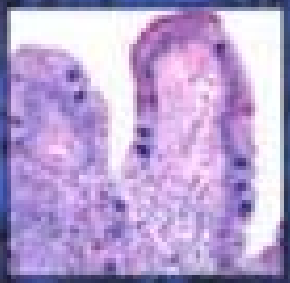


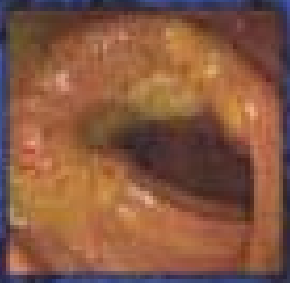


Atlas of Gastrointestinal Endoscopy and Related Pathology

Klaus F. R. Schiller
Roy Cockel
Richard H. Hunt
Bryan F. Warren
Foreword by Peter B. Cotton

Second Edition

Blackwell Science

Atlas of Gastrointestinal Endoscopy and Related Pathology

To Sidney Truelove

President, British Society for Digestive Endoscopy at its foundation,
and later President, British Society of Gastroenterology, gastroenterologist,
teacher, colleague and friend, in appreciation and with thanks.

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WITH THE COLLABORATION OF
Martin G. Lombard
Anthony I. Morris
A. John Morris
Thomas Rösch

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Editorial Assistant: Elizabeth Callaghan
Production Editor: Charlie Hamlyn
Production Controller: Kate Charman

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Contents

Contributors

Chief contributors and editors

Klaus F.R. Schiller DM, FRCP
Consultant Physician and Gastroenterologist (Emeritus), St. Peter's Hospital, Chertsey, Surrey and Acland Nuffield Hospital, Oxford; Former Vice-President (Endoscopy), British Society of Gastroenterology

Roy Cockel MA, MB, FRCP
Consultant Physician and Gastroenterologist, University Hospital Birmingham (Selly Oak Hospital), Birmingham; Senior Clinical Lecturer, University of Birmingham, Birmingham

Richard H. Hunt FRCP, FRCPEd, FRCPC, FACG
Professor, Division of Gastroenterology, McMaster University Medical Centre, Hamilton, Canada

Bryan F. Warren MB, ChB, FRCPath
Consultant Gastrointestinal Pathologist, John Radcliffe Hospital, Oxford; Honorary Senior Lecturer in Pathology, Oxford University, Oxford

Contributors

Martin G. Lombard FRCPI, FRCP
Consultant Gastroenterologist and Honorary Senior Lecturer in Medicine, Royal Liverpool University Hospitals and University of Liverpool, Liverpool

Anthony I. Morris FRCP
Consultant Physician and Clinical Director of Gastroenterology, Royal Liverpool University Hospital, Liverpool

A. John Morris MB, ChB, FRCP
Consultant Gastroenterologist and Honorary Senior Lecturer, Glasgow Royal Infirmary, Glasgow

Thomas Rösch MD
Director of Gastrointestinal Endoscopy, Department of Internal Medicine II, Technical University of Munich, Munich

Foreword

Like the authors of this book (and this writer), endoscopy has changed considerably over the last three decades. The early years of youthful excitement, pioneering (and some mistakes) led to a period of adult confidence and reasonable competence. Now, in maturity, there is the fun and obligation of reflection, and perhaps the beginnings of wisdom. What are now the main issues?

Endoscopy has become mainstream as other exciting new diagnostic and therapeutic techniques emerge and evolve towards practicality. Whilst we must embrace any developments which may have benefit for our patients, the imperative for endoscopy leaders must be to encourage enhanced efficiency and quality in endoscopic services. We are all aware that there are widespread problems of omission and commission, and that not all patients are optimally served.

There are three fundamental elements in this agenda: initial training, continuous quality improvement and patient empowerment. Initially we need to learn how to do endoscopy properly, and then continuously to strive to improve our efficiency and outcomes, and to make our patients partners in these endeavours.

Training programs are gradually becoming more thoughtful and structured, with less reliance on learning 'by osmosis' at the possible expense of our patients. Understanding what can be done, and what we are doing, is being facilitated by the increasing availability of community outcomes data derived from the wider use of structured endoscopy reporting systems. The fact that we and endoscopy are not perfect must be shared openly with our patients. Not even the experts claim 100% success and safety. Patients deserve to know more about the practice and competencies of individual endoscopists so that they can make informed choices. I strongly support the use of 'report cards', using fairly simple quality metrics, and the development of practice benchmarks.

Where does this new book fit in? Clearly, it is an important contribution for endoscopic trainees, and for all those involved in the endoscopy process. It is a clear and vividly illustrated guidebook to endoscopic appearances and to the major procedures, and the endoscopist will be well served by the inclusion of so much pathological material and the helpful introduction to endoscopic ultrasound. This book will undoubtedly find a place amongst other available learning resources.

I congratulate the authors on bringing this work to completion, and recommend it to the endoscopy community.

Peter B. Cotton
Director, Digestive Disease Center
Medical University of South Carolina, USA

Preface

In 1986, three of us (KFRS, RC and RH) produced a volume entitled *A Colour Atlas of Gastrointestinal Endoscopy* which was published under the imprint of Chapman & Hall Medical. Since then there have been many developments in gastroenterology and endoscopy. We therefore felt that the time had come to update and to expand the previous Atlas and also to broaden our approach. With the addition of BFW, the original trio has become a quartet and we have a new title for this second edition, *Atlas of Gastrointestinal Endoscopy and Related Pathology*. We also have a new publisher, Blackwell Science.

As endoscopy is now universally established we felt that it was inappropriate to include, in an Atlas, any discussion on such topics as the principles of endoscope design, methods of recording visual data, the design of endoscopy rooms or the organization of an endoscopy service. These and other matters, including detailed descriptions of technique and of endoscope sterilization, are covered in other publications, many of which are listed in the section on Bibliography and Other Information.

A fuller discussion of the diseases mentioned, their differential diagnosis and of the various methods of investigation and treatment available is also beyond our remit. We do not dwell in depth on the merits of endoscopy versus classical barium radiology or the newer non-invasive radiological techniques. We do, however, recognize the contribution of endoscopic ultrasonography to clinical practice and a chapter is devoted to this subject.

The place of enteroscopy remains uncertain: it may never become established in all hospitals but is likely to find a permanent niche in reference centres. A chapter on this growing subject has therefore been included.

We could have added a chapter on Growing Points. This might, for example, have included such topics as virtual colonoscopy, endoscopic fluoroscopic spectroscopy, non-visual biosensors, the possible uses of very small robots, and experimental therapeutic techniques such as endoscopic gastroplasty. Every practising endoscopist should be aware of what is new in endoscopy, but we felt that none of these techniques had yet been sufficiently developed to merit further discussion in an Atlas.

Most endoscopists will not regard themselves simply as expert technicians but as members of a gastroenterological team carrying clinical responsibility during endoscopy and also subsequently when major decisions are made on all available evidence, including that from endoscopy. This principle is generally accepted the world over, and for this reason training programmes for gastroenterologists not only set targets for endoscopy but also insist on a working knowledge of gastrointestinal pathology.

To accommodate these developments the most important differences between the original Atlas and the present volume are the inclusion of a chapter on how the pathologist can help the endoscopist, and the presentation of histopathological appearances alongside endoscopic images. No attempt has been made to write a

comprehensive textbook of gastrointestinal pathology, but sufficient data are presented to underline the relevance to the endoscopist of some knowledge of pathology. This is reflected in the new title.

The views obtained when using fibre endoscopes and newer video endoscopes are generally similar, as the same regions and lesions are being surveyed. The older images, whether square or round, were often of excellent quality. In the event, most endoscopists now use electronic equipment and are more used to viewing a screen, and recording images on video tape or as video prints. There seemed little point in attempting to replace the better pictures of the original Atlas but we have tried to supplement these as appropriate with images obtained by the use of video endoscopes.

Although this Atlas includes some material from the original work, the text has been almost entirely rewritten and expanded, many images have been replaced and many new ones added, and each chapter has been restructured to suit the new contents and purposes of this venture. While this Atlas is a second edition, we present it to our readers as a new work. It certainly seems so to us, especially taking into account the amount of time and effort expended, we hope to good effect.

For whom is this Atlas intended? As we stated in the Preface to the original Atlas, we aim, firstly, at the less experienced endoscopist so that he or she may gain confidence by having available a range of appearances from which to learn and with which to compare findings. Secondly, more experienced endoscopists may wish to broaden their horizons and may be stimulated by seeing a wider spectrum of appearances than those with which they are familiar. We hope that this Atlas will find a place in the endoscopy room as a bench book, as we are told the previous edition did. Radiologists, pathologists and non-specialist physicians and surgeons may also be interested to examine what the endoscopist actually sees and does during diagnostic and therapeutic procedures. Furthermore, we hope that this publication will lead to a wider understanding of the place of endoscopy in gastroenterology, that it will help decide which patients are most appropriately referred for endoscopy, and not least that it will reveal some of the limitations of the technique. We believe that this new book should be of value to clinical students and their teachers during discussions on gastroenterological topics. With the increasing involvement of interventional radiologists in endoscopic procedures, we wish for them to be among our readers. Histopathologists are also very much a part of the team so it may be of help and interest to them to have a ready access to a collection of endoscopic appearances when handling the fruits of endoscopy. And, last but not least, there is the nurse endoscopist. The number of practitioners in this new specialty has risen rapidly and their breadth of experience has expanded. With increasing acceptance of the new role the number of such specialty nurses will continue to rise. To this new group of potential readers we also extend our welcome and hope that they will find the Atlas useful.

Our own experience, and with it our collection of endoscopic images, has grown over the years. Nevertheless, we could not have undertaken this task without the help of others, as acknowledged elsewhere. Producing an Atlas, like the successful practice of gastroenterology, relies heavily on the support of a good team. To all our collaborators and colleagues we express our thanks.

KFRS
RC
RHH
BFW

Acknowledgements

Our first acknowledgement goes to our collaborators, Dr Martin Lombard, Dr Anthony Morris and Dr Thomas Rösch for their chapter on endoscopic ultrasound, and Dr John Morris for his overview of enteroscopy.

Some illustrations have previously appeared in *A Colour Atlas of Gastrointestinal Endoscopy*, edited by three of us and published by Chapman & Hall Medical; in this Atlas we listed and thanked a number of endoscopists for their loan of images, some of which are now reused. We must reassure our readers that the majority of the figures that appear in this second edition originate in our own units. Nevertheless, we have been offered a wealth of additional material by old and new friends and colleagues.

We must extend special thanks to Dr Mark van Blankenstein and colleagues, Endoscopy Unit of Rotterdam University Hospital Dijkzigt, Prof. Neil Mortensen, Department of Colorectal Surgery, John Radcliffe Hospital, Oxford and Dr Christopher Williams, Wolfson Unit, Northwick Park and St. Mark's Hospital, London.

We also gratefully acknowledge help from Dr Monzur Ahmed, Prof. Duncan Colin-Jones, Prof. Massimo Crespi, the late Dr Jack Davies, Dr Richard Dickinson, Prof. Brian Gazzard, Mr Bruce George, Dr Peter Golding, Dr Godman Greywoode, Dr Rebecca Harrison, Dr. Stefan Hubscher, Prof. Gunar Jänerot, Dr Blair S. Lewis, Dr Duncan Loft, Dr Nick Mahy, Dr Tony Mee, Dr Peter Millard, Dr David Mutimer, Dr Simon Olliff, Dr Adam Padel, Dr Marios Panos, Dr Juan Piris, Dr Howard Rigby, Mr Alan Roe, Dr David Rowlands, Dr Scott Sanders, Dr Brian Saunders, Prof. Neil Shepherd, Prof. Janaka de Silva, Dr Subbaramiah Sridhar, Dr Tim Stephenson, Prof. Paul Swain, Dr Robin Teague, Dr Naomi Uemura, Dr Marjorie Walker and Dr Peter Willoughby.

A small number of images have been reproduced, with permission, from *Illustrated Case Reports in Gastroenterology*, the *Journal of Clinical Pathology* and the *American Journal of Surgical Pathology*.

It takes a long time to put together a useful collection of endoscopic images, and over this time we have had much help from medical, nursing, technical and secretarial staff of our Units. They deserve more than routine thanks. We must also thank KeyMed (Medical and Industrial Equipment) for the loan of some excellent equipment; Helene Beard for expert preparation of histological figures and Molly Harwood for help with the images drawn from the collection held in the Department of Colorectal Surgery, John Radcliffe Hospital, Oxford. The Department of Medical Illustration, Selly Oak Hospital, Birmingham, prepared many of the photographs of equipment. Special mention must be made of Robin Roberts-Gant, Medical Informatics Unit, Nuffield Department of Clinical Laboratory Sciences, University of Oxford, who so expertly handled a melange of old and new, round and square transparencies, prints, photographs and digital images of all sizes, and also helped in

the preparation of the greyscale images and many other illustrations. The line drawings in Chapter 6 were prepared by Jane Fallows.

The staff of Blackwell Publishing were throughout encouraging and supportive. In particular we must list Charlie Hamlyn, Marcela Holmes, Audrey Cadogan and Sally Lane and last but not least Andrew Robinson who was in overall charge, and ever helpful and patient.

When there are four editors, all of whom are also contributors, the manuscript inevitably has a troubled gestation, a difficult birth and a traumatic childhood. We could not have produced this Atlas without the patient, sustained and efficient input (in her spare time) from Ginny Schiller at the keyboard.

We cannot thank everyone who helped us enough. If the result is worthwhile it is in great measure due to their unstinting support.

KFRS
RC
RHH
BFW

Abbreviations

AIDS	acquired immunodeficiency syndrome
APC	argon plasma coagulation
AVM	arteriovenous malformation
CBD	common bile duct
CLO	columnar lined oesophagus
CMV	cytomegalovirus
CT	computerised tomography
DALM	dysplasia-associated lesion or mass
EATL	enteropathy-associated T-cell lymphoma
EGC	early gastric cancer
ERCP	endoscopic retrograde cholangiopancreatography
ESWL	extracorporeal shock wave lithotripsy
EUS	endoscopic ultrasonography
FAP	familial adenomatous polyposis
FB	foreign body
FNAB	fine needle aspiration biopsy (also FNA)
GAVE	gastric antral vascular ectasia
GIST	gastrointestinal stromal tumour
GORD	gastro-oesophageal reflux disease
HPF	high power field
MALT	mucosa-associated lymphoid tissue
MRC	magnetic resonance cholangiography
MRCP	magnetic resonance cholangiopancreatography
MRI	magnetic resonance imaging
Nd-YAG	neodymium-yttrium aluminium garnet
NSAID	non-steroidal anti-inflammatory drug
OGD	oesophago-gastro-duodenoscopy
PAS	periodic acid Schiff
PCR	polymerase chain reaction
PEG	percutaneous endoscopic gastrostomy
PSC	primary sclerosing cholangitis
PTC	percutaneous transhepatic cholangiography
REAL	revised European and American lymphoma classification
TTS	through-the-scope
UC	ulcerative colitis
US	percutaneous ultrasonography

CHAPTER 1

Getting the Most out of your Pathologist

This chapter discusses handling of biopsy, endoscopic resection and cytological specimens and how to present them to the pathologist to realise their optimum diagnostic potential. Mention is made of some techniques used by pathologists with which endoscopists should be familiar. Throughout this chapter emphasis is laid on ways clinicians and pathologists can work together. Views expressed in this chapter are based on practice in one of our centres. Readers in practice elsewhere will bear this in mind, for example regarding the section headed 'How quickly can I have an answer?'

Information

What do I tell the pathologist?

The pathology request form is a request for a specialist opinion and as such must include adequate information about patient details including symptoms, results of other relevant investigations, endoscopic findings and, above all, the sites from which the samples have been taken. The request may be made on a simple card or a computerized document including part of the endoscopy report. An integrated request form for endoscopists (Fig. 1.1, adapted from D. Jenkins, 1988) has been developed by the British Society of Gastroenterology Guidelines Group for

COLORECTAL BIOPSY

CLINICAL DATA SET FOR SUSPECTED CHRONIC IDIOPATHIC IBD

Patient Name: Hospital:
Date of birth: Sex: F ❑ M ❑
Date of examination:

Main symptoms:

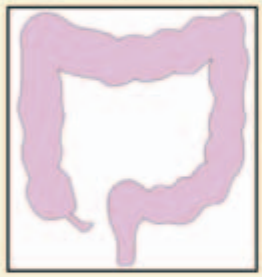
Diarrhoea: watery ❑ bloody ❑

Total duration of disease:

Duration of present episode:

Present clinical state: active ❑ inactive ❑

Sigmoidoscopic appearance: mucosal features:



extent of disease:

- rectum ❑
- rectum & sigmoid ❑
- left side ❑
- substantial ❑
- extensive ❑
- pancolitis ❑

pattern:

- continuous ❑
- segmental ❑

Site of biopsy/biopsies:

Clinical opinion:

ADDITIONAL INFORMATION (IF APPROPRIATE)

Include previous gastrointestinal surgery, therapy, results of stool culture, other diseases present

1.1

Inflammatory Bowel Disease; this form serves as a good example of the use of tick boxes and may be used either as a sheet of paper or as part of a computerized request.

The exact site of origin of samples is often crucial, as for example in the stomach when gastritis of the antrum and pangastritis have quite different clinical connotations and risk potential; again, metaplasia in body-type mucosa as a consequence of inflammation and loss of specialized cells can cause it to look identical to antral mucosa. Another example is in the diagnosis of Barrett's oesophagus where it is essential to know whether the biopsy is truly from the oesophagus. Although the presence of underlying oesophageal mucous glands or ducts (Fig. 2.141) may help in identification they are not always present. If it is known with certainty that the biopsy came from the oesophagus and not from an hiatal hernia or the stomach, disorderly glandular mucosa with or without intestinal metaplasia is enough to corroborate the endoscopic diagnosis.

Relevant previous surgical operations must be mentioned on the request form as it is an intellectual challenge to the pathologist if a form is labelled

'oesophageal biopsy ?inflamed' when the patient has had an oesophagectomy with colonic interposition. Similarly, small bowel metaplasia might be reported in a defunctioned rectum when there had been a previous colectomy with ileo-colic anastomosis. Without knowledge of the previous anastomosis the biopsy taken from a remnant of normal small bowel would be misinterpreted.

It is helpful to include a copy of the endoscopy report and perhaps an endoscopic picture: pathologists find macroscopic appearances invaluable. This could be supplemented by occasionally inviting the pathologist to see lesions *in situ* at endoscopy. Such an approach improves working relations and makes the pathologist feel part of the team, with consequent increased enthusiasm for endoscopic specimens.

Suggestions for obtaining biopsy and cytological specimens

Certain general recommendations can be made. For example, for lesions where malignancy is suspected or is a possibility, whether such a lesion is raised, flat, depressed or ulcerated, up to 12 biopsy specimens should be collected. The target sites should include as many aspects of the lesion as possible, for example the rim as well as the ulcerated centre. When a solid lesion appears to be submucosal, superficial biopsies are often unhelpful and other methods of obtaining material, e.g. 'large particle'/snare biopsy, fine needle aspiration or the use of hot biopsy forceps should be considered.

When the interest is centred on an observed or possible mucosal abnormality, the biopsy forceps must of course be directed as appropriate but in any case multiple specimens should be sought as mucosal abnormalities are often patchy and may not be visible endoscopically. It is difficult to recommend a useful minimum number of specimens; suffice it to say that your interested, co-operative and involved pathologist will prefer too many to too few.

The requirements for screening and surveillance will differ from those for diagnosis. For example, a diagnosis of Barrett's oesophagus will be confirmed when one biopsy from a lower oesophagus clearly lined with columnar mucosa is positive in this respect. Conversely, when a patient with known Barrett's oesophagus attends for screening for dysplasia, it is recommended that quadrantic biopsies should be taken at 2 cm intervals along the length of the Barrett's oesophagus. Again, in the follow-up of a patient with inflammatory bowel disease known to have had pancolitis, it is advisable to take biopsies at 10 cm intervals from caecum to rectum, although this is not always practicable.

When specimens for cytological study are collected, a sheathed brush must be used. Contamination of the biopsy channel of the endoscope with foreign material can lead to irreparable complications in patient management and to serious medicolegal problems. Other aspects of cytology are discussed later in this chapter.

The above is no more than a set of general recommendations. More specific and more detailed recommendations appear as appropriate in succeeding chapters.

How do I send the biopsy specimens?

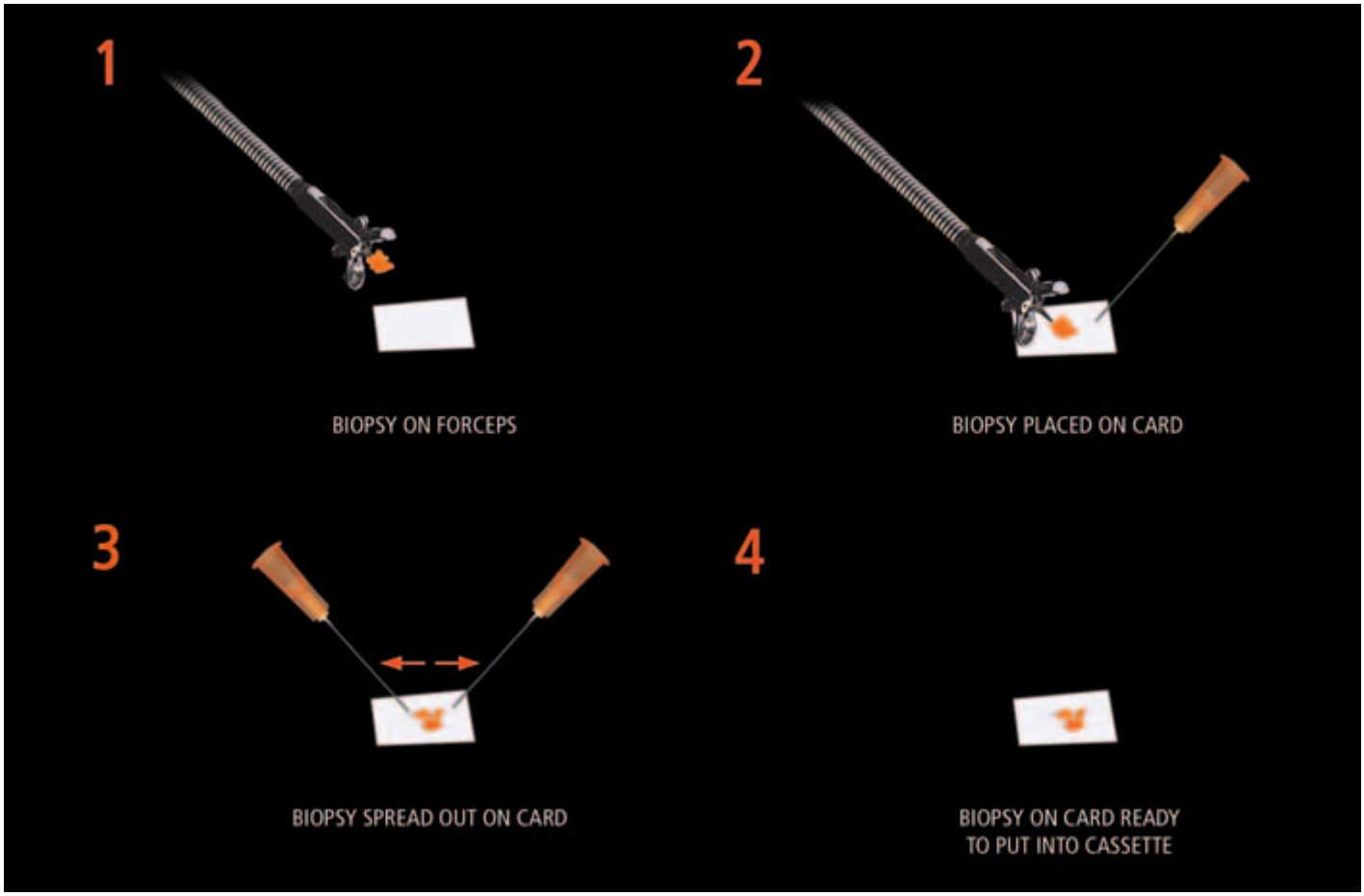
Biopsies from different anatomical sites or lesions must be submitted in separate, labelled containers. The impossibility of distinguishing atrophic gastric body-type mucosa from antral mucosa has already been mentioned. The distribution of a colitis is a great diagnostic aid which is lost if all samples are floating in the same pot.

Similarly, a diagnosis of dysplasia or invasive malignancy is only of practical use if the site is known: a dermatologist would not place odd looking naevi from different sites in the same pot, in case one of the naevi should be a malignant melanoma needing further excision. Each pot should be labelled individually and placed in a plastic bag with the request form in a separate plastic pocket within the bag (Fig. 1.2) to avoid smudging and contamination of the form with potentially infected body fluids.

1.2

Most material for pathological study can be put into 10% buffered formalin, the amount of fixative required being a minimum of five times the volume of the tissue to be fixed. Tissue fixation involves a complex set of chemical reactions which are slowed by cooling. Thus there is no logic in the common practice of placing specimens in formalin in the refrigerator overnight. Glutaraldehyde is a good fixative for electron microscopic studies. Bouin's fixative gives better preservation of neuroendocrine cells but is not used in routine endoscopic diagnostic biopsy practice.

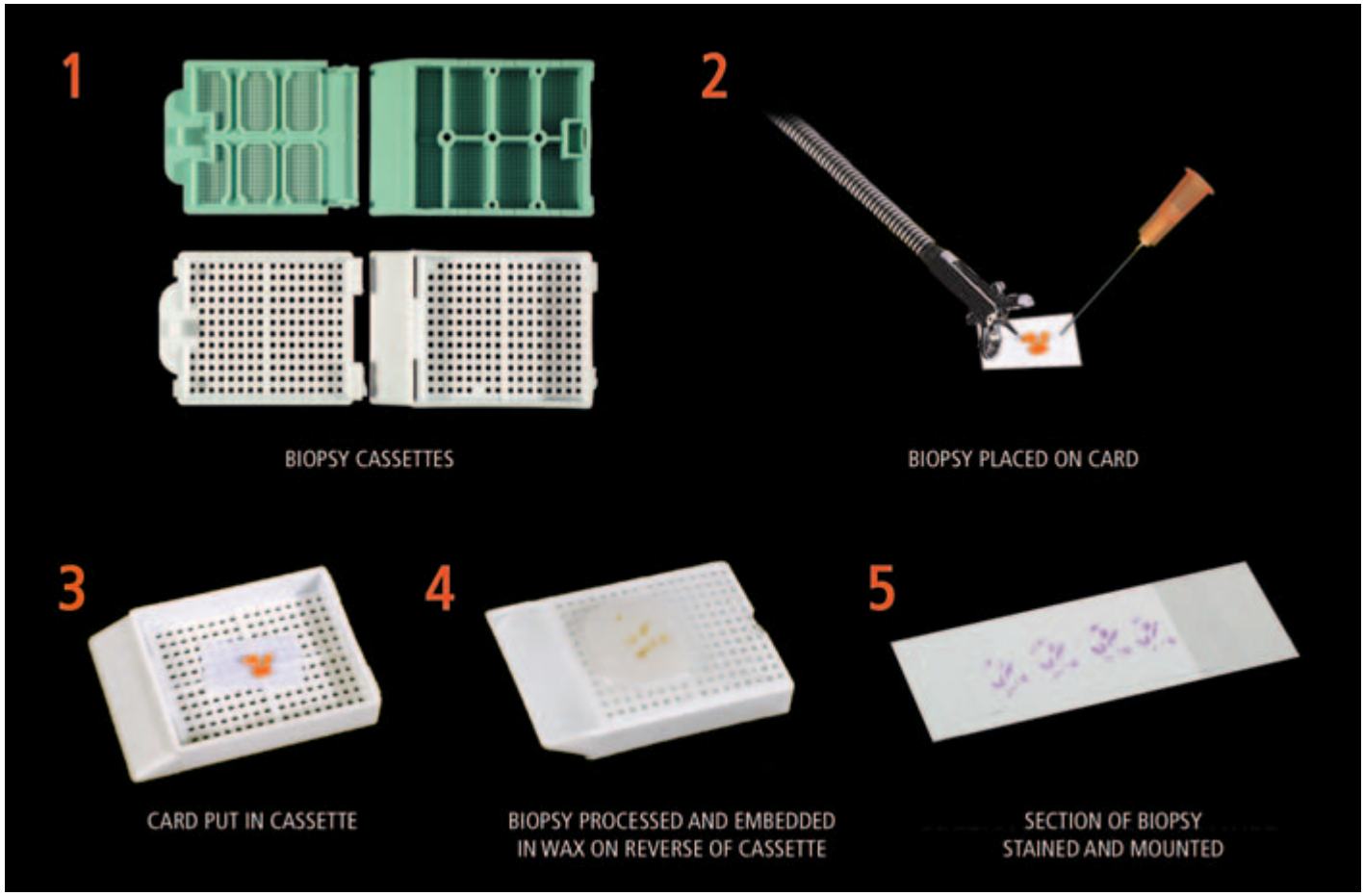
Biopsies should be extracted gently from the forceps using a needle. Although it takes more time, this is best done in a Petri dish of physiological saline to prevent drying artefacts. In a non-orientated mucosal biopsy contraction of the muscularis mucosae results in curling of the tissue with the mucosa on the outside of the ball. Teasing of very small biopsies in an attempt to orientate them may be difficult and potentially disrupting to the tissue, but orientation of larger, more easily visible pieces may be helped by placing the material on a strip of thin card, muscularis mucosae side down (Fig. 1.3). The biopsy will adhere to the card and will not roll into a ball as the muscularis mucosae contracts with fixation. Multiple biopsies may be put on one strip of filter paper so long as the strip is carefully labelled. This permits some degree of orientation at the postfixation stage. However some laboratories do not use this method routinely and rely on cutting an adequate number of sections at different levels through the block, which usually allows a well orientated view of the mucosal architecture. Orientation is especially important for accurate assessment of villous architecture, inflammation and dysplasia. In the absence of correct orientation, proper assessment of villous architecture in small bowel biopsies is impossible. Similar problems occur with recognition of inflammation in the large bowel and dysplasia anywhere in the gastrointestinal tract. Lymphocytes, plasma cells and eosinophils are normally present only in the superficial part of the lamina propria of the large bowel. An increase in chronic inflammatory cells is recognized by



1.3

noting the presence of plasma cells at the level of the muscularis mucosae and loss of the normal gradient of cell density between the upper and lower parts of the lamina propria. This is impossible if the view is of a transverse section of only the superficial part of the mucosa at more than one site. Recognition of dysplasia depends on many subtle and less subtle changes in the crypt epithelium. One reliable feature is failure of nuclear maturation and the presence of abnormal nuclear detail throughout the full length of the crypt. If the full length of the crypt cannot be viewed the presence and grade of dysplasia cannot be assessed.

Exceptionally, biopsies need to be presented fresh. Under such circumstances it is best to make arrangements for the pathologist to attend in person. Freshness is essential, for example, in the investigation of motility disorders, such as Hirschsprung's disease in children or slow transit constipation in adults so that specimens are in the best state prior to freezing. Fresh specimens are also useful to assess excision in larger polyps, in transanal endoscopic microsurgical excision and when schistosomiasis is suspected. Crushing a biopsy between two glass microscopy slides and viewing the unstained biopsy with ordinary light microscopy and between paired polarizing lenses may reveal the refractile wall of the schistosomes. When successful, this gives a very rapid answer; when unsuccessful, a potentially useful rectal biopsy has been destroyed. A duplicate specimen should always therefore be submitted in routine fixative.



1.4

Histological processing cassette

Automated histological processing machines are now in common use. Tissue is placed into a small perforated plastic cassette. Figure 1.4 shows two types, one with six small compartments and one with a single large compartment. When processed, the tissue is embedded in paraffin wax and the wax block is adhered to the back of the tissue cassette. The cassette is then used to mount the block on a microtome for sectioning and subsequently for storage. The cassette is labelled with a unique identifying laboratory number. Laboratory handling is facilitated by the use of such cassettes in the endoscopy room. When this step is not used in the endoscopy room biopsies have to be extracted from a pot of formalin and placed in a cassette in the laboratory. Repeated transfer of tissue from one container to another may lead to fragments of tissue from one patient's specimen contaminating another. One way to reduce the number of specimen handling and transfer steps is for the endoscopist to place the biopsy in a labelled, lidded cassette, as described above, in the endoscopy room. Endoscopists may care to discuss this approach with their pathologist.

Frozen section diagnosis

Frozen sections are rarely used in gastrointestinal endoscopic biopsy practice, with the exception of the very few specialized investigations, usually research orientated immunohistochemistry, with reagents which are not yet validated or are known not to work on paraffin processed material. As mentioned earlier, it may be useful in motility disorders.

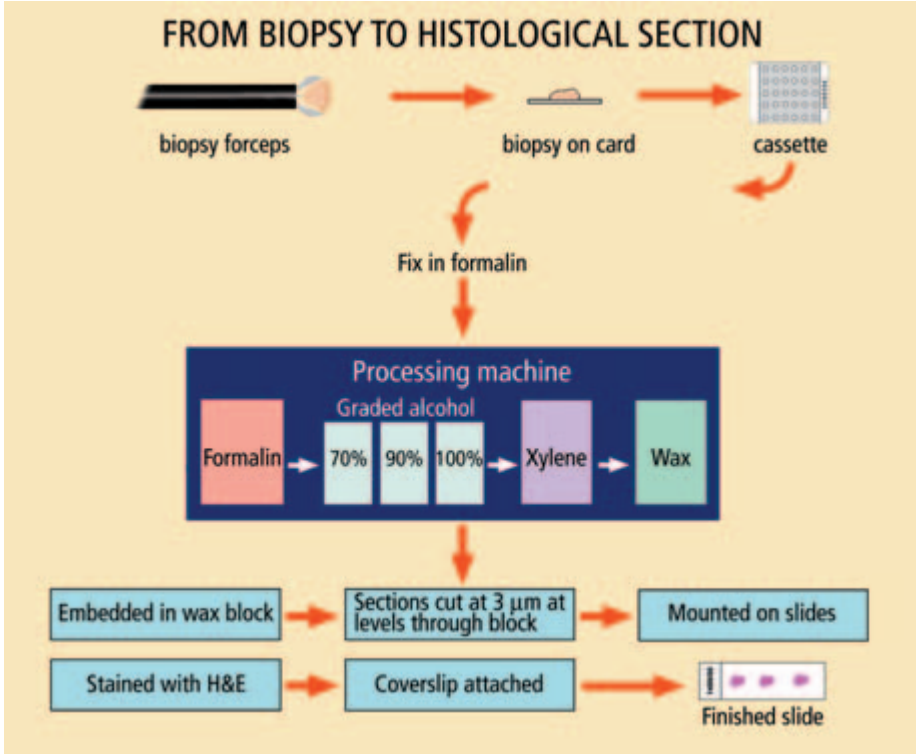
How quickly can I have an answer?

This depends primarily on the size of the specimen sent to the laboratory, and the time of day it is sent. With very small biopsies, it is possible to provide at least a provisional report within the working day or overnight, depending on the time of receipt. If special staining techniques are required the process will be prolonged as described below. Cytological smears can often be reported on within 10–20 min of receipt by the pathology laboratory.

What does the laboratory do with the specimens?

A flow chart illustrating the routine handling of a biopsy specimen appears below (Fig. 1.5).

In the first place this involves adequate fixation of the tissue, and processing of the specimens through to paraffin wax. Sections are then cut and stained appropriately for microscopic examination. Most gastrointestinal diagnoses are made using a routine haematoxylin and eosin stain, but specialized staining will cause delays. Simple tinctorial staining, e.g. for mucin, can be done on the same day and it is recommended that this should be performed routinely on all gastric, Barrett's and duodenal biopsies as isolated signet ring adenocarcinoma cells may be so easily missed. More complex staining, such as for immunohistochemistry, may take a further day. Initial immunohistochemical staining may indicate that further immunohistochemical stains are necessary, and this will take usually another full day.

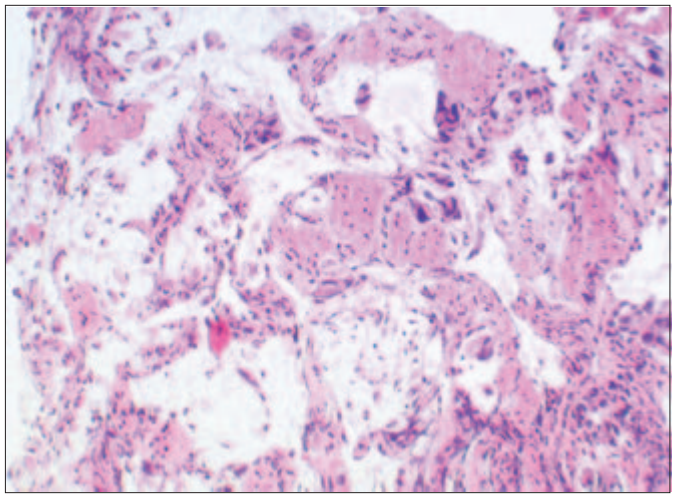


1.5

Special techniques

Additional commonly used tinctorial stains

Alcian blue combined with periodic acid–Schiff (PAS) staining is essential in the evaluation of biopsies from the oesophagus, stomach and duodenum for identification of acid mucin/neutral mucin. Without this technique intestinal metaplasia and individual signet ring cancer cells may very easily be overlooked. In Fig. 1.6 the section has been stained with haematoxylin and eosin. When stained with Alcian blue and PAS the signet ring cells are easily seen (Fig. 1.7).



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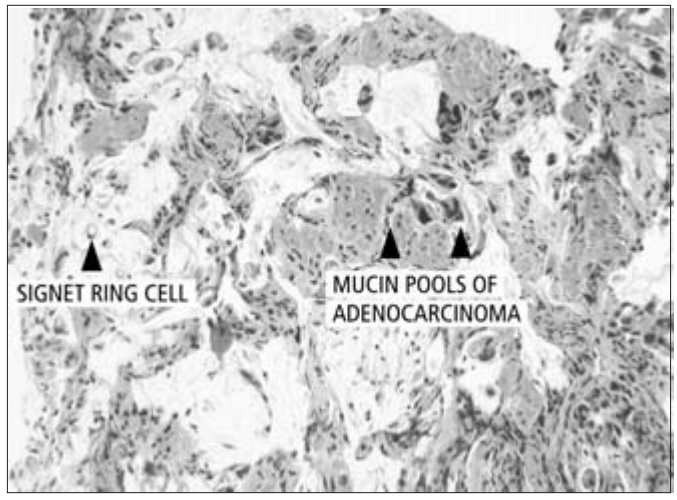

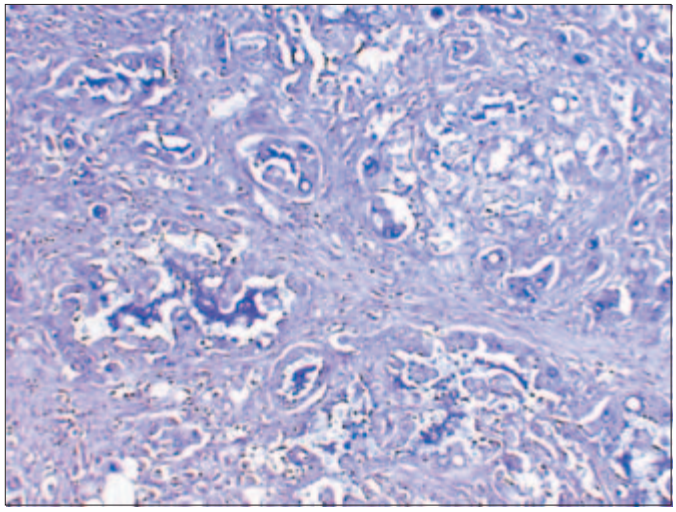

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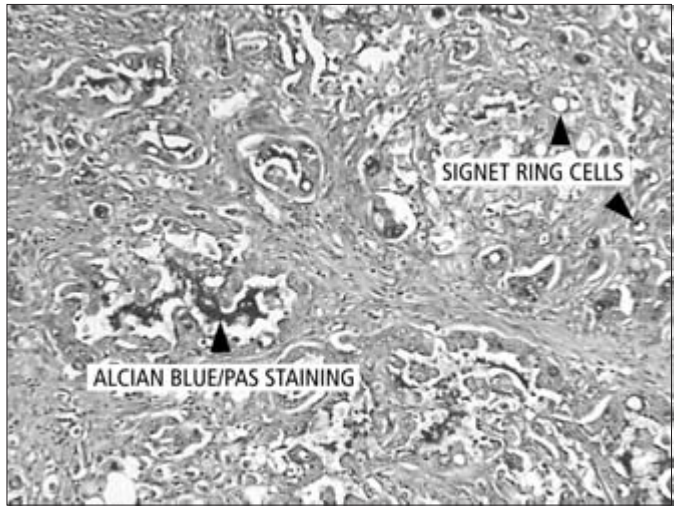
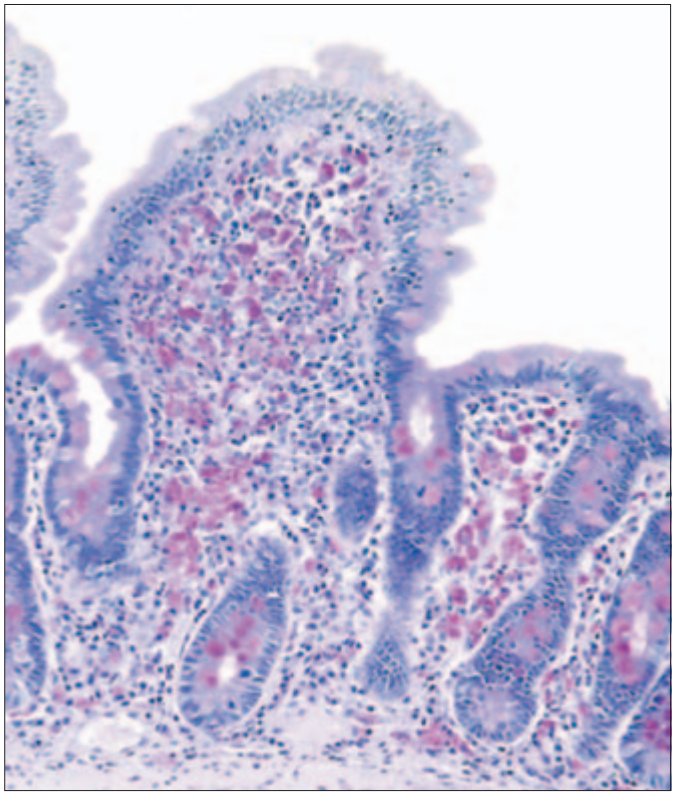


The PAS part of this combination also stains fungi, macrophages in Whipple's disease (Fig. 1.8) and amoebae in large bowel biopsies. Trichrome stains are useful for collagen if collagenous colitis is suspected. Sections stained with Sirius red or in this case with Congo red, when viewed under crossed polarizing lenses identify amyloid by the presence of apple green birefringence (Fig. 1.9).



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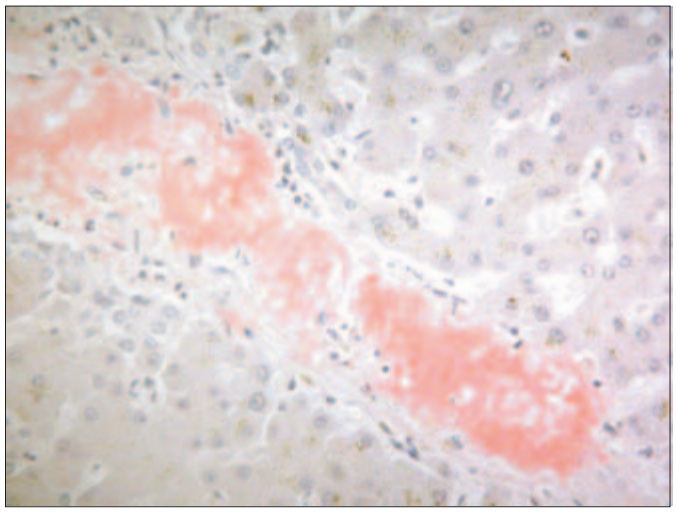

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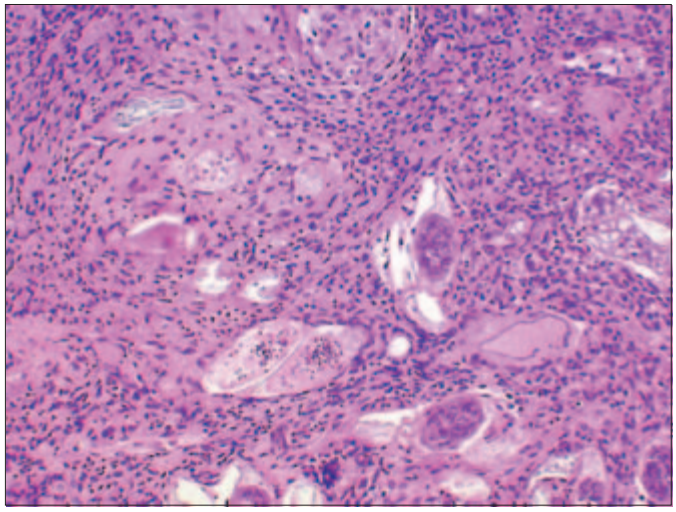
Ziehl–Neelsen stain, well known as a means of identifying mycobacteria, also stains schistosomes (Fig. 1.10). Grimelius is a silver stain for neuroendocrine cells (Fig. 1.11). There have been few new tinctorial stains but the recently introduced Genta stain shows mucin and *Helicobacter* as well as demonstrating tissue morphology.



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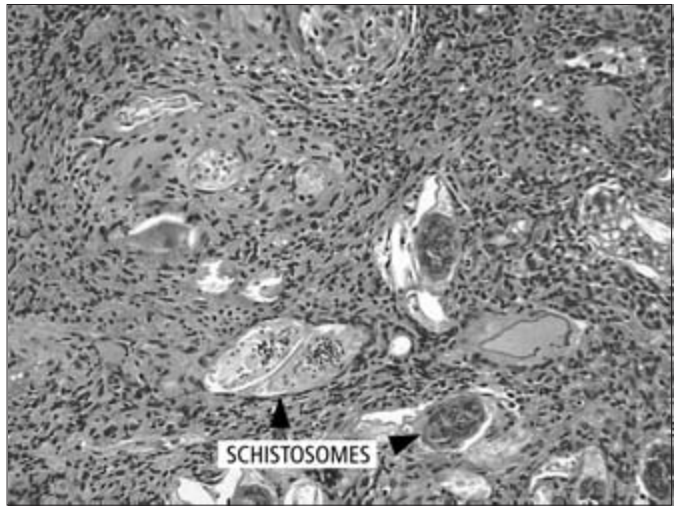

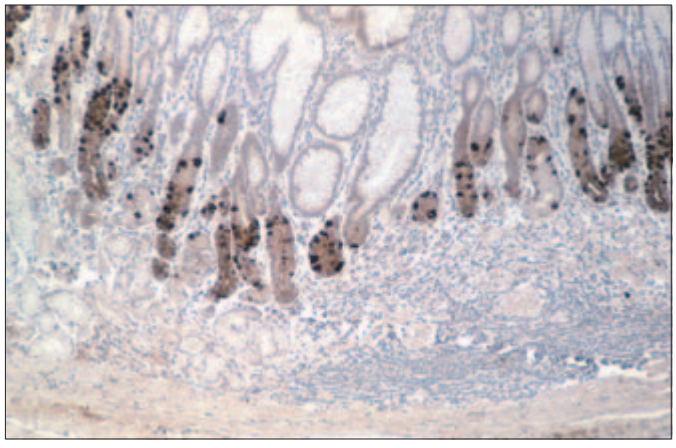

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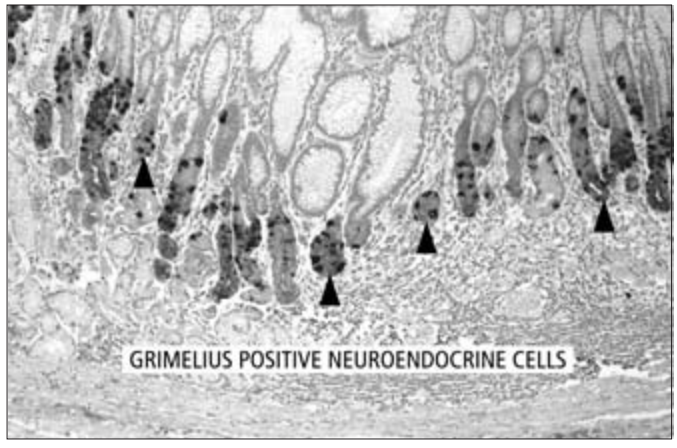
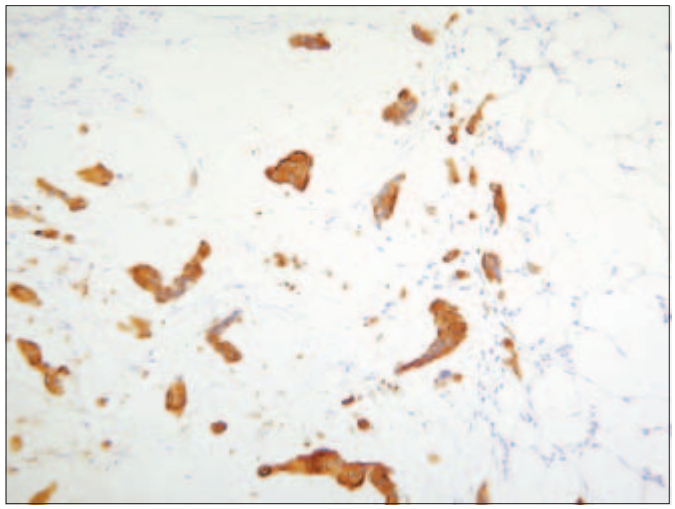


Immunohistochemistry

Immunohistochemical stains for cytokeratins, epithelial membrane antigen and carcinoembryonic antigen are used to detect abnormally sited epithelial cells when diagnosing invasive malignancy. Some caution is needed in interpretation since some cytokeratin stains may attach to new fibroblasts in ulcer bases as well as staining epithelial cells. Many cytokeratin stains are available and may be used to overcome this pitfall. Cytokeratin 20 is a sensitive marker for gastrointestinal adenocarcinoma cells (Fig. 1.12), whereas cytokeratin 7 is a sensitive marker for gynaecological adenocarcinomas; this pair of cytokeratin stains is therefore very useful for the distinction of a primary gastrointestinal carcinoma from a metastatic ovarian or endometrial carcinoma (Fig. 1.13). Lymphomas are best evaluated immunohistochemically. This will enable identification of T or B cells and will by staining for kappa and lambda light chains assess clonality in B cell infiltrates where the diagnosis of lymphoma is subtle. Cytokeratin staining will highlight the crypt epithelium and will enable the destructive lymphoepithelial lesions of a MALT lymphoma to be identified more easily. These are areas of crypt epithelium which are infiltrated by B lymphocytes causing epithelial destruction at that site. Chromogranin A will identify more than 90% of neuroendocrine cells. This is especially useful for carcinoid tumours. Immunohistochemical staining gives confusing results in



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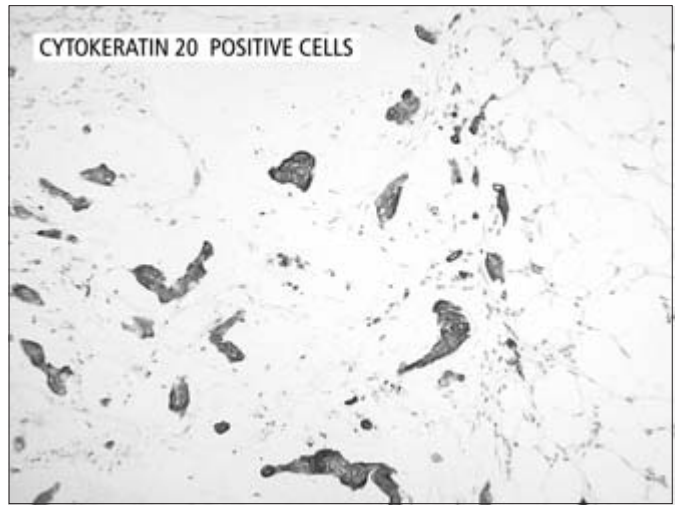

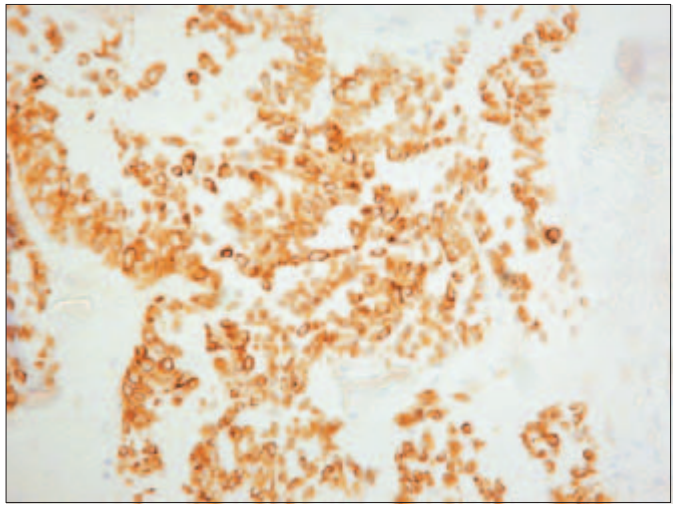

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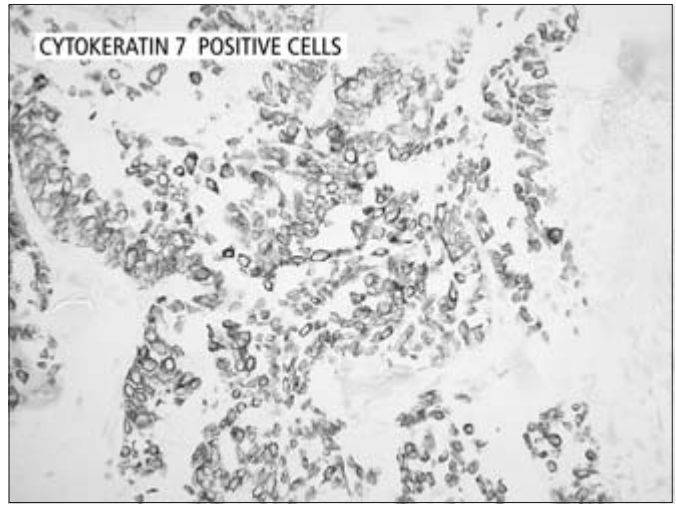
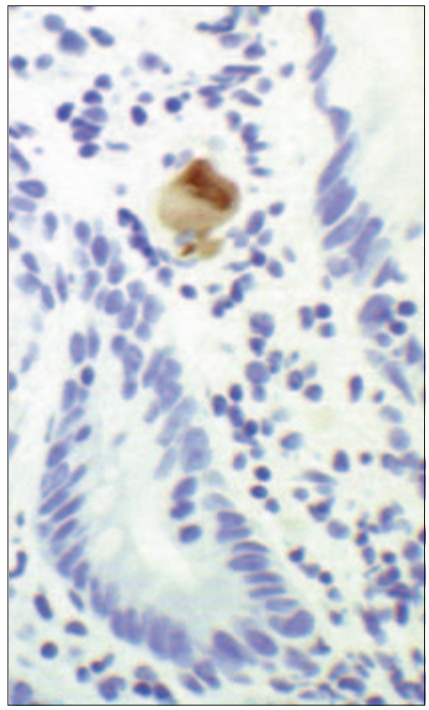




1.14

gastrointestinal stromal tumours (GISTs) but, whether they appear predominantly neural, muscular, mixed or neither on immunohistochemistry, most are positive with CD34, a vascular endothelial cell marker. Nearly all GISTs including those negative with CD34 will stain with antibody to Ckit proto-oncogene, which is thought to indicate a possible origin of these tumours from the interstitial cells of Cajal (whose normal function is as 'pacemaker cells' in the intestine). Different patterns of immunohistochemical staining in GISTs have sometimes been correlated with prognosis, but it is probably better to count mitotic figures as a guide to prognosis (Fig. 2.415 and Table 2.10).

Immunohistochemical stains for organisms such as cytomegalovirus (Fig. 1.14) are an essential part of the work-up of a biopsy from an immunocompromised individual. *Herpes simplex* antibody staining is useful in suspected herpes oesophagitis.

Electron microscopy

This is rarely used in gastroenterological practice but may help in identification of *Microsporidia* spp. in immunocompromised patients. It may occasionally be useful to subclassify rare tumours. Figures 5.50 and 5.51 demonstrate microerosions in NSAID-related enteropathy.

Flow cytometry

Flow cytometry has found favour particularly in Sweden for the early diagnosis of dysplasia in ulcerative colitis by the detection of aneuploidy. It has not as yet become routine in most laboratories.

In situ hybridization

In situ hybridization is used for demonstration of abnormal DNA, RNA and other abnormal protein products, and in the diagnosis of some viral infections.

Polymerase chain reaction (PCR)

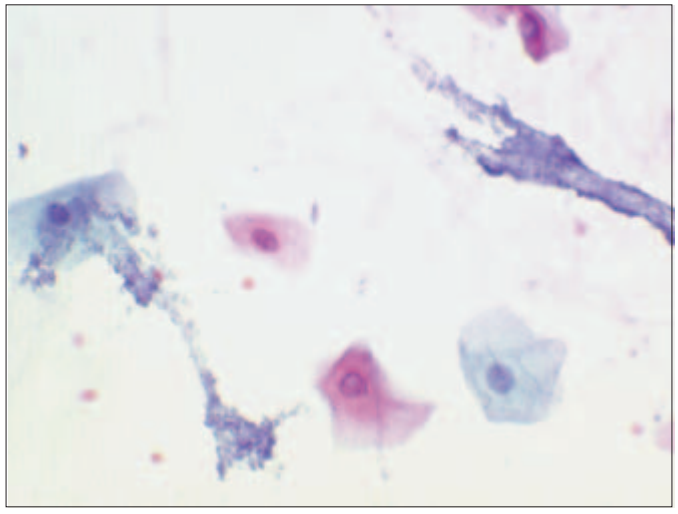
PCR demonstrates small amounts of protein, as for example in the tissue detection of *Yersinia* or *Mycobacterium paratuberculosis*.

Tissue typing

HLA tissue typing is useful in evaluation of 'carry over', when tissues may inadvertently originate from two patients. Anxiety, mismanagement or litigation may result if, for example, carcinoma or dysplasia is seen in a single fragment of blocked tissue when such a diagnosis was not expected. The problem may be resolved by referral of the block or sections to a laboratory specializing in tissue typing of small fragments.

Cytological specimens

Brush specimens taken for cytological examination are mainly employed in upper gastrointestinal tract diagnosis. The addition of cytology to biopsy may improve the positive diagnostic yield, especially for carcinoma of the oesophagus when compared with biopsy alone. Figure 1.15 shows the cytology from normal oesophagus and Fig. 1.16 from squamous cell carcinoma. Bile duct cytology is useful for lesions out of reach of biopsy forceps. Figure 4.43 illustrates brushings from a normal bile duct, in contrast to those taken from a bile duct carcinoma (Fig. 4.44). Anoscopy and cytology with a spatula and brush have become standard in some centres with a special interest in anal intraepithelial neoplasia. Brush cytology specimens generally have a higher yield than washings. It is essential that the pathologist should instruct the endoscopist and assistants in the technique of slide preparation. In particular, different staining techniques will require the appropriate method of slide preparation. Giemsa staining needs air dried slides, whereas most other stains need immediate smear fixation.



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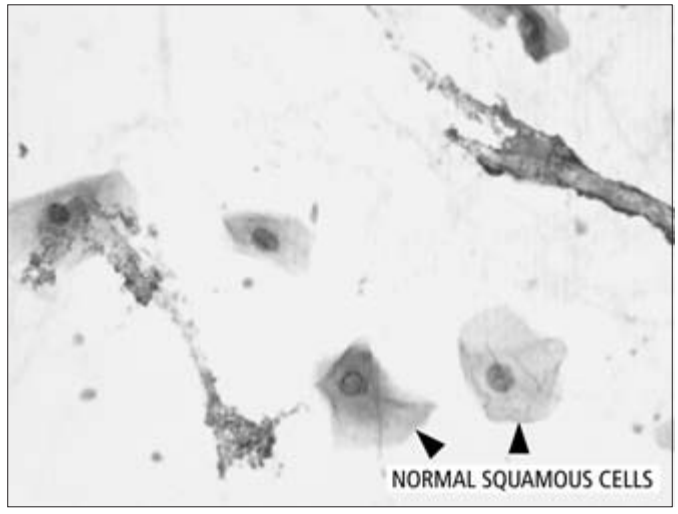

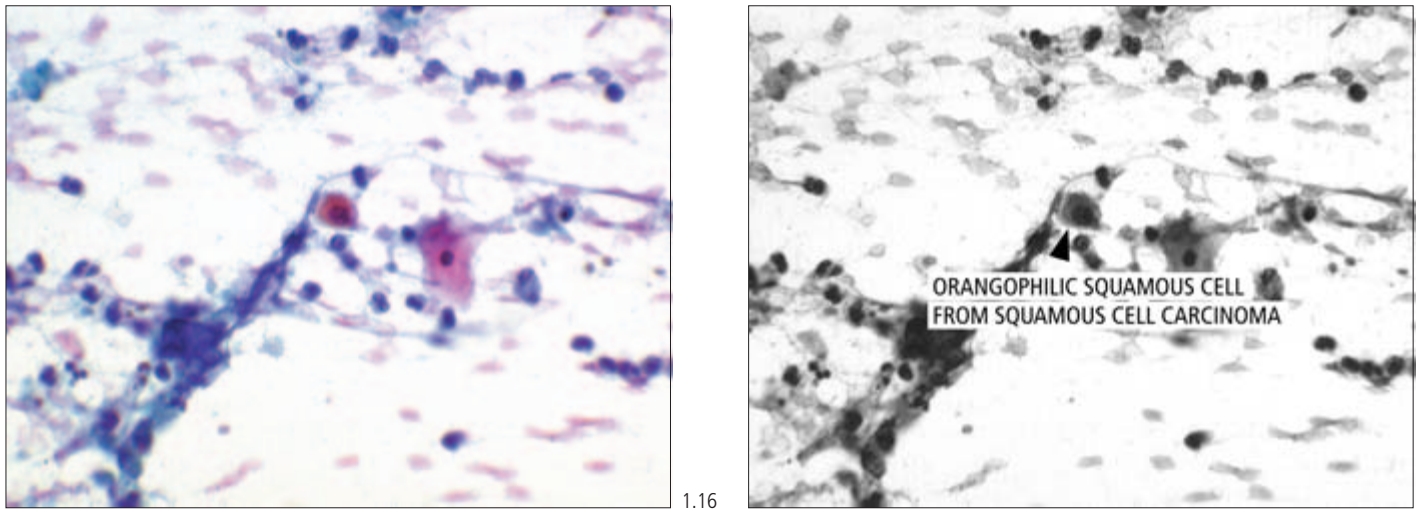

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Fine needle aspiration cytology is not commonly used by endoscopists but may be of value in sampling lesions beneath the mucosa both for localized tumours and for attempting to improve the chances of tissue diagnosis in suspected linitis plastica.

Endoscopic resection specimens

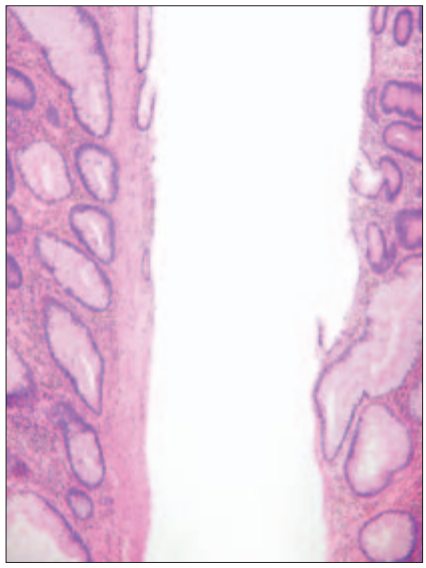
Polypectomy

The problems here are of orientation, diathermy artefact in the assessment of polyp type, stalk invasion and completeness of excision. A pedunculated polyp will shrink when fixed in formalin. Normal mucosa in the stalk shrinks more than the adenomatous mucosa which results in a stalk that was easy to visualize in the fresh state, retracting and disappearing into the polyp such that it is difficult or impossible to identify on the following day. Some endoscopists mark the site of excision on the excised polyp with ink or with a pin. If a pin is used it should pass from the stalk to the polyp, because insertion from the polyp towards the stalk may cause problems of misinterpretation of pseudoinvasion due to misplacement of epithelium into the stalk by passage of the pin, as shown in Fig. 1.17.

Transanal endoscopic microsurgery

Specimens from this technique need especially careful handling to provide the information required for subsequent patient management. The operation produces a square of full thickness rectal wall including tumour. Such specimens should be received fresh and pinned on to cork, to identify the narrow but crucial margin of normal mucosa which represents the mucosal resection margin.

Transanal endoscopic microsurgery requires the use of specialized equipment and cannot be performed via standard rigid or flexible endoscopes.



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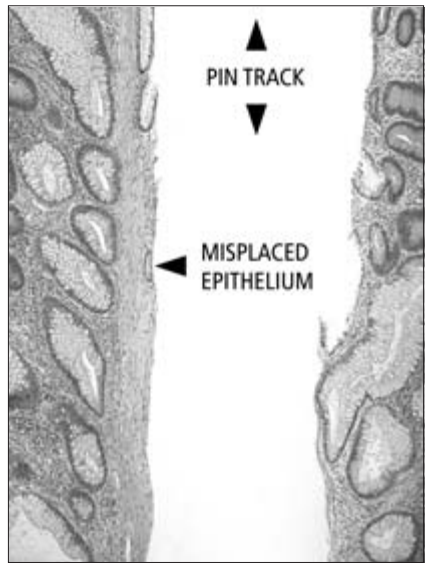
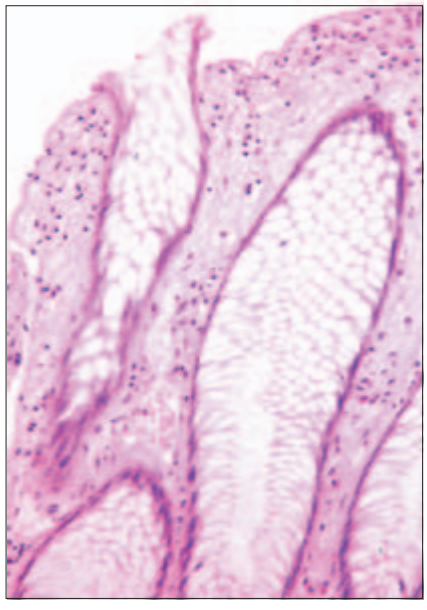


Artefacts

It has been said that the histopathologist is merely a viewer of artefacts, i.e. that pathological specimens and stained histological sections are in themselves almost artefacts. To these must be added, for example, the damage to tissue during collection, handling and processing. These are some of the challenges to which the histopathologist tries to rise.

One of the commonest problems is the heat induced artefact seen after diathermy or the use of hot biopsy forceps. This makes normal mucosa look like a metaplastic polyp (Fig. 1.18).



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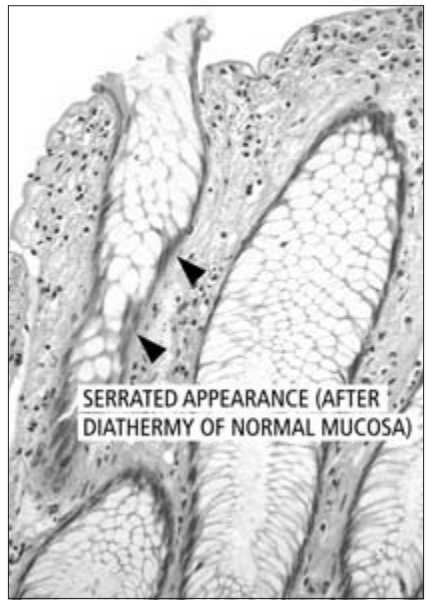

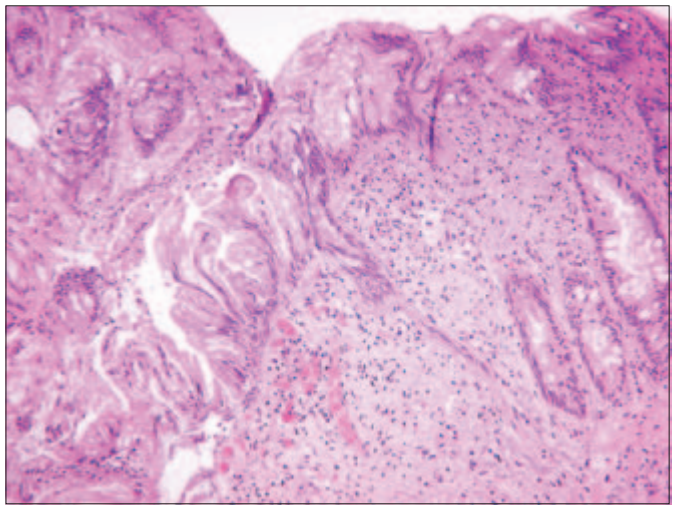

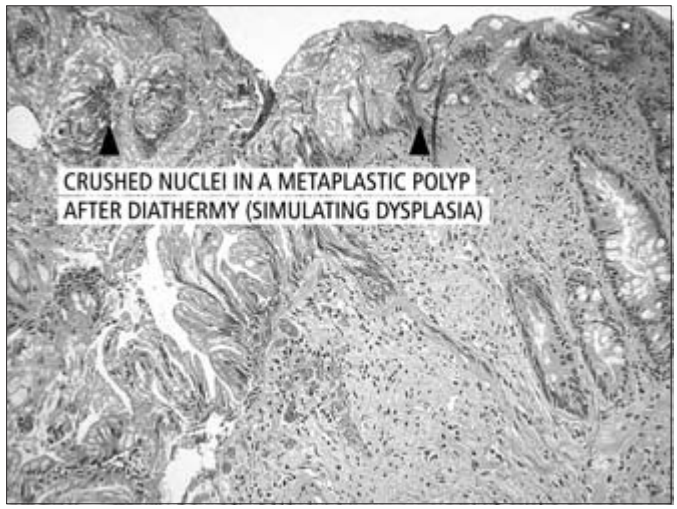

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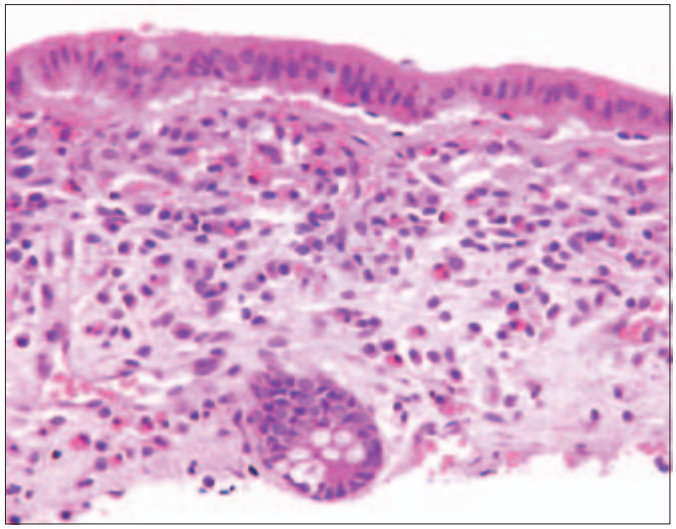

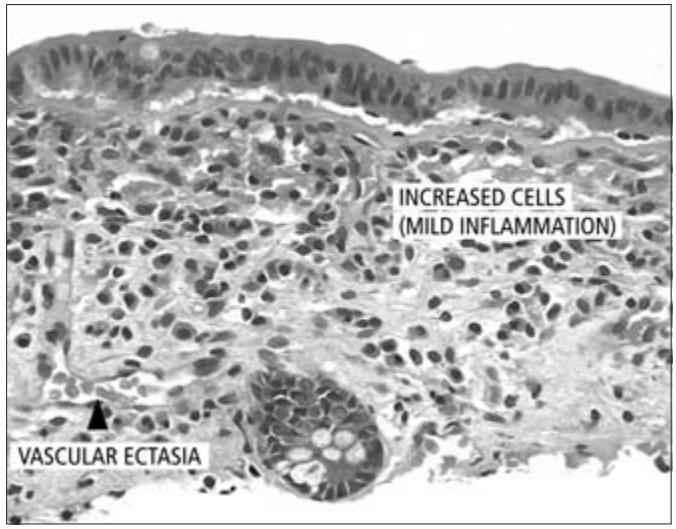

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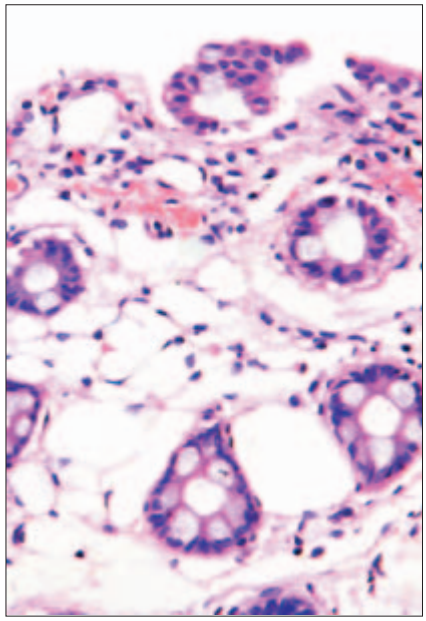
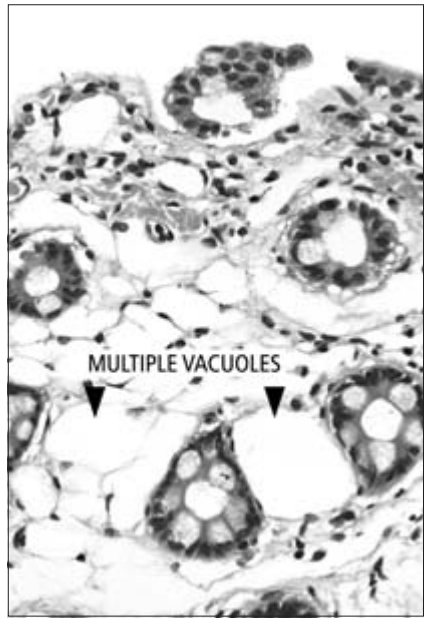
Metaplastic polyps may look like adenomas by crowding the nuclei together and simulating dysplasia (Fig. 1.19).

Following the use of heat, there may also be difficulty in the assessment of the completeness of excision of an adenoma or of a carcinoma within an adenoma.

Preparation of the bowel for endoscopy using hyperosmolar solutions results in oedema and mucin depletion, and some authors report occasional appearances of inflammation, although this is not our experience. Mild inflammation may occasionally be seen following irritant enemas (Figs 1.20 and 3.336). White mucosal patches seen during the withdrawal of the colonoscope (Fig. 3.337) may be the result of hydrogen peroxide, occasionally used in endoscope cleaning, which causes a vacuolated appearance in the lamina propria (Fig. 1.21).





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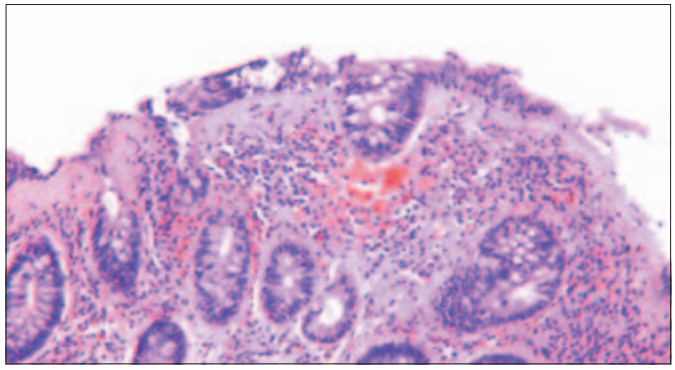
Disruption and telescoping of glands and separation of epithelium result in much confusion. Separation is a useful artefact in collagenous colitis when during processing and sectioning the surface of the epithelium lifts away from the abnormal collagen band (Fig. 1.22). Separation becomes a nuisance in the duodenum where pseudo-lymphangiectasia may result (Fig. 1.23); however, unlike those of true lymphangiectasia (Fig. 1.24), these spaces are not lined by endothelium. Retraction spaces also occur around tumour deposits which may make the diagnosis of vascular invasion difficult; special stains for vascular endothelial cells such as CD34 will usually resolve this problem.



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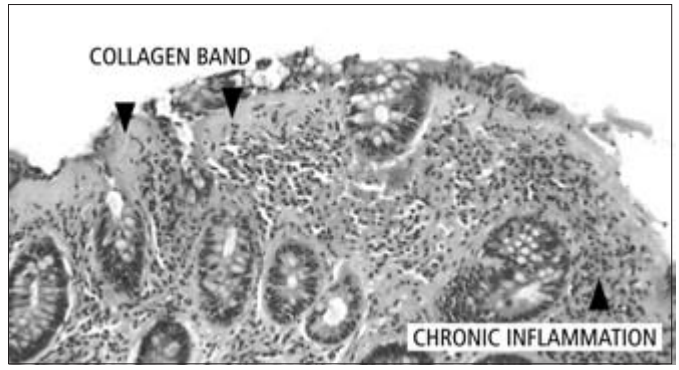

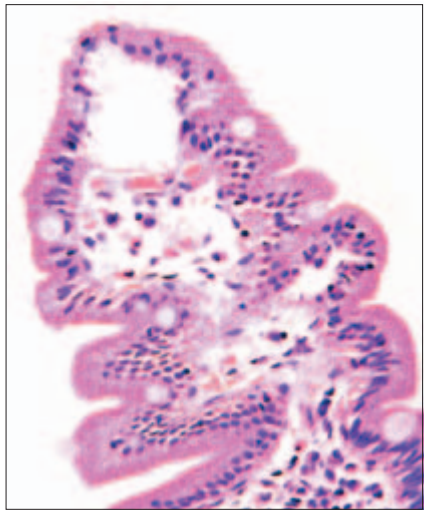

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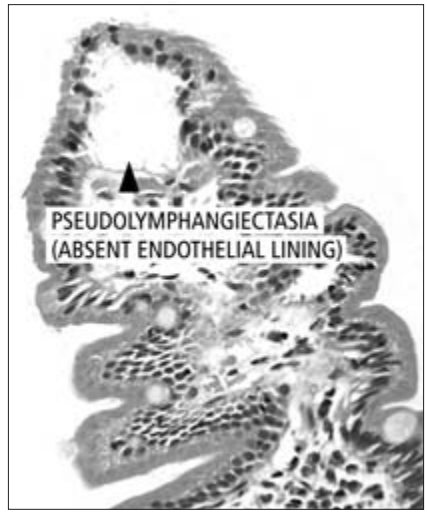

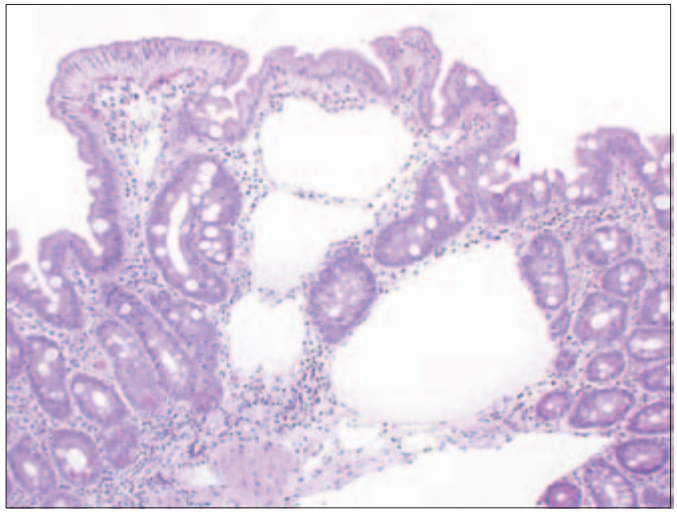

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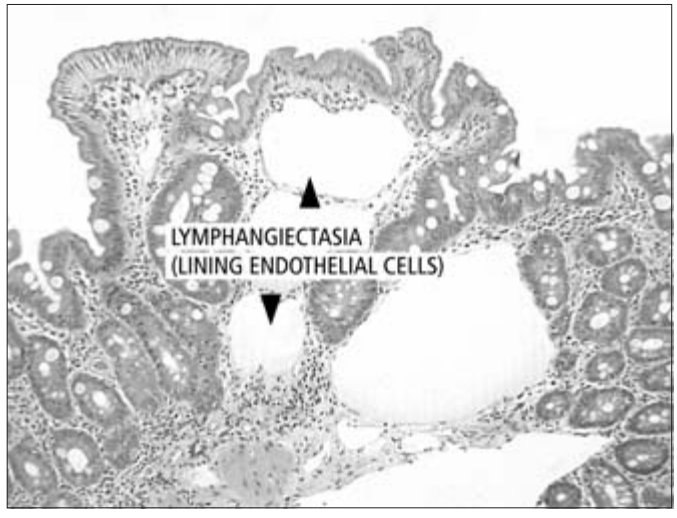
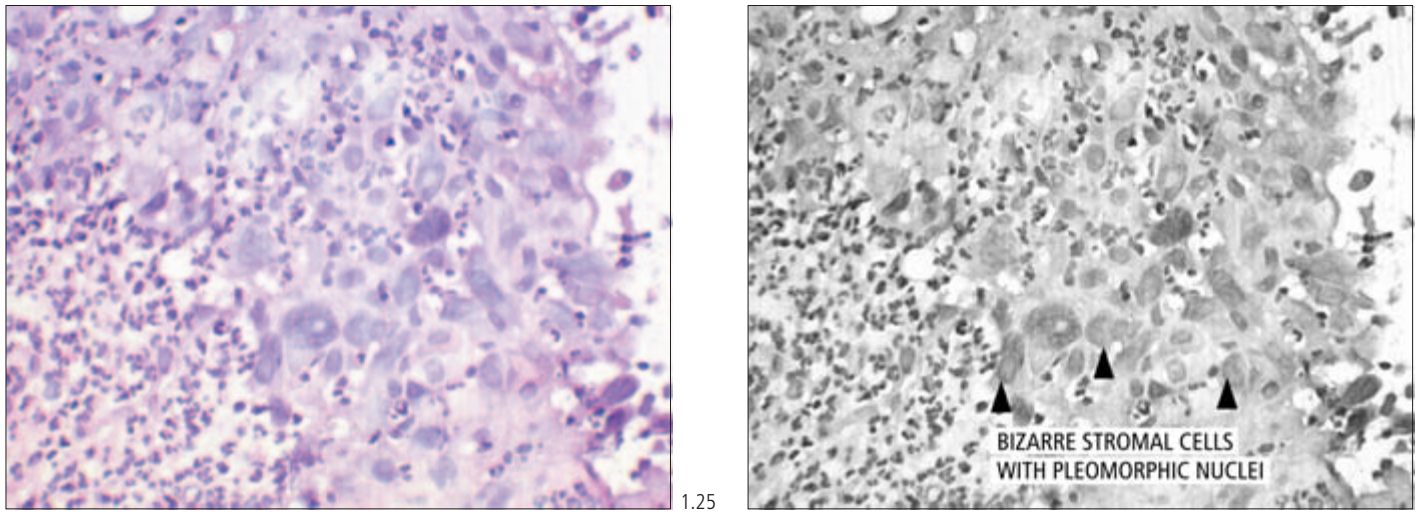


The most diagnostically dangerous artefact is crushing which in the biopsies from the stomach and oesophagus results in glandular crowding and nuclear pleomorphism simulating malignancy (Fig. 1.25). Whenever possible samples from the oesophagus should be taken before bougienage of strictures and if this is not feasible the pathologist should be informed appropriately. Smooth muscle tumours may be simulated by crush artefact producing a ball of smooth muscle from the muscularis mucosae.



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Clinicopathological meetings

Regular meetings should be held between clinical gastroenterologists, both physicians and surgeons, and their pathologist colleagues for clinical, educational and audit purposes. It may also be helpful if a radiologist can attend. At such meetings, routine material should be discussed as well as material from patients with unusual or rare conditions and those where clinical and pathological features do not correspond. The pathologist may wish to revise his/her diagnosis in the light of evidence emerging during discussion, or may be prompted to further investigation such as more levels or stains or to obtain previous biopsy material from his/her own file or from that of another hospital where the patient had previously been treated.

The presence of a formally timetabled clinicopathological meeting should never be a barrier to informal consultation in person. The gastroenterologist should be a welcome visitor to the pathology department at all times. Likewise, the pathologist should be an equally welcome visitor in the endoscopy suite, to familiarize him/herself with the challenges and limitations of endoscopy, to see interesting appearances, to help select biopsy numbers and sites, to collect unusual or urgent specimens, and to emphasize the close cooperation between clinician and pathologist which is essential to obtaining the best results.