MUCOSAL BIOPSY OF THE GASTROINTESTINAL TRACT

Second Edition

Volume 3 in the Series
MAJOR PROBLEMS IN PATHOLOGY

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Editor's Foreword

A relatively short while ago gastrointestinal mucosa for histologic examination was limited to that which could be obtained from the stomach by blind suction biopsy or from the colon within easy reach of the proctoscope. However, the technology for mucosal biopsy has advanced very rapidly in just the last few years. Now with the aid of the fiberoptic panendoscope, the entire stomach as well as the first and second portions of the duodenum are easily visualized and biopsies of specific lesions or anatomic sites are readily obtained. Similarly the fiberoptic colonoscope has made it possible to view and biopsy the entire colon and often the terminal ileum. Intervening intestinal mucosa, not accessible to fiberscopic biopsy instruments, is routinely sampled by blind suction biopsy.

In the five years since the first edition of *Mucosal Biopsy of the Gastrointestinal Tract* was published, use of the endoscopic biopsy technique has become widespread. The ability to obtain biopsy specimens from all areas of the gastrointestinal tract without having to resort to laparotomy has greatly expanded our knowledge of the pathogenesis, evolution and response to therapy of nearly every form of intestinal mucosal disease. This technologic advancement has also made it imperative that pathologists become familiar with the interpretation of mucosal biopsies from all levels of the gastrointestinal tract.

Dr. Whitehead who is well known for his many important contributions to the recent advances in gastrointestinal pathology has managed to pull together the vast literature on this subject into a beautifully written and remarkably complete monograph. The second edition has been updated and expanded with the addition of a substantial amount of new text and illustrations. The pathologist, the gastroenterologist and the surgeon who deals with gastrointestinal disease will find the new edition of *Mucosal Biopsy of the Gastrointestinal Tract* eminently readable and an invaluable comprehensive reference. It contains everything one needs to know about the mucosal biopsy.

JAMES L. BENNINGTON, M.D.
Consulting Editor

Preface

In the five years that have elapsed since the first edition of this book the practice of gastrointestinal endoscopy has become commonplace. Consequently a tissue diagnosis in fresh biopsy material is now possible in an ever increasing number of disease states which in a previous era were investigated by indirect and less accurate methods. As a result this edition not only includes significant additions that up-date the previous topics covered, but also sections that concern recently recognised or better defined disease entities. The text is increased by over one half the original, and there are 47 new illustrations and 200 new references.

Like the first edition, the book is primarily concerned with the diagnosis and significance of non-neoplastic lesions of the mucosa of the stomach, and small and large intestine. In the main it concerns ordinary light microscope appearances, but where relevant, information relating to histochemical or electron microscope observations are included. Consideration of the oesophageal mucosa is again omitted since arguably it has a greater similarity of pathology to the oral mucosa and skin. In any case the impact of fibreoptic endoscopic biopsy in the diagnosis of oesophageal lesions has proved disappointing, owing to the fact that rigid oesophagoscopy examination meant that sighted biopsy of oesophageal lesions was not an entirely new possibility. Although fibreoptic instruments made examination of the oesophagus technically easier, the biopsies produced unfortunately are small and superficial.

I remain indebted to many of my previous colleagues in the Radcliffe Infirmary, Oxford, since the bulk of the material upon which this book is based was collected during the years I spent there. In particular I wish to express my gratitude to Dr. S. C. Truelove, who was head of the gastroenterology unit, and to Dr. A. H. T. Robb-Smith, who until recently was the Director of Pathology. My thanks are also due to Mr. Barry Gormley and the other scientific staff of my own department for their painstaking efforts in slide preparation, and I greatly value the expertise of Mr. Peter Graffe who developed and printed the additional photomicrographs. Mrs. Elizabeth Stephens and Mrs. Eugenie Efinger have shown great patience in their careful preparation of the manuscript and reference lists, and it is with great pleasure that I acknowledge their assistance.

Dr. Robert Riddell, who currently works in the department of anatomic pathology at the University of Chicago, U.S.A., is to a large extent responsible

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for clarifying the issues concerning precancerous states in ulcerative colitis. It is he who has kindly provided Figs. 13.14 to 13.20 and I acknowledge this gesture with sincere gratitude.

W. B. Saunders Company have given me every possible consideration, which has made the task of preparing this second edition so much easier. I wish to thank the editor of *The Journal of Clinical Pathology* for permission to include Figs. 1.1, 1.3, 1.4, 1.9–1.11, 2.10, 2.15–2.19, 6.5, 9.1, 9.2, 10.1–11.3, 11.7–11.10, 11.12 and 11.13, and Blackwell Scientific Publications Ltd. for permission to use Figs. 8.8, 8.9, 8.11 and 8.12 which appeared in the *British Journal of Haematology*.

RICHARD WHITEHEAD

General Comments on Procedure

The biopsy specimens produced by either suction or forceps techniques are inevitably small, but with technical expertise informative sections can usually be prepared. Rapid fixation is essential, and handling should be the minimum necessary to ensure that sections are cut at right angles to the mucosal surface. On the whole suction biopsy specimens are easier to manage, because sections in the ideal plane are produced simply by cutting the disc-shaped piece of tissue at right angles to its long axis. When difficulty is experienced, for example with the small biopsies obtained by some of the fibreoptic instruments and especially if the specimen is irregular in shape, stereoscopic microscopy will often help in orientation. The easy recognition of the mucosal surface comes with practice, and, this achieved, the biopsy can be flattened onto a piece of filter paper, mucosal surface uppermost. Fixed flat in this way the specimen is nearly always roughly laminar, and when sectioned at right angles to its long axis the required result is produced. Forceps biopsies received already fixed are often rolled up into a ball reminiscent of a hedgehog having encountered an adversary. The mucosa is always outermost and the specimen cannot be flattened without producing a good deal of traumatic artefact. It is better to process these specimens as they are received, and although inevitably the first few sections will be tangential, deeper cuts nearly always produce sections at right angles to the mucosal surface.

Detailed stereo-microscopic examination of the biopsy specimens can give helpful preliminary information which later can be correlated with histology, particularly in small bowel diseases. It should never take the place of a proper histological examination, and delay in fixation, extra handling and the wiping away of adherent mucus in order to achieve a good stereoscopic view should be rigorously avoided, because of the inevitable distortion, smearing and crushing artefacts that may result. Frequently the net result is a loss of much more information than is gained by the stereoscopic study.

Pathologists will vary in their preference of fixatives, and some insist that the inclusion of mercury is an advantage. It probably matters little for ordinary haematoxylin and eosin (H & E) staining, but it is as well to remember that alcoholic and heavy metal fixatives containing mercury should be avoided if it is desirable to study enterochromaffin cells, and that Paneth cell granules are

soluble in acetic acid and other acid fixatives. In this laboratory all biopsies are fixed in 10 per cent neutral buffered formal-saline and embedded in paraffin wax after passing through an automatic processing machine.

In addition to H & E, any of the many specialised staining methods applicable to paraffin-embedded material may be employed. Individual pathologists often have their own preferences, and circumstances will sometimes dictate which particular method is used.

For gastric biopsy specimens the author uses routinely, in addition to H & E, a reticulin stain (James 1967). This particular method was chosen because the sections are left in the silver solution for about half an hour, and this is a convenience when staining many sections at the same time. Toning in gold chloride is not normally employed, but this is a personal preference. Also, as a standard procedure for gastric biopsy specimens, a Maxwell stain is used (Maxwell 1963) but in a modified form (Burns 1971, personal communication), as follows:

Modified Maxwell Stain

1. 1 per cent alcian green in 3 per cent acetic acid for 5 minutes.
2. Wash in distilled water.
3. 1 per cent periodic acid for 5 minutes.
4. Wash in distilled water.
5. 10 per cent of normal hydrochloric acid in 10 per cent sodium or potassium metabisulphite for 5 minutes.
6. Wash in distilled water.
7. 1 per cent alcian yellow in 3 per cent acetic acid for 15 minutes.
8. Wash in distilled water.
9. Harris's haematoxylin for 2 to 5 minutes.
10. Blue sections in tap water.
11. 2 per cent pyronine Y, ½ to 1 minute.
12. Rinse in distilled water.
13. 1 in 10 dilution of 1 per cent light green in 1 per cent acetic acid, ½ to 1 minute.
14. Rinse in cold tap water, gently blot. Rinse in acetone twice. Rinse in xylol twice and mount.

(Excessive orange colour can be removed by rinsing in alcohol after xylol, and then, after further rinsing in xylol, mount.)

The results are

Nuclei	blue.
Superficial gastric epithelial mucin	yellow.
Intestinal mucin, i.e. in goblet cells	green.
Chief cells	purple/red.
Parietal cells	turquoise/green.

For jejunal and other small intestinal biopsy specimens, an H & E, a periodic acid–Schiff (PAS) preparation and a phosphotungstic acid–haematoxylin

or phloxine tartrazine stain are advocated. The PAS stains the brush-border particularly well and is easier to handle than the alternative alkaline phosphatase method. Either of the remaining two procedures allows the easy identification of Paneth cells. If early collagenisation of the subepithelial zone is suspected, a connective tissue stain such as a van Gieson is useful.

A PAS preparation is also used as a mucin stain in rectal biopsy examination because it doubles as a screen for the detection of amoebae. A better staining method for amoebae in the author's view, however, is the Goldner modification of Masson's trichrome method (Goldner 1938). Perls' method used as a stain for haemosiderin is often useful in the confirmation of ischaemic lesions.

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SECTION ONE

Gastric Biopsy

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CHAPTER ONE

Normal Appearances in Gastric Biopsy Specimens

The pyloric glands occupy a roughly triangular area in the lower third of the stomach. On the greater curve they occur only in the immediate pyloric region, but on the lesser curve the upper limit—although usually in the region of the incisura angularis—is more variable and rarely extends to the oesophagus. The cardiac glands are found distal to the cardio-oesophageal junction for a distance of about 1 cm and the remaining mucosa is of body type. The junction between the three areas may be abrupt but is often occupied by a narrow transitional zone. At the pylorus-body junction in the lesser curve region the width of the transitional zone is variable, and rarely the whole lesser curve shows a transitional appearance.

BODY MUCOSA (Fig. 1.1)

Throughout the stomach the superficial epithelium is composed of a single layer of cells with a basal nucleus below a typical cup-shaped column of clear or faintly granular mucin. Occasionally one sees an intraepithelial lymphocyte which has insinuated itself between adjacent epithelial cells. The surface epithelium dips to form shallow gastric pits, into which open approximately four gastric glands. These are simple straight tubules, tightly packed together, roughly the same length, which occupy three-quarters of the thickness of the mucosa. Most cells lining the upper part of the glands are parietal cells. These are eosinophilic and triangular with a central nucleus. Their longest side is applied to the basement membrane, and sometimes intracellular canaliculi are visible. At the junction of the glands with the pits, scattered amongst the parietal cells and sometimes deeper, there are the mucin-secreting neck cells which, like the superficial epithelium, contain PAS-positive and diastase-resistant mucin. The lower half of the glands contain the chief cells which mingle with the parietal cells in the region of the middle third. Chief cells have a basal nucleus and a cytoplasm filled

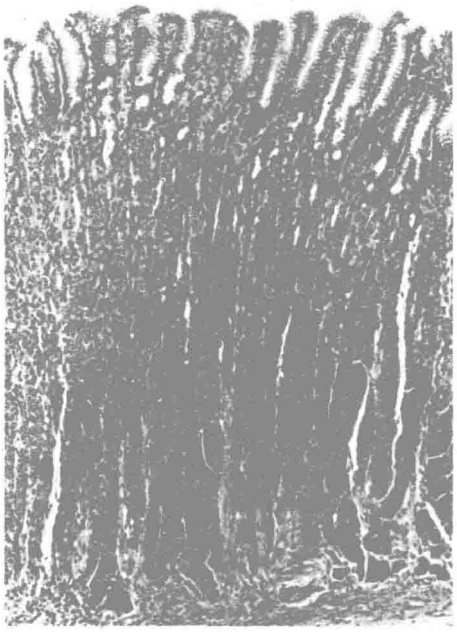

Fig. 1.1. Normal body mucosa. Note regular superficial epithelium, short gastric pits, predominance of parietal cells in upper half and chief cells in lower half of tubules. (H & E × 75)

with basophilic pepsinogen granules. The degree of basophilia of the granules varies from biopsy to biopsy. Occasionally, towards the base there is an argentaffin (enterochromaffin) cell. Rarely the basal parts of the tubules are cystic (Fig. 1.2) but the flattened cells always retain their special staining characteristics. This is in contrast to the cystic change which occurs in Ménétrier's disease, in which the cysts are invariably larger and associated with abnormalities of the muscularis mucosa *(see later)*.

The lamina propria is most obvious between the gastric pits where it contains a small number of plasma cells, lymphocytes, eosinophils and histiocytes, together with a fine capillary plexus and non-myelinated nerves. There are no objective guidelines as to what constitutes a normal number of cells in the lamina propria. This of course is only a reflection of the difficulty firstly, in defining a normal population and secondly, in acquiring biopsy specimens from such a group. Illustration of this is seen in the study of Myren and Serck-Hanssen (1975) who found what they considered subjectively to be an increase in inflammatory infiltration in the interfoveolar lamina propria of four antral, three lesser curve and two greater curve biopsies from ten healthy medical student volunteers. At intervals along the mucosa the muscularis mucosa sends infrequent groups of smooth muscle fibres upwards between the glands. In reticulin preparations (Fig. 1.3) the basement membrane of the surface epithelium, pits and glands appears as a single layer of fibres, between which a delicate mesh of strands represents the supporting connective tissues between and in relation to the capillaries. The sharp division between the reticulin pattern of the lamina propria

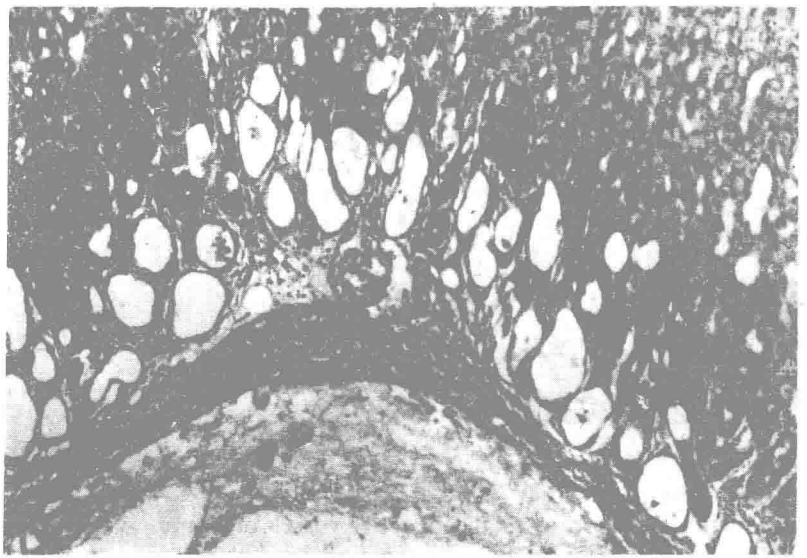

Fig. 1.2. Body mucosa showing cystic change at the base of the gland tubules. (H & E × 188)

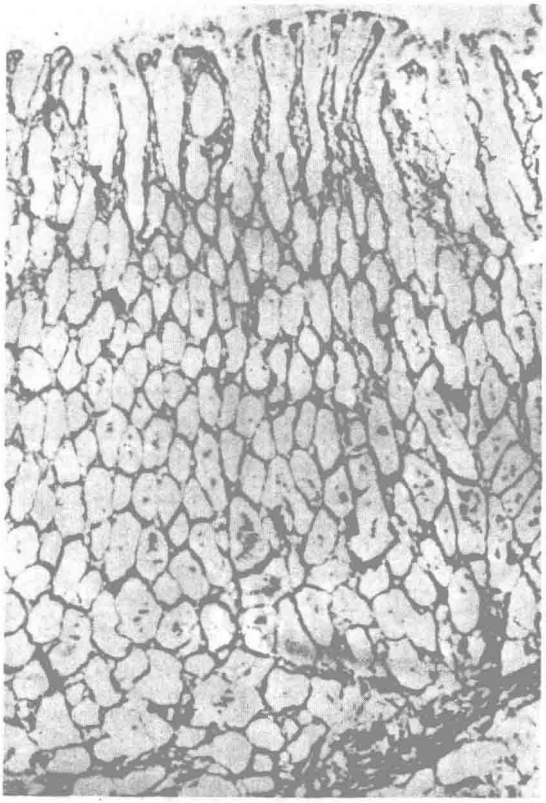

Fig. 1.3. Normal body mucosa with typical reticulin distribution. (Reticulin stain × 102)

and the denser network around the fibres and vessels of the muscularis mucosa is broken only by occasional tent-shaped skeins of fibres which surround the upward muscular prolongations.

PYLORIC MUCOSA (Fig. 1.4)

Here the gastric pits are deeper and sometimes branched, and the glands are shorter, less tightly packed than in the body, ending at different levels and occupying one half or less of the mucosal thickness. They are simple or branched coiled tubules, composed of mucin-secreting cells and occasional parietal cells (Fig. 1.5) especially near transitional zones. The incidence and distribution of parietal cells in antral mucosa was studied in an autopsy series by Tominaga (1975). They were found in 98.3 per cent of cases throughout the range from newborn to old age, and were considered to be a normal constituent of antral mucosa and not due to either metaplasia or dystopia. Very infrequently an isolated zone of chief cells may also be seen (Fig. 1.6). Argentaffin cells are more

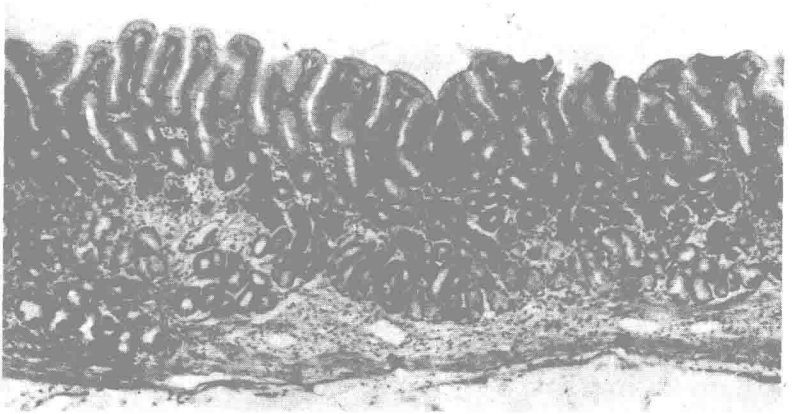

Fig. 1.4. Pyloric mucosa. The pits are relatively longer and some branch; they occupy one half the thickness of the mucosa. The glands are of simple mucin-producing type. (H & E × 75)

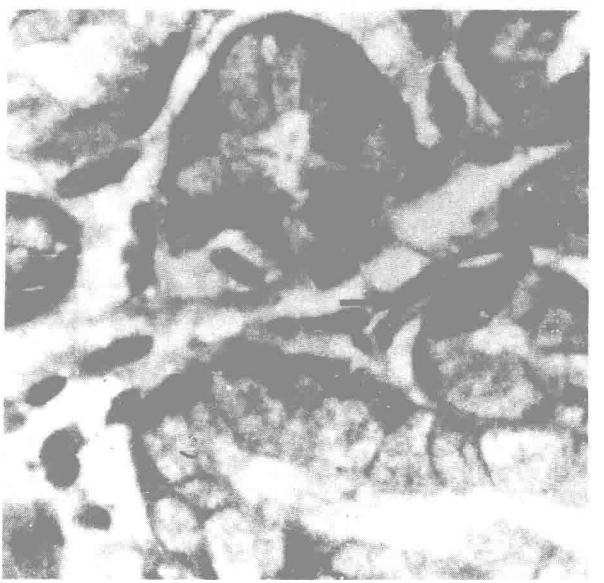

Fig. 1.5. Pyloric mucosa. Occasional parietal cells in otherwise typical mucin-producing pyloric glands. (H & E × 400)

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