Morson and Dawson's Gastrointestinal Pathology

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FOURTH EDITION

Blackwell
Science

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Foreword

Until about the time of World War II knowledge of Gastro-intestinal Pathology was largely based on autopsy studies which were often erroneous because of tissue autolysis. Although the years beteeen the two World Wars saw the beginnings of an increase in the number of surgically resected specimens, after the second World War there was a huge increase in the number of gastrectomies and intestinal resections. New techniques of gastric biopsy, small bowel biopsy and colonoscopic biopsy followed and added to the abundance of tissue available to pathologists for the diagnosis and the study of the pathogenesis of gastrointestinal disease. Today gastrointestinal pathology is accepted as one of the largest sub-specialties within general histopathology. There has been a steady movement away from old style morbid anatomy and histology during the past 50 years to a greater appreciation of cellular pathology. This has been particularly fruitful in the study of mucosal infiltrates and malignant tumours especially lymphomas and connective tissue tumours. Although new techniques, in particular immunocytochemistry and molecular analysis, have become popular, especially in research, it is remarkable how the old technique of haematoxylin and eosin staining remains the standard for diagnostic purposes.

This book was conceived during the late 1950s by myself and the late Professor I.M.P. Dawson. We were a happy and productive partnership but it took many years before the first edition was published in 1972. A second edition came out in 1979 but it was becoming clear that the scope of gastrointestinal pathology was increasing so fast that additional authors were essential for a third edition, published in 1990. That trend has continued. It is a real pleasure for me to see so many of my former trainees, research fellows and visitors to the Pathology Department of St Marks Hospital contributing to this fourth edition.

This book was conceived at its very beginning as a source of reference to both pathologists and gastroenterologists. It is patient orientated in the sense that it aims to provide information of value in the clinical management of gastrointestinal disorders.

Comparison of this edition with previous ones illustrates how the revolution in the technology of publishing, both text and illustrations, has advanced. The quality of illustrations is particularly important as they add so much to a reading of the text.

Basil C. Morson
August 2002

Preface to Fourth Edition

It is twelve years since the last edition of *Morson and Dawson* was published, and during this period there have been considerable advances in our knowledge and understanding of the pathology of gastrointestinal diseases. Some of these advances have resulted from developments in basic science that have allowed molecular diagnostics on fixed tissue to become a reality or the armamentarium of monoclonal antibodies for immunohistochemical diagnosis and classification to be continually expanded. Changes in our approaches to gastrointestinal lymphomas and stromal tumours are good examples. However, other important advances have come from more careful observation and clinico–pathological correlation, either of routine histological sections (such as the identification of new forms of colitis) or of resection specimens (such as the recognition of the clinical relevance of circumferential margin involvement in rectal cancer). Concomitant advances in therapy have produced 'new' pathological lesions induced by drugs, radiotherapy, or surgery. The scope of *Gastrointestinal Pathology* has therefore enlarged significantly since the last edition of this book.

The twelve year interval has also seen the retirement of Dr Basil Morson from clinical practice at St Mark's Hospital and the sad death of Professor Ian Dawson. This edition therefore represents the first in which the two original authors have played no direct part in the revision. Their four 'disciples' from the third edition have been joined by four additional authors, Professor Neil Shepherd, Dr Jimmy Sloan, Professor Ian Talbot and Dr Bryan Warren, all of whom have had a special interest in gastrointestinal pathology for many years, and it is a great pleasure to welcome them to the team. Dr Basil Morson and the late Professor Ian Dawson have had a profound influence on the professional lives of all eight of us, and we all feel privileged to be entrusted with this new edition. We are delighted that the names Morson and Dawson continue to be incorporated into the title of the book, and we are especially grateful to Dr Basil Morson for his unfailing interest and encouragement, and for honouring us by writing the foreword to this edition.

The overall structure of this fourth edition is similar to the last three, in that diseases are considered in relation to the anatomical regions of the gut. However, within these sections we have made changes to the way we deal with tumours. Our increasing understanding of the relationships between benign and malignant tumours makes it inappropriate to deal with them any longer in separate chapters. Tumour-like lesions, on the other hand, have been given their own chapters in the stomach, small and large intestines. We have not included a section on the pathology of the peritoneum in this edition, because many of the diseases affecting this structure fall outside the purview of gastroenterology, and a number of excellent publications covering this area have appeared during the last twelve years. The chapters covering gastritis, peptic ulcer disease and gastric neoplasia have been completely re-written, as have those on inflammatory disorders of the small intestine and non-epithelial tumours in all parts of the gut. Many others have been extensively modified, but we have endeavoured to maintain the style of the previous editions. It is probably the inclusion of a large number of colour illustrations for the first time that has made the greatest impact on the overall appearance of the book. There were eight in the last edition, we now have 402. Nevertheless, we have retained about 150 black-and-white figures, mainly because we have been unable to better them in colour, and the overall number of figures has increased by more than 120.

We would like to place on record our gratitude to all our colleagues and friends, too numerous to mention individually, who have supplied us with the material on which this book is based. We do, however, give special thanks to Dr Nick Francis for providing many of the illustrations of the more 'exotic' infectious diseases. A book of this kind is inevitably dependent on the skills of very many laboratory staff, medical photographers, secretaries and experts in information technology. To them all we offer our sincere thanks, but we would like to acknowledge particularly Mr R. Roberts-Gant, Mr R. Creighton, Mr N. Garraghan, and Mrs B. Kelly. Andrew Robinson, Alison Brown, Elizabeth Callaghan, Fiona Pattison and the staff of Blackwell Publishing have carefully and patiently steered us through the various stages of the publication process and it is a pleasure to express our gratitude for their huge efforts on our behalf.

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October 2002

Preface to First Edition

Gastroenterology is a rapidly developing and expanding branch of medicine in which histopathology plays an important role in diagnosis and treatment no less than in research. Surgical specimens and biopsy material from the gastrointestinal tract account for a considerable proportion of all the material seen in any department of general histopathology. This book is the outcome of our special interest in both the histopathology and histochemistry of the gastrointestinal tract over the last 20 years during which we have been fortunate enough to have access to a large amount of surgical and biopsy material related to our earlier appointments at the Bland-Sutton Institute of Pathology, the Middlesex Hospital, London, the Department of Pathology in the University of Leeds, and particularly to our current posts at St Mark's Hospital and within the Westminster Hospital Group. We have directed our attention particularly to pathologists who require a reference book for use in the laboratory but we hope this book will also be useful to those studying for the final MRCPath or its equivalent. It contains sufficient related clinical and radiological detail, with references, to interest physicians, surgeons and radiologists who either require ready access to information on pathology for diagnostic, teaching or research purposes, or are interested in pathology for its own sake.

Progress in gastroenterology has been so rapid that a textbook can become out of date in some aspects between writing and publication. We have provided lists of references for diseases or groups of diseases at the end of each chapter which are reasonably comprehensive up to the end of 1970; where possible we have selected those references which themselves contain valuable reviews or give a more detailed account of the subject. No one, however, can entirely encompass the rapidly growing literature in gastroenterology and there may inevitably be some omissions.

We would like to record our sincere thanks to all those clinical colleagues without whose help we could not have documented our experience; and we are grateful to all the pathologists, physicians and surgeons who have referred difficult or interesting material to us over the years, providing experience of a special character. We owe particular thanks to Dr Arthur Spriggs who has contributed the chapter on Cytology from his own vast experience; to Dr H.J.R. Bussey who has given such stalwart support over many years and to Mr Norman Mackie, Senior Photographer at St Mark's Hospital whose technical and artistic skill is evident in many of the photographs. Every histopathologist and histochemist is dependent on the skill of his laboratory technicians and we would like to thank all those who have helped, especially Mr Lloyd Soodeen, AIMLT, Mrs Bhanu Patel, AIMLT, and Miss Jane Hepple.

We are grateful to the following for providing illustrations: Dr R.J. Sandry, Dr Barbara Smith, Dr R. Whitehead, Dr Jane Burnett, Dr H. Lederer, Dr D. Spencer and the photographic department of Westminster Hospital Medical School. Dr J. Gleeson kindly advised us on the value of radiology in malabsorption syndromes. The photographs and charts of carcinoma of the stomach are reproduced by permission of the Editor of the *British Journal of Surgery*. If we have inadvertently omitted any acknowledgement we offer our apologies. Our special thanks must go to Miss Jill Ashby (now Mrs Griffith Jones) who typed and retyped the seemingly endless pages of semi-legible manuscript efficiently and cheerfully; and to Mrs Marion Cook who compiled the index for us. We are indebted to Mr Per Saugman and Mr J.L. Robson of Blackwell Scientific Publications for their courteous and enthusiastic cooperation. Finally, as our dedication appropriately indicates, we thank our wives and children who will be no less glad than we are that 12 years of reading, writing and proof reading have come to an end.

London   B.C.M.
1972   I.M.P.D.

Examination and reporting

1 Reception and examination of biopsy and surgical specimens

Pathology reporting procedures

The primary aim of examining pathology specimens is to provide physicians and surgeons with essential diagnostic and prognostic information allowing the best clinical management of the individual patient. However, this is not the only purpose of pathological examination. Biopsy and surgical resection specimens are a very important source of material for research into disease processes and for teaching. Moreover, advances in information technology and word processing in recent years have meant that pathology records are being used more and more for epidemiological studies and for medical audit. It is important therefore that valuable pathological material is dealt with in such a way as to give as much information as possible for each of these purposes.

The best clinical pathology service can only be obtained when there is close co-operation and understanding between the pathologist and the laboratory staff on the one hand, and physicians and surgeons with their paramedical staff on the other. In order to report on gastrointestinal specimens the practising pathologist should not only have a good basic knowledge of clinical gastroenterology but also know the various clinical procedures used for obtaining specimens, so that difficulties in obtaining adequate biopsies are appreciated. Similarly, the clinical gastroenterologist, surgeon, endoscopist or radiologist should have an understanding of the fundamentals of histopathological diagnosis so that they not only know which tissue samples are most likely to yield the most useful information but also appreciate the limitations of histopathology. Recent advances have made this even more important. Endoscopy, diagnostic imaging and therapeutic instrumentation have each made a great impact on the management of gastrointestinal disease and have provided pathologists with biopsy material from sites that hitherto were inaccessible without major surgery. Similarly, in the pathology laboratory, new techniques in enzyme histochemistry, immunocytochemistry, morphometry, flow cytometry and molecular biology have provided important new methods for diagnosis, which are of great relevance to all physicians and surgeons. More than ever before, the way in which tissue is resected, handled and presented to the laboratory determines and limits the information potentially available.

We believe that there should be an agreed routine between physicians and surgeons, pathologists and technical staff as to how all the common suspected conditions should be handled, in both resected and biopsied material. Regular meetings and discussions are essential to explain what the pathologist can and cannot demonstrate given the optimal material, and to permit the pathologist to appreciate more fully what physicians and surgeons need to know. In few fields of medicine will such co-operation pay greater dividends. In particular, suspected unusual or rare conditions should always be discussed *before* any tissue is removed.

General principles

Each specimen for pathological examination must be accompanied by a request form which, apart from the obvious details of the patient's name, age, sex, hospital number, ward or out-patient clinic, date and the name of the clinician, should give a summary of the *relevant* clinical information and an indication of what specific information the physician or surgeon is seeking. For biopsies it is essential to know the site of biopsy and the endoscopic appearances, while for surgical specimens it is necessary to know the type of operation carried out and, for any non-routine procedure, a diagram illustrating the surgical anatomy of the specimen. Details of previous biopsies and previous treatment, such as radiotherapy, are invaluable. The importance of the information given on the request form in diagnostic interpretation cannot be underestimated — frequently it provides the only information available to the

pathologist. All too often the filling in of forms is regarded as a chore by clinical staff, so that the information given is either sketchy, incomplete or indecipherable; or the job is delegated to a junior member of the paramedical staff who is unfamiliar with the patient and unaware of the reason why pathological examination is being requested. Unless the pathologist goes to some considerable length to get the necessary information in such cases, there is always the possibility of diagnostic misinterpretation and serious harm to the patient.

It is also important that the pathologist be allowed to examine the *whole* of any specimen submitted for pathological diagnosis, in order that tissue blocks for histology may be selected from those areas which are known by experience to yield the greatest amount of information. No prior interference should be allowed and specimens should most certainly not be divided, or samples taken for some other purpose such as research, without the pathologist's express agreement. On the other hand, the pathologist should always endeavour to co-operate with the requirements of any reasonable research project.

Generally speaking, therefore, it is best that all surgical specimens for pathological examination reach the laboratory in the fresh, unfixed and unopened state. Because of their small size, gastrointestinal biopsies dry out quickly and should be orientated and placed in an appropriate fixative as soon as possible. Knowledge and understanding of pathological techniques by those submitting the specimen to the laboratory is particularly important here, because whilst most specimens may be fixed in a standard formaldehyde-based laboratory fixative, those needing special study with enzyme histochemistry or electron microscopy may require quenching or immersion in a special fixative (see p. 8).

Laboratory investigation of gastrointestinal biopsies

Most gastrointestinal mucosal biopsies are just tiny samples of a large surface area of suspected disease or volume of abnormal tissue. It is important therefore that they are taken from the right place, and are of sufficient size, number and quality to be representative of the lesion under investigation. Care must be taken to minimize crushing of tissues by biopsy forceps or coagulation during diathermy, since these artefacts make interpretation difficult or impossible.

The vast majority of diagnostic biopsies will be conventionally orientated, fixed, processed and sectioned. In this section we comment on these. Special techniques are described in Chapter 2.

Orientation, fixation and embedding

Biopsies that include muscularis mucosae are usually orientated easily with the naked eye or using a hand lens since the muscularis contracts and the mucosal surface becomes convex. To avoid this phenomenon of curling up, the clinician or endoscopy assistant taking the biopsy should place it mucosal surface upwards onto a flat sheet to which it can adhere during fixation. Some units use either a piece of frosted glass or smooth-surfaced card. Strips of cellulose nitrate ('Millipore') filter material are particularly useful because they can be sectioned without removing the biopsy tissue and permit a series of up to eight or even 10 endoscopic biopsies to be sectioned and mounted in one procedure on a single microscope slide, thus saving time and laboratory resources and easing the task of the pathologist in reviewing the whole series [1]. The normal serum exudate will stick the biopsy to the mounting vehicle, provided that the latter is dry. The biopsy is then placed in a standard fixative, according to the preference of the individual pathologist; 10% aqueous formal saline, buffered at neutral pH, is widely used. However, some laboratories claim better preservation of morphology with unbuffered formal saline, despite the fact that different batches of fixative may vary quite considerably in their pH. Improved nuclear preservation is often obtained when 2% acetic acid is added to the formal saline, or when Bouin's fixative is used. However, the latter suffers the disadvantage of destroying Paneth cell granules, and acid fixatives cause fragmentation of DNA and RNA, seriously hampering molecular biological investigation. Examination with a dissecting microscope, which provides a three-dimensional view of the mucosal architecture of small intestinal biopsies, is rarely done nowadays.

Smaller biopsies, particularly those taken at upper gastrointestinal endoscopy, do not always contain muscularis mucosae and can be impossible to orientate in the operating theatre or clinic. They should be placed in a square of porous non-soluble paper tissue (not gauze), wrapped and placed in fixative. Specimens from different sites must always be kept separate and specifically labelled.

The pathologist or a trained technician should check the orientation of large biopsies before embedding in paraffin wax, using a dissecting microscope if necessary. Tiny biopsies can sometimes be correctly orientated in this way; they should ideally be embedded singly since this allows reorientation if necessary, which is impracticable in a whole block containing three or four specimens in different planes. When biopsies are particularly badly curled and distorted they may be flattened for orientation by embedding in heated 1% aqueous agar precooled to 5°C, keeping the biopsy flat while the agar sets [2]. Processing and double embedding in paraffin wax can then be undertaken.

Sectioning and staining

It is a wise precaution, when sectioning a paraffin block of a small mucosal biopsy, to mount and stain one section as soon as the knife begins to cut into the tissue, so that the orientation may be checked before the block is partly cut through or any

step-sectioning is done. At this early stage, reorientation is still possible if sections prove to be transverse or tangential. Biopsies of any size should always be step-sectioned at three different levels and stained routinely with haematoxylin and eosin (H&E). Intervening sections should be kept for further investigation if necessary. With gastric biopsies, in addition to staining with H&E, we routinely stain for *Helicobacter*-like organisms, using either crystal-fast violet or a modified toluidine blue. We do not use any special stains routinely on other biopsies, since occasionally they result in valuable sections being 'wasted' on inappropriate stains. When serial or semiserial sections are being stained using a number of techniques, however, it is wise to stain the first and last with H&E to check that any lesion is present at the beginning and end of a run.

Laboratory examination of surgical specimens

All surgical specimens should be received in the laboratory fresh, unfixed and unopened by operating room staff, including the surgeon. If a specimen has been removed outside normal laboratory working hours it can be safely placed unfixed in a plastic bag in a refrigerator (not in a deep freeze compartment) overnight for examination next morning. It is important that specimens be examined in the fresh, untouched state so that they may be prepared, if necessary, for colour photography and samples taken for special histological techniques such as electron microscopy or histochemistry. Photography of fresh, unfixed specimens has a number of disadvantages. Their shiny, glistening surfaces give rise to 'highlights' and reflections which detract from the quality of photographs obtained, and the delay in fixation, along with the drying effect of photographic lights, has a detrimental effect on tissues which may compromise good histology. It should therefore be confined to cases whose special appearances may become lost or modified by the process of specimen preparation or fixation.

Fresh specimens should be opened and examined carefully, using a hand lens if necessary, in a good light. The specimen is then fixed in 10% formal saline in such a way as to preserve the normal and pathological anatomy for photography. No amount of macroscopic description is any substitute for good photographs, which are easily reproducible for records and teaching purposes. The advent of digital cameras has greatly simplified the photography of specimens and permits the incorporation of a printout of a photograph to be incorporated in the computer-generated report. Although colour can be restored by immersing fixed specimens in 70% ethyl alcohol, we find this unnecessary in most cases.

Most gastrointestinal specimens are best prepared by opening them longitudinally with scissors, gently washing the mucosal surface with cold water or formal saline to remove any blood or excess mucus, and pinning them out flat on a cork board, mucosal surface uppermost. In the case of rectal tumours situated below the pelvic peritoneal reflection, where it is important to preserve the anatomy of the circumferential resection plane for assessment of completeness of excision, it is recommended that the lower rectum is left to fix unopened, so that serial 5-mm slices can be cut in a horizontal plane once the specimen is well fixed. This avoids disturbance by the pathologist of the anterior resection plane, which could obscure its true position. When in such circumstances the lumen of the lower rectum is stenosed, it is helpful to place a wick of formalin-soaked paper tissue, to speed penetration of the fixative. After the specimen has been carefully labelled, the board is then floated, face downwards, for 24 h in a tank of 10% formal saline covered by a close-fitting lid. The specimen is then unpinned, and kept in fixative until blocks for histology are taken. However, if the disease has produced an annular, tight, thick-walled stricture, or if there are diverticula or there is an intussusception, it is better that the specimen be distended with formalin and tied off at both ends as close to the limits of excision as possible; after 24 h fixation it can then be cut longitudinally over a probe passed through the lumen of the stricture so that the pathology is demonstrated at the correct level. Gastrectomy specimens should usually be opened by cutting along the greater curve because this will avoid most gastric pathology which is centred on the lesser curve; small intestinal and colonic specimens opened by cutting along the antimesenteric border; and rectal specimens (other than those removed for carcinoma) opened anteriorly. Specimens from Whipple's operation for tumours of the ampulla of Vater, common bile duct and carcinomas of the head of the pancreas include the duodenal loop which can be opened along its lateral aspect, thus leaving the ampulla intact. Cannulae may be passed through the ampulla in the fresh specimen as far as possible into the common bile duct and pancreatic duct to enable the anatomy of the fixed specimen to be easily assessed.

Before blocks for histological study are taken it is important to make a macroscopic description of every resection specimen and, if necessary, a diagram on which the site of each tissue block can be marked. We use photocopies of a set of simplified diagrams for this purpose, an example of which is shown in Fig. 1.1. The advent of more sophisticated computer systems for handling histopathology data will permit incorporation of such diagrams in printouts of reports. The description should give the length of the specimen, including both greater and lesser curvatures of gastrectomy specimens, and a concise account of all pathological lesions to include their size and appearance, and their position relative to the resection lines, the gastric curvatures, the mesenteric attachments, or other anatomical landmarks such as the ileocaecal valve for right hemicolectomy specimens or the dentate (pectinate) line for abdominoperineal excisions of the rectum and anus. With rectal specimens removed by anterior resection, when
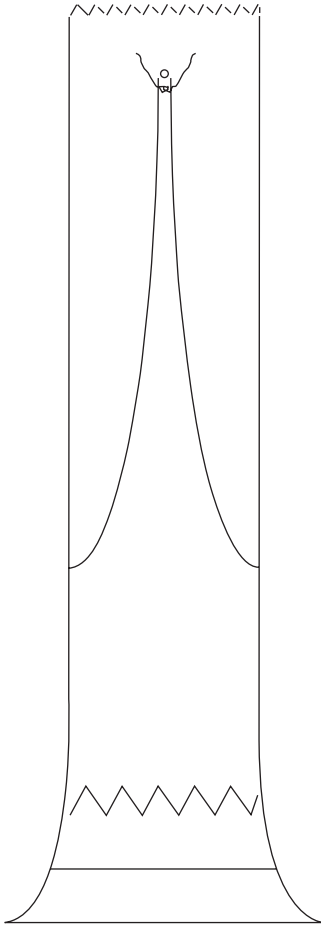
Fig. 1.1 An example of a simplified diagram of an abdominoperineal resection of rectum on which the site of the tumour and of tissue blocks taken for histology can be marked.

the anus is not included in the specimen, a useful anatomical landmark is provided by the pelvic peritoneal reflection, which lies on the anterior surface at approximately the mid-point of the rectum (the average length of the rectum being 15 cm). When the peritoneal reflection is present, it is therefore possible to judge that approximately 7.5 cm above it the colon ends and the rectum begins.

The taking of blocks for histology is obviously a very important step in the examination of any pathology specimen, and it is essential that sufficient samples are taken so that the maximum possible amount of information about the disease process under study can be obtained from the specimen. Nowadays the great majority of gastrointestinal resections are for tumours, polyps or inflammatory bowel disease and the methods used for these will be described in some detail.

Resections for neoplasia

The gross description of macroscopic appearances should include the position of the tumour relative to anatomical landmarks, its size and morphology, and its distance from the surgical limits of excision. The selection of blocks is geared towards assessing the nature of the tumour, the extent of local spread, involvement of the peritoneal surface, the presence of vascular invasion and the degree of lymphatic spread. It is usual to take between two and four blocks of the tumour, making sure that any parts with different macroscopic appearances are sampled, and also the junction between the tumour and the adjacent mucosa. Blocks should also be taken to establish the extent of spread through the bowel wall and, for advanced tumours, whether or not there is invasion of adjacent serosal surfaces, omentum, mesentery, soft tissues or other organs.

It is also important to establish that the tumour has been excised completely, since this is obviously a predictor of local recurrence. Transverse sections through both proximal and distal resected ends of oesophagectomy or gastrectomy specimens should always be examined for direct spread or lymphatic permeation; experience shows that for resections for colorectal cancer this is only necessary when the tumour is within 3 cm of one end [3]. In recent years the use of stapling devices for surgical anastomoses provides two 'doughnuts' of tissue, which represent the resection ends. Histological examination of transverse sections of these provides an excellent method of assessing the completeness of excision in the longitudinal plane. Assessment of the deep (so-called circumferential) planes of excision, around the advancing margin of the tumour, is also important when dealing with excisions of oesophageal and colorectal carcinomas because they may predict local recurrence. This is most easily done by cutting 5-mm slices transversely through the tumour to identify macroscopically the slice with the deepest penetration and least surgical clearance. This slice should then be subjected to histological examination for confirmation or otherwise of total excision [4,5]. It may be helpful to paint the deep or circumferential excision plane with Indian ink or some other marker that withstands tissue processing.

Venous invasion, especially when present in extramural vessels, has a profound effect upon the prognosis of gastrointestinal carcinoma and should always be sought macroscopically and microscopically The veins in the neighbourhood of a tumour should be carefully inspected by dissection of the adjacent mesentery or omentum, and any suspicious vessels examined histologically. More frequently venous invasion is occult and is best discovered in blocks taken from adjacent to the tumour, and tangentially to it.

For cancers of the gastrointestinal tract from the oesophagus to anus it is an important principle that all regional lymph nodes, however small, should be removed for microscopic examination. There is ample evidence that prognosis after surgical treatment of both gastric and colorectal cancer is profoundly altered not only by the presence of lymph node metastases but also by the numbers of nodes involved. There is evidence that the effect on prognosis is in direct proportion to

the distance of involved nodes from the primary tumour, it therefore being important to examine all lymph nodes in the field of drainage between the primary tumour and the surgeon's vascular tie [6]. The 'highest' lymph node (the one nearest the tied vascular pedicle) in resections for colorectal cancer should be taken separately, as this allows proper Dukes' classification into C1 or C2 cases. Careful dissection of the omentum or mesentery adjacent to gastric or colorectal cancers usually allows the lymph nodes to be identified, but in difficult cases, when they are obscured by abundant adipose tissue or situated around the lower rectum, an alcohol-xylene clearance technique may be useful [7]. As an alternative, blocks of perigastric or perirectal fat from places where lymph nodes would be expected to lie can be processed and sectioned for microscopic examination. Charts showing the anatomical position of the primary growth and the position of regional lymph nodes may be helpful to the surgeon and useful for the concise presentation of data at multidisciplinary cancer management meetings.

Polypectomy specimens

Resected polyps of any substantial size are usually adenomas of the large bowel, which are preinvasive neoplasms in which it is vital to establish whether or not invasive malignancy has developed and total excision has been achieved. Although polyps from other sites and of different types exist, it is best to deal with them all in the same way. It is first necessary to identify the stalk of the polyp because this gives a guide to the correct plane for trimming and must be sectioned so that it is correctly orientated in relationship to the head of the polyp. It is also the only place where completeness of excision can be established histologically. If the stalk cannot be identified it will be necessary to trim all the more carefully so that multiple step-sections into the lesion will display it satisfactorily. Trimming of a polyp should be minimal and confined to two sides of the lesion so that it can rest on either side with the intact stalk projecting from one end. One should never cut across a polyp but, as far as is possible, embed the whole lesion in one wax block and then cut multiple step-sections (keeping spares for special stains) through the head until the level of the stalk is reached.

Resections for inflammatory bowel disease

The examination of these specimens can be a laborious and time-consuming task because of the considerable amount of tissue that may be resected and because of the complicated surgical anatomy, with strictures, fistulae and abscesses, that may result from the inflammatory process, especially in Crohn's disease. Preparation of the specimens, either by open-

ing or by inflating with fixative, is a particularly important step in identifying the various complicated lesions that occur and good specimen photographs often make a much more informative record than lengthy macroscopic descriptions. When taking blocks, it is important to sample areas representative of the different macroscopic features. It is preferable to take them all from the same plane, usually along the longitudinal axis of the intestine, since these allow a better survey of the extent of the disease process. It is also important to label the blocks accurately and, in complicated cases, to make a diagram of the sites from which each is taken, so that macroscopic and microscopic appearances may be correlated. It is difficult to generalize as to how many blocks should be taken. For a total colectomy anything less than six blocks from different parts of the colon is probably insufficient and as many as 12 may be essential for accurate diagnosis. The regional lymph nodes should also be sampled, although it is unnecessary and probably impracticable to remove all of them.

When colonic resection is undertaken for dysplasia or carcinoma in chronic inflammatory bowel disease the examination must be particularly exhaustive. Blocks should be taken from all recognizable mucosal lesions, and numerous random samples taken from the background 'flat' mucosa. If they are taken from the entire length of the large bowel in two parallel 'tramlines', foci of dysplasia can be mapped. It is vital to establish whether invasive malignancy has developed at any of the sites of dysplasia, and if so, its extent of spread should be assessed as described above for carcinoma specimens.

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2 Special investigations

The days are now past when the only way to process a biopsy or a tissue block from a resected specimen was to fix it in formalin, embed it in paraffin wax, stain the sections with haematoxylin and eosin and examine them under the light microscope. Many new and sophisticated techniques are now available which, when correctly chosen, provide valuable additional information, and most hospital laboratories are equipped to undertake at least some of them. A number require other than conventional processing and handling of tissues and it is vital to establish, *before* a biopsy or a resected specimen is immersed in fixative, what the clinician considers the likely diagnosis to be, whether a particular investigation is required and which techniques the pathologist should use to provide the most useful information. Closer co-operation has led to a surge of interest in gastrointestinal problems and a higher standard of diagnosis. This chapter affords a brief description of the more important techniques available and their value. Details of methodology are available in standard textbooks and reviews [1–8].

Gastrointestinal biopsies, except for those from the small intestine, are now normally taken under direct vision and visible lesions can be sampled directly. The various biopsy techniques and the orientation of specimens are described in Chapter 1.

Preservation of tissues

There are two principal and two subsidiary techniques for tissue preservation [1–3].

Fixation and embedding

This, followed by light microscopy, is still the most widely used and valued technique. Fixation is usually in 10% buffered formaldehyde. There are three possible embedding techniques (Figs 2.1–2.3).

1 *Embedding in paraffin wax.* This is easy, all laboratories are equipped for it, pathologists are used to it, and it allows large blocks to be taken from resected specimens and some crude electron microscopy is possible after reprocessing thick sections. It allows some special investigations but precludes others (Fig. 2.1).

2 *Embedding in acrylic resin.* This is suitable only for small pieces of tissue; it is technically more difficult to cut the thin 2-µm sections, is expensive and the material cannot be used for electron microscopy as acrylic resin disintegrates in the electron beam. It has the advantage that much cellular and intracellular detail can be made out (Fig. 2.2).

3 *Embedding in epoxy resin.* This, normally preceded by fixation in freshly prepared buffered glutaraldehyde, is the method of choice for electron microscopy. Tissue blocks are small, but semithin 1-µm sections can be taken from them, stained with toluidine blue and examined by light microscopy. This provides a fine-detail image of cellular structure that bridges the gap between light and electron microscopy (Fig. 2.3).

Quenching without prior fixation

This technique involves plunging small pieces of fresh tissue into an inert liquid such as isopentane, precooled in liquid
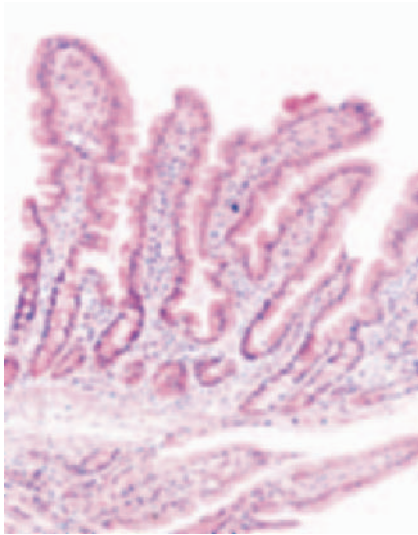
Fig. 2.1 Normal jejunum. Tissue fixed in buffered formaldehyde, followed by embedding in paraffin wax. Villous outlines are well preserved and the nuclear staining is crisp and clear; cell boundaries are distinct. The villi have, however, probably undergone some shrinkage.
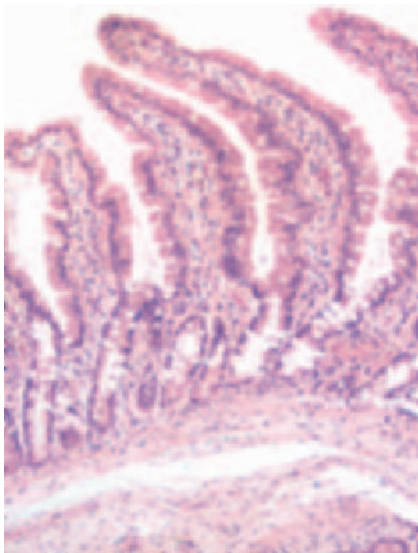

Fig. 2.2 Normal jejunum. Tissue fixed in formal calcium, followed by embedding in acrylic resin (JB4 glycol methacrylate, section 2 μm). Appearances are comparable with Fig. 2.1, but the thinner section allows more nuclear and cytoplasmic detail to be recognized.

nitrogen, followed by the cutting of sections at 5–6 μm in a cryostat at about –25°C, thus avoiding the use of a fixative. Once cut, sections can be postfixed if necessary. It allows the histochemical demonstration of those enzymes that do not tolerate fixation and the identification by immunocytochemical methods of antigenic components that are altered by routine fixatives. It may also provide a rapid answer for the surgeon in
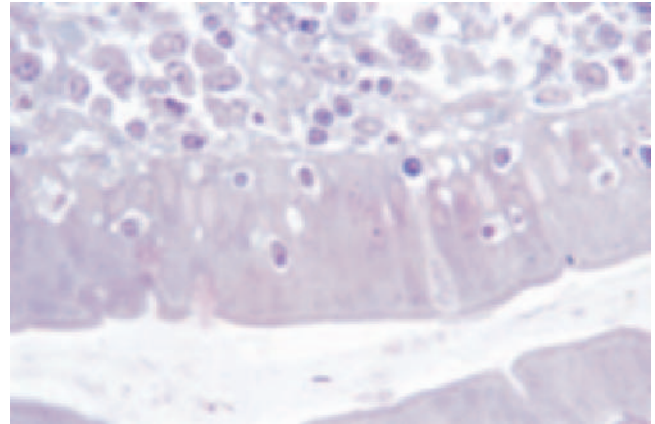

Fig. 2.3 Normal jejunal villus. Tissue fixed in buffered glutaraldehyde followed by embedding in epoxy resin (araldite, section 1 μm, toluidine blue). Cell outlines are perhaps slightly less clear than in Fig. 2.2 but there is further improvement in the intracellular nuclear and cytoplasmic detail and the brush border can be clearly seen.

problems such as Hirschsprung's disease. The technique's principal disadvantages are the need for constant supply of liquid nitrogen for quenching, difficulty with subsequent storage of the quenched tissue, and a certain loss of fine cellular detail after staining, though this can be improved by postfixing the section. It is often useful to divide a sufficiently large sample to allow both conventional fixation and quenching.

Refrigeration at 4°C

This method is useful when tissue is being retained for biochemical analysis. Unfixed surgically resected specimens can be stored in this way overnight when removed after laboratory hours.

Freeze drying

This technique, followed by vapour fixation, is a valuable research method particularly for the identification of biological amines. It has little place in routine diagnosis.

Choice of technique

This depends on three factors.
1 *The nature of the information required*. Are there specific factors to be looked for, and if not how can the tissue be best preserved so that as wide a range of investigations as possible can be done later if necessary?
2 *The size of the piece of tissue available*. Is it divisible and if so, will each piece be representative of the whole?
3 *The laboratory facilities available*. What can be offered locally or through access to more specialized facilities elsewhere, provided that the tissue is initially properly preserved?

If there is a particular problem to be solved—e.g. a survey of mucosal nerves in Hirschsprung's disease—this clearly governs the technique of preparation used. When there is no such indication, the size of the piece of tissue is likely to be the determining factor. If it is divisible into three, it is probably best to fix one piece in formaldehyde, one in fresh buffered glutaraldehyde and quench the third. If divisible only into two, omit the glutaraldehyde; if indivisible the pathologist must choose between formaldehyde fixation and quenching (with postfixing for haematoxylin staining) according to preference; the first is probably the preferred technique for biopsies as rebiopsy is often possible.

Morphometry, quantification and stereology

Morphometry is the measurement of the size and shape of objects. It allows calculation of area, perimeter and other variables.

Quantification is the measurement of the number of structures or features per area or length of tissue or the amount of a particular material present using microdensitometry techniques.

Stereology is the calculation of three-dimensional properties of an object from two-dimensional planes.

Such studies can be performed on paraffin- or resin-embedded sections and to some extent on postfixed cryostat sections.

Three types of morphometric technique are available for direct light microscopy without automation. All of them can be applied to photomicrographs, electron micrographs, projected slides or transparencies. They are:

1 the use of a camera lucida attachment or a projection microscope to project an image onto standard thickness paper or card. A tracing is then made of the cells under study and their areas are either measured using a planimeter or calculated by cutting out the shapes of individual cells and weighing them;

2 the use of either a linear intercept eyepiece graticule to express the area of a particular tissue component as a percentage of the total tissue area or a hexagonal line graticule to give a surface : volume ratio; and

3 the use of a point counting graticule.

Many laboratories now possess some form of image analysing equipment. Simpler quantification can be undertaken using tracings and photomicrographs as above. More sophisticated versions allow automated analysis of size, shape, texture or staining intensity from images captured on monitor screens. Such techniques (which may be used to measure villus height and thickness, determine the relative numbers of cell types present, or assess nuclear changes in epithelia showing disordered growth and differentiation) have an important place in research and evidence-based practice

[7,8] and will be referred to in appropriate chapters. Certain basic principles must be observed to ensure that valid and reproducible results are obtained.

1 There must be sufficient tissue to provide an adequate number of microscopic fields for sampling.

2 The biopsy must be representative of any lesion present; not all disease processes are evenly distributed throughout a section.

3 Section orientation must be accurate and avoid factors such as tangential cutting which distort surface areas and volumes.

4 There must be no artefacts such as those produced in a small bowel biopsy which does not include muscularis mucosae, as a result of which 'spreading' of villi can occur.

5 Sections must be of uniform thickness.

Electron microscopy

Electron microscopic techniques fall under the following two headings.

Scanning electron microscopy

A scanning electron microscope creates three-dimensional representations of unsectioned specimens which have been fixed, dried and coated (Fig. 2.4). This is predominantly a research technique [5] and it will not be further discussed here.

Transmission electron microscopy

Light microscopes cannot resolve particles smaller than about 2 μm. Transmission electron microscopes allow resolution down to about 2 nm (about × 1000 that of their light counterparts). They are used diagnostically to evaluate changes in microvilli, to demonstrate subcellular particles such as mitochondria, lysosomes and Golgi apparatus, to show
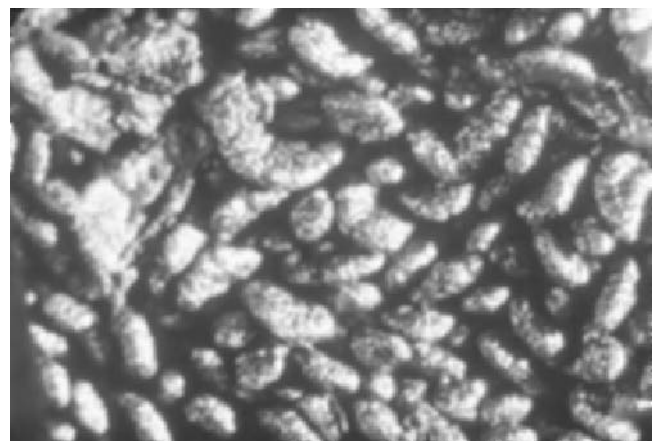

Fig. 2.4 Normal jejunum. Scanning electron micrograph, gold sputter coated. This provides a three-dimensional surface view of villi but does not yield a great deal of useful diagnostic information.
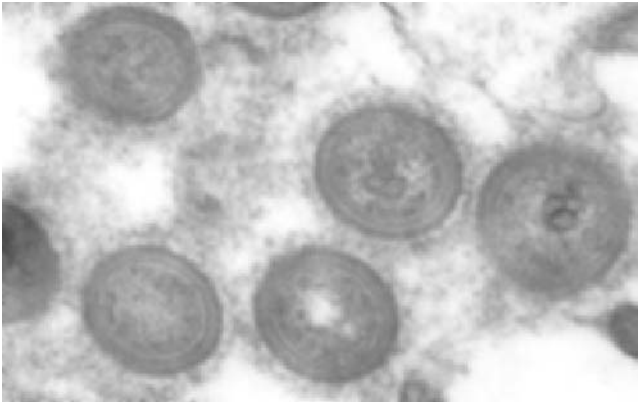
Fig. 2.5 Transmission electron micrograph in Whipple's disease showing the causative bacillary organism (*Tropheryma whipelii*) in cross-section.

Fig. 2.6 Normal jejunum. Acid phosphatase, cryostat section (6 μm) postfixed in buffered formaldehyde, pararosanilin technique, methylene blue counterstain. The location of the enzyme within digestive sacs and lysosomes is clearly visible. Some is also present in macrophages in the lamina propria.

cytoplasmic inclusions such as bacteria, and to demonstrate intracellular granules [6] (Fig. 2.5). Some techniques for histochemistry and immunocytochemistry are possible at ultrastructural level.

Histochemistry

Histochemistry is the use of chemical techniques on tissue sections, usually to identify specific substances within cells or to define sites of enzyme activity. In gastrointestinal pathology they are of value in three main fields: (i) the identification of particular enzymes within cells or nerve fibres; (ii) the detailed investigation of mucosubstances; and (iii) as a method for identifying and partially classifying intracellular cytoplasmic granules.

Enzyme histochemistry

Histochemical techniques for enzymes do not stain them directly. When a section is incubated with a substrate specific for a particular enzyme under controlled conditions and the enzyme is present in active form in the section, a breakdown product (the primary reaction product or PRP) is formed (or, in the case of oxidoreductases, an electron transfer takes place). The PRP (or the electrons) are 'captured' by the addition of a suitable chemical to produce an insoluble coloured end-product, the location of which indicates the site of enzyme activity. Methods of tissue preservation are particularly important since some enzymes are relatively unaffected by fixatives while others are inactivated. Examples of useful techniques are those for alkaline and acid phosphatases, aminopeptidases, disaccharidases (particularly lactase and sucrase; Fig. 2.6), and cholinesterase (Fig. 2.7). However, as monoclonal or polyclonal antibodies directed at enzymic epitopes have become available, many of these often
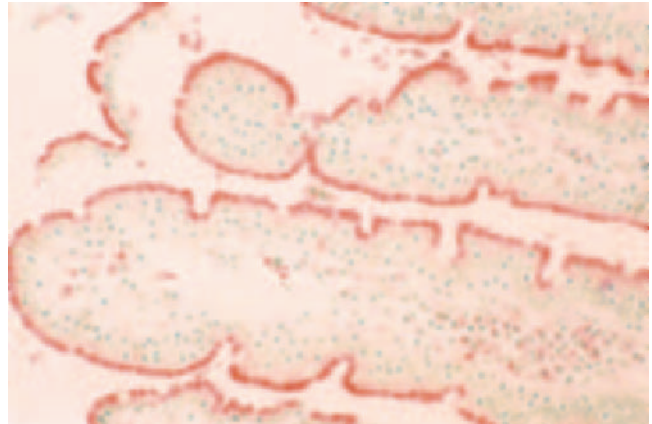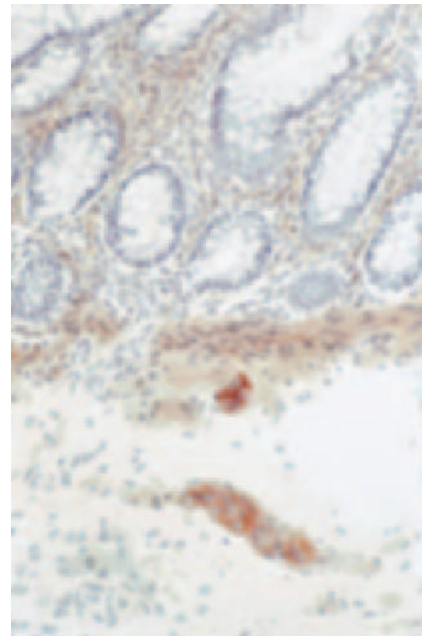

Fig. 2.7 Normal large intestine. Acetylcholinesterase (AChE), formaldehyde fixation, gum sucrose impregnation, cryostat section (6 μm). Individual cholinesterase-positive nerve fibres can be seen in the lamina propria and there is heavy 'staining' of ganglion cells. Some AChE-positive material is also present in the muscularis mucosae.

capricious histochemical techniques have been superseded by immunohistochemistry.

Mucin (glycoprotein) histochemistry

Reliable techniques, which can be performed in any routine laboratory on formalin-fixed material, allow the separation of

Fig. 2.8 Intestinal metaplasia of gastric mucosa. Goblet cells stain deep blue with alcian blue/dPAS, being reactive with both alcian blue (due to the presence of sialic acid) and dPAS. Residual gastric columnar cells are dPAS reactive (magenta), secreting only neutral mucins.
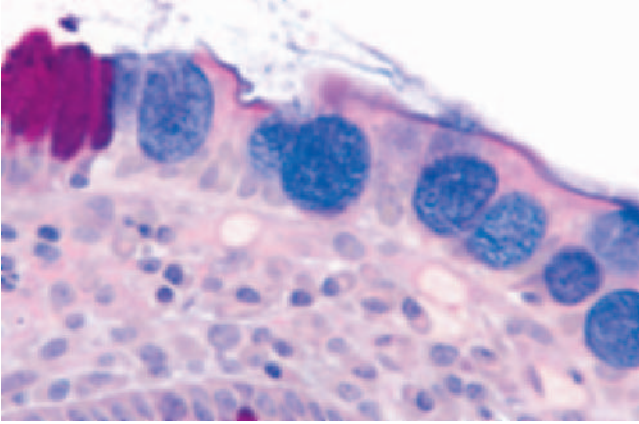

Fig. 2.9 Lymphoid aggregate within normal colorectum. Proliferative cell nuclear antigen (PCNA) immunohistochemistry reveals nuclear positivity in germinal centres and within crypt bases.

mucosubstances into *neutral mucins* (diastase digestion, followed by a periodic acid–Schiff (dPAS) technique); *acid non-sulphated* sialomucins (stained by alcian blue at pH 2.5); and *acid sulphated* sulphomucins (stained by alcian blue at pH 0.5 or by high iron diamine, or Gomori's aldehyde fuchsin) [9–11]. These techniques can be combined to demonstrate more than one pattern of mucin in a single section (Fig. 2.8). They can be made more discriminating by pretreatment of sections with selected enzymes such as neuraminidase, by the use of saponification methods for deacylation, by the use of alternative oxidizing agents to periodic acid, and by varying the concentrations of magnesium chloride in the incubating medium. More precise identification of glycoproteins can also be obtained by the use of specific lectins in a peroxidase technique. Their use and value have been critically discussed [12], and they are referred to where appropriate in subsequent chapters.

Silver precipitation techniques

Most silver 'stains' consist of aqueous or alcoholic solutions of silver nitrate which probably bind to anionic side-chains and disulphide groups. They must then be reduced to visible silver, either by a reducing substance already present in particular cells (argentaffin reaction) or by the addition of a reducing agent (argyrophil reaction). The only intracellular reducing agents capable of giving an argentaffin reaction in ordinary preparations of gut tissues are the compounds formed between 5-hydroxytryptamine (5-HT) and aldehyde fixatives. Other biological amines such as histamine or noradrenaline, which are capable of forming reducing complexes, diffuse away rapidly in aqueous fixatives. A positive argentaffin reaction can therefore be regarded as demonstrating 5-HT. The granules of other endocrine cells are, by contrast, argy-
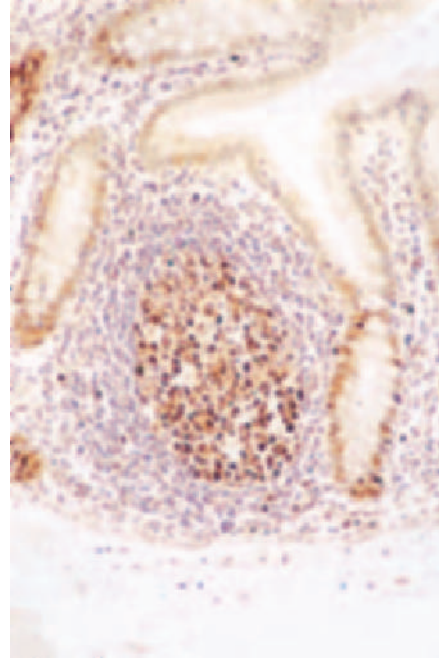
rophil. However, different argyrophil techniques have different sensitivities for the granules of the various cell types. For example, the Grimelius technique, probably the most widely used, does not consistently identify somatostatin-containing D cells, while the Sevier–Munger technique does. One study has suggested that the Churukian–Schenk technique is the most sensitive overall [13].

Basic and metachromatic dyes

These cationic dyes bind to anionic side-chains in polypeptides and staining is often improved by acid hydrolysis; they can be used, along with lead haematoxylin, as alternative staining methods for endocrine cell granules.

Immunocytochemical techniques

All of these depend on the ability to raise and purify a specific antibody to an antigenic component of a cell. Examples of diagnostically useful antigens that can be recognized include endocrine cell and neuronal granules, vesicles and peptides, cytokeratins and intermediate filaments, leucocyte markers, immunoglobulins, oncofetal antigens, proliferation markers [4], mucin core proteins and numerous enzymes (Fig. 2.9). Suitable methods of tissue preservation that do not alter antigen combining power, antigen retrieval protocols and adequate controls are essential. These techniques are reliable and are continually being improved and extended; they repre-

sent one of the great advances in histopathology in the last 25 years.

Investigation of the innervation of the gut

Modern concepts of the innervation of the gut have stemmed from the ability of histochemists and immunocytochemists to classify different types of nerve fibres and ganglia by demonstrating the polypeptides that they contain and the enzymes that they synthesize.

Pioneer studies in the 1970s showed that satisfactory demonstration of the distribution of nerve fibres and ganglia in myenteric and submucosal plexuses requires thick (20–100-µm) sections cut in the plane of the plexuses, parallel to the bowel wall, from large (20–100-mm) blocks of tissue, fixed for a week in 10% formaldehyde [14]. It is clear that such studies can have no place in routine work, but the results obtained from them can be applied by practising pathologists once the experimentalist has indicated which fibres or ganglia are important in which disorders. A classic example is the use of cholinesterase techniques on mucosal biopsies from suspected Hirschsprung's disease (Fig. 2.10).

Five main groups of neurotransmitters are now recognized: acetylcholine, the biological amines adrenaline and noradrenaline, gamma aminobutyric acid, neuropeptides (an ever expanding family that includes substance P, vasoactive intestinal polypeptide (VIP), somatostatin and bombesin) and
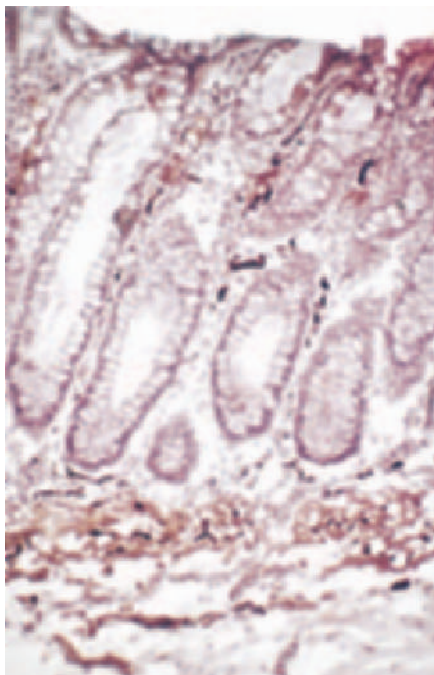

Fig. 2.10 Rectal biopsy in Hirschsprung's disease showing the more numerous, thickened fibres present in the lamina propria. Compare with Fig. 2.7, same technique.

nitric oxide. These may be identified using appropriate histochemical or immunocytochemical techniques [15], and are likely to prove of increasing importance as our knowledge of gastrointestinal innervation increases. The functions of these neurotransmitters and the independence of the gut-associated intrinsic nervous system are more fully discussed in the appropriate sections of this book.

Flow cytometry

Until recently, histopathologists have been content to rely on the microscopic examination of tissue sections for diagnosis, supplemented when necessary by those special techniques already described above. Techniques that do not rely on tissue sections are now being increasingly used for both research and diagnosis. Examples are flow cytometry which provides a rapid quantitative means of measuring a variety of cell constituents and can be adapted to analyse the cell cycle, and the measurement of nuclear DNA content which can provide important insight into the nature and behaviour of precancerous and cancerous lesions [16]. Flow cytometry can be modified to sort and count cells labelled with a fluorescent antibody.

DNA technology in gastrointestinal pathology

Recombinant DNA technology has provided an important investigative tool in the field of gastrointestinal pathology. Its value has been most obvious in explaining the pathogenesis of neoplastic disorders, but DNA technology will increasingly be employed to augment tissue diagnosis. Important examples are the hereditary disorders that happen to involve the gastrointestinal tract. For a number of these the underlying gene has been cloned, allowing affected persons to be identified through germline mutation analysis. Of particular relevance in gastrointestinal pathology are methods that can be applied directly to tissue sections such as *in situ* hybridization and methods that utilize DNA or mRNA extracted from tissue samples. Small groups of cells or even single cells can be isolated from tissues by laser capture microdissection [17]. Such methods have been of particular value in uncovering the genetic basis of neoplasia, for which bowel cancer has served as an important model for solid tumours. The principles and practical uses of recombinant DNA technology are described briefly below.

Restriction endonucleases

Around 500 different restriction endonucleases are available. These bacterially derived enzymes recognize particular base sequences and cut the DNA molecule at specific sites deter-

mined by the presence of the relevant base sequences. The ability to cut DNA at specific sites is crucial for many aspects of recombinant DNA technology. Being of different length and physical structure, restriction fragments will have a characteristic electrophoretic mobility in agarose gel and may therefore be separated. However, each individual carries maternally and paternally derived chromosomes that are not identical, so that 'equivalent' restriction fragments may not be of identical length or electrophoretic mobility. This difference or polymorphism has been used for linkage analysis and to demonstrate loss of heterozygosity (LOH) [18,19].

Southern blotting

This refers to the transfer of electrophoretically separated fragments of denatured DNA from agarose gel to a nitrocellulose membrane. Fragments of special interest are detected with radiolabelled probes of complementary DNA. This classical approach has been used for linkage analysis and to demonstrate loss of heterozygosity [18,19], but has been largely superseded by PCR-based methodologies (see below).

DNA microsatellite markers

Non-coding repetitive tracts known as microsatellites may show considerable variability and have been used to establish an individual's DNA fingerprint. DNA probes to these regions provide a powerful approach to haplotyping (i.e. characterizing and distinguishing maternally and paternally derived alleles) and demonstrating loss of heterozygosity.

Polymerase chain reaction (PCR)

Molecular biological analysis of DNA derived from tissue is limited by the amount and quality of the extractable DNA. Viral DNA may have integrated with a fraction of the cells and/or be present as a small number of copies. Mutation detection requires analysis of relatively large amounts of DNA of a particular quality. However, if the structure of the DNA sequence is known, single-stranded DNA can be replicated within the laboratory by means of a pair of primers and the enzyme DNA polymerase. The process can be repeated until the DNA has been replicated a millionfold, hence the designation polymerase chain reaction (PCR) [20]. PCR bridges the gulf between histopathology and molecular genetics by allowing DNA to be extracted from formalin-fixed paraffin-embedded tissues that may be merely selected, microdissected or extracted at the level of individual cells by laser capture microdissection [17]. For example, human papilloma virus has been demonstrated within amplified DNA samples obtained from fixed tissues [21]. The method has also been used to detect subtle mutations within oncogenes and loss of tumour suppressor genes, and for the demonstration of DNA micro-

satellite instability (see below). Messenger RNA expression can be studied by means of reverse transcriptase (generating cDNA) PCR (RT-PCR) using a commonly expressed housekeeping gene (e.g. glyceraldehyde 3-phosphate dehydrogenase) as an internal control. The prospect of developing *in situ* PCR marks the total integration of histopathology and molecular biology [22].

Linkage analysis

The ability to distinguish between equivalent but genetically polymorphic fragments of DNA of maternal and paternal origin allows the inheritance of a particular restriction fragment length polymorphism (RFLP) or haplotype determined by DNA microsatellite markers to be traced through a family. Linkage means that a gene causing a disease is on the same chromosome and physically close to the fragment of DNA that is being traced, i.e. that the disease and the linked DNA marker or haplotype are consistently coinherited. Demonstration of linkage of a disease to an inherited RFLP or haplotype proves that the disease is genetically determined and identifies the approximate locus of the disease causing gene. It was on the basis of linkage analysis that genetic loci for familial adenomatous polyposis [18], hereditary non-polyposis colorectal cancer [23] and Peutz–Jeghers syndrome [24] were identified.

Demonstration of loss of heterozygosity (LOH) and DNA microsatellite instability (MSI)

An individual shows heterozygosity when maternally and paternally derived allelic pairs can be resolved into bands with differing electrophoretic mobility. Tumours may show loss of heterozygosity (LOH), meaning that one or other band has been lost. The loss is generally due to a mitotic error leading to loss of the entire chromosome or a part of the chromosome containing the DNA fragment. It has been established that when LOH for a particular locus occurs on a consistent basis in a particular type of tumour, then the chromosome involved will contain a tumour suppressor gene that is implicated in the pathogenesis of that tumour [19]. Colorectal cancer shows consistent LOH for chromosomes 5, 17 and 18 corresponding to the tumour suppressor genes *APC*, *TP53* and possibly *DCC* [25]. Tumour suppressor genes act recessively at the somatic level, meaning that both genes need to be non-functioning before there is a full transforming effect. The first gene may be non-functioning because of an acquired somatic mutation or as a result of an inherited mutation.

Using DNA microsatellite markers, tumour DNA may sometimes show not band loss, but additional bands (bandshifts) indicating mutation of the microsatellite region. This serves as a marker of defective DNA mismatch repair that may be inherited or acquired [26] (Fig. 2.11).