MUCOSAL BIOPSY OF THE GASTROINTESTINAL TRACT

Volume 3 in the Series
MAJOR PROBLEMS IN PATHOLOGY
JAMES L. BENNINGTON, M.D., *Consulting Editor*

MUCOSAL BIOPSY OF THE GASTROINTESTINAL TRACT

Volume 3 in the Series

MAJOR PROBLEMS IN PATHOLOGY

JAMES L. BENNINGTON, M.D., *Consulting Editor*

Chairman, Department of Pathology
Children's Hospital of San Francisco
San Francisco, California

1973

W. B. Saunders Company Ltd London · Philadelphia · Toronto

W. B. Saunders Company Ltd: 12 Dyott Street
London WC1A 1DB

West Washington Square
Philadelphia, Pa. 19105

833 Oxford Street
Toronto 18, Ontario

Mucosal Biopsy of the Gastrointestinal Tract

ISBN 0-7216-9303-2

Printed at The Lavenham Press Ltd, Lavenham, Suffolk, England.

Print Number: 9 8 7 6 5 4 3 2 1

Other monographs in the series,
Major Problems in Pathology:

Published

Evans and Cruickshank: *Epithelial Tumours of the Salivary Glands*

Mottet: *Histopathologic Spectrum of Regional Enteritis and Ulcerative Colitis*

Forthcoming

Beckwith and D'Angio: *Renal Tumors in Infants and Children*

Hughes: *Pathology of Muscle*

Kempson: *Uterine Tumors*

MacKay: *Soft Tissue Tumors*

Melnick: *Histochemistry Applied to Pathology*

Sagebiel: *Histopathologic Diagnosis of Melanotic Lesions of Skin*

Striker, Cutler and Quadracci: *Use and Interpretation of Renal Biopsy*

Thompson: *Oesophageal Lesions*

Thurlbeck: *Chronic Obstructive Lung Disease*

Richard Whitehead, M.D., M.R.C.Path.

Consultant Pathologist,
The Radcliffe Infirmary and the United Oxford Hospitals

Clinical Lecturer, The Medical School,
University of Oxford

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.

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.

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 pathologist, the gastroenterologist and the surgeon who deals with gastrointestinal disease will find "*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

The mucosa of the gastrointestinal tract undergoes extremely rapid postmortem autolysis and it is not surprising that relatively minor lesions in the more inaccessible regions have gone unobserved until recent times. The advent of biopsy via flexible tubes which can be passed well into the gastrointestinal tract and the detailed histological examination of the fresh tissues obtained have been responsible for rea advances in our understanding, diagnosis and treatment of many of its afflictions. This is particularly true of the non-neoplastic conditions, especially when they involve the stomach and small intestine, but it is also true as far as the inflammatory and ischaemic lesions of the colon are concerned. For a variety of reasons it does not apply to the oesophagus which, like the rectum, has been accessible via the available biopsy instruments for a much longer period. It has, however, a less specialised epithelial lining, and the number of non-neoplastic clinical situations associated with distinct histology are few. The author therefore makes no apology for its exclusion from consideration in this book which is thus primarily concerned with the non-malignant diseases of the stomach, small and large intestines. Only in those instances where there has been recent significant advance will malignant conditions be considered.

The practice of diagnostic mucosal biopsy is spreading and is now performed widely outside specialised centres. With the introduction of more sophisticated instruments, all areas of the gastrointestinal tract can be sampled under direct vision. The availability of these fresh tissues at a minimum risk and discomfort to the patient has also created new and exciting opportunities for research, since it allows examination by immunological, histochemical and electron-microscopic methods. These newer techniques have not as yet superseded older established methods as routine diagnostic procedures, and it is true to say that only in isolated areas has their use been responsible for significant advance in our knowledge. With increasing use and experience there is little doubt that this will be rectified but it is clear that there is still much to be observed and learned from the examination of ordinary tissue preparations by light microscopy. This is especially true if it is combined with reasoned clinical correlation. Observations that can be made by the light microscope using ordinary techniques in tissue preparation are those that will be considered. The primary aim of this book is thus to meet the needs

of the practising histopathologist who is being asked more and more frequently to pass opinions on biopsy specimens taken from the gastrointestinal tract. It is hoped that it will also be of value to physicians and surgeons who have a particular interest in gastroenterology.

I have been particularly fortunate to have collaborated as part of a team with Dr S. C. Truelove at the Radcliffe Infirmary, Oxford, an eminent international figure in the field of clinical gastroenterology. The bulk of the material upon which this book is based comes from his clinics. Our many discussions concerning clinico-pathological correlation have contributed greatly to my education for which I wish to express my most sincere gratitude. I also wish to acknowledge the encouragement and advice given to me by Dr A. H. T. Robb-Smith, Director of Pathology at the Radcliffe Infirmary, in whose department I have enjoyed first-class laboratory facilities. Without the valued assistance of the photographic laboratory in which Dr T. Parry and Mr D. Luckett have spent many hours on my behalf, this book would never have been completed. I also wish to make a special tribute to Mrs Maria Lobban and Miss Ysanne Jacobs who, with a high degree of skill, have been responsible for the technical work involved in the handling of the biopsy specimens and in subsequent preparation of tissue sections. It gives me great pleasure to thank Mrs Rachel Hunt for her great patience in typing the manuscript.

W. B. Saunders Company, the publishers, have given me every consideration for which I am greatly appreciative. I wish to acknowledge the editor of *The Journal of Clinical Pathology* for permission to include Figures 1, 3, 4, 9–11, 19, 21, 26–30, 58, 84, 85, 90–100, 104–107, 109 and 110, and Blackwell Scientific Publications Ltd for permission to use Figures 78, 79, 81 and 82 which appeared in the *British Journal of Haematology*.

CONTENTS

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 can later 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 which 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 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 haematoxylin and eosin, 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 haematoxylin and eosin (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.

References

Goldner, J. (1938). A modification of the Masson trichrome technique for routine laboratory purposes. American Journal of Pathology *14*, 237–243.

James, K. R. (1967). A simple silver method for the demonstration of reticulin fibres. Journal of Medical Laboratory Technology *24*, 49–51.

Maxwell, A. (1963). The alcian dyes applied to gastric mucosa. Stain Technology *38*, 286–287.

Section I

GASTRIC BIOPSY

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—whilst 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)

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. The majority of 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 with basophilic pepsinogen granules. The degree of basophilia of the granules