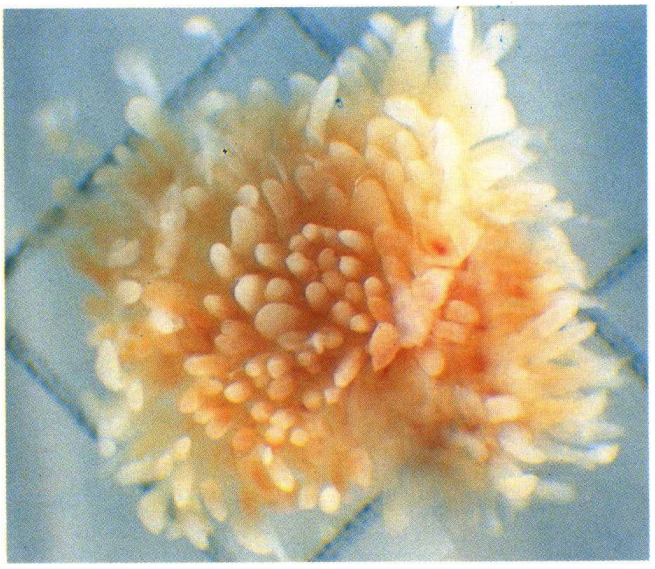
2 Histological section of a well-orientated rectal biopsy showing a metaplastic polyp. The relation between the mucosa and submucosa is clearly demonstrated.

3

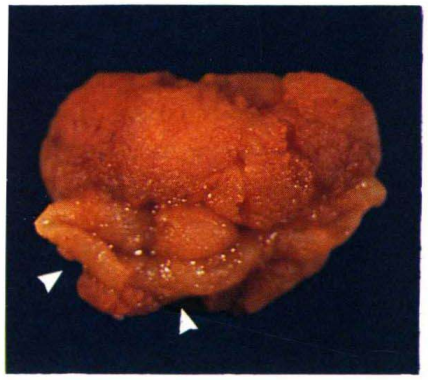


3 Large bowel biopsies arranged on a cellulose acetate strip in the same sequence as in **1**. The pointed end of the strip, by convention, indicates the most proximal biopsy, in this case from the hepatic flexure.

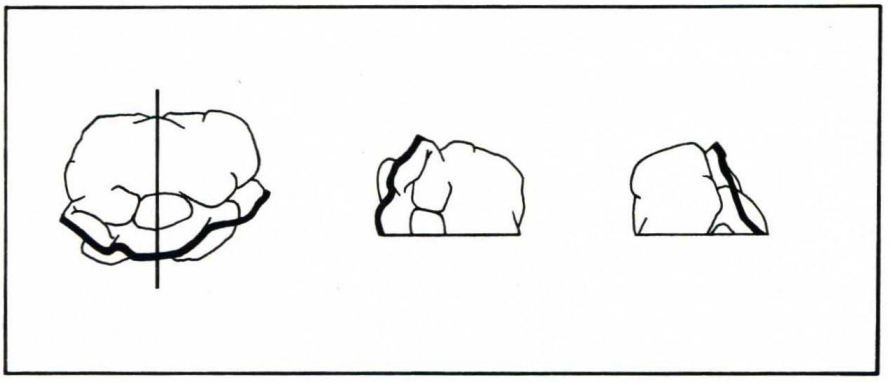
4



4 Small bowel mucosa viewed under a dissecting microscope in order to investigate malabsorption. The microscope reveals normal finger-like villi.

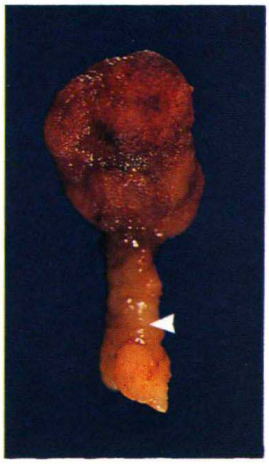


5b

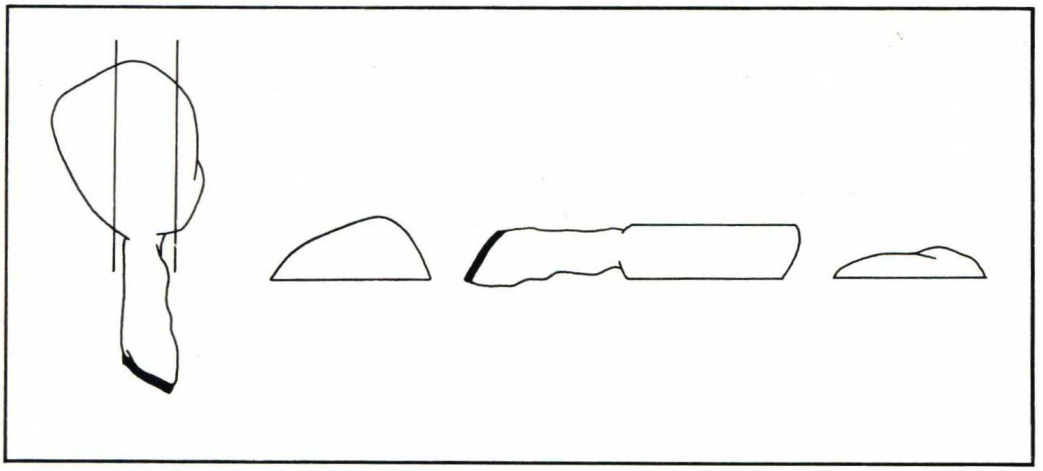


5a A sessile polyp of the large bowel surrounded by a short skirt of normal large bowel mucosa (▶).

5b A sessile polyp bisected and both halves embedded cut side down.

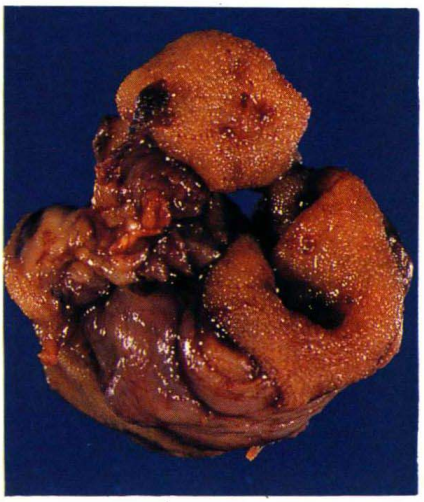


6b

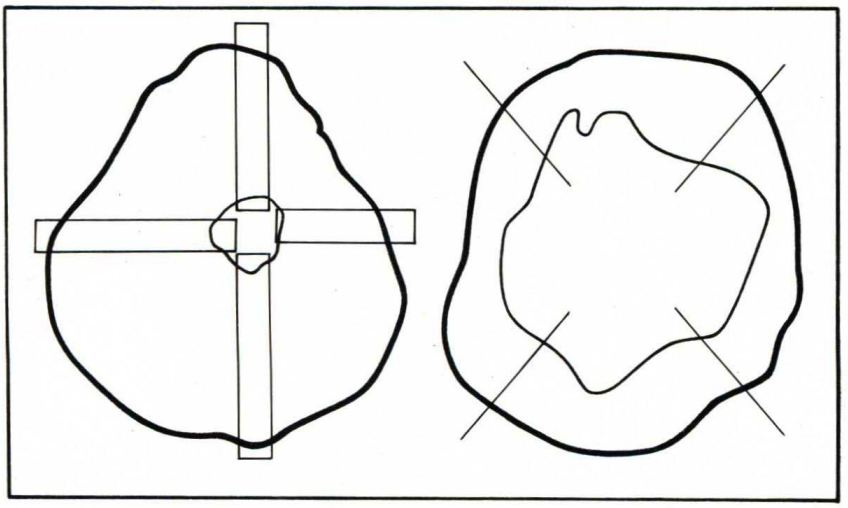


6a A pedunculated polyp with a long stalk of normal mucosa over a fibrovascular core of submucosa (▶).

6b Blocks from a pedunculated polyp. Those with slender stalks should be trimmed as shown, leaving the stalk intact. Several histological sections should then be cut through the block that contains the stalk.



7b



7a Ring from the excision end of the bowel taken at a colectomy operation (in this case the ring is relatively thick).

7b Dissection of rings of bowel wall. Sutures and staples must be removed before the tissue is blocked. Depending on the size of the ring, dissection can be by parallel longitudinal blocks, useful if the ring is thick, or by radial longitudinal cuts to make circumferential blocks from short rings.