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Gross examination of the large intestine

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Introduction

Specimens from the large intestine provide a substantial proportion of the work in many histopathology departments. Rapid autolysis due to digestive enzymes aided by the faecal flora make it essential that both the doctor who takes the biopsy specimen and the pathology department deal with it appropriately to ensure ideal fixation. All too often colonic resections arrive at the pathologist a day late, half-opened, filled with faeces, and showing evidence of autolysis, making the subsequent macroscopic and microscopic assessment difficult and sometimes inconclusive. Many of the problems with specimen handling can be avoided by good communication between clinician and pathologist. In this broadsheet we describe the handling and dissection of specimens as performed in our specialist institute. However, both of us have experience of busy general departments and all the methods are equally appropriate for a district general hospital laboratory. It is our opinion that these methods in the long term save time. An extra five minutes spent in examining a specimen will save time in the reporting room and often avoid return visits to the dissecting table for further blocks. We shall confine our discussion to the handling of biopsy and resection specimens.

Biopsy specimens

ROUTINE PARAFFIN WAX SECTIONS

With the advent of the colonoscope and the increasing number of physicians and surgeons able to use it, histopathologists are now seeing large numbers of endoscopic colonic biopsy specimens. The introduction of the flexible sigmoidoscope to outpatient departments will further increase biopsy numbers. Orientation of biopsy specimens greatly aids diagnosis and endoscopists should place them on to a flat surface before fixation to prevent subsequent curling. We provide our outpatient department with a supply of ground glass slides for this purpose. Small pieces of thin card are equally effective.

Multiple biopsy specimens from different sites, such as those from a colonoscopic examination, provide further problems. To include all of them in the same pot prevents the localisation of individual lesions in the colon. Some laboratories request that each biopsy specimen is sent separately. This permits accurate localisation of each specimen, but is wasteful and time consuming for technicians and pathologists. To avoid these problems we use a cellulose nitrate filter sheet (pore size 0·8 μm). The filter (supplied as discs by Sartorius GmBH) can be cut with scissors into a rectangular shape and has a pre-printed grid on one surface. A corner is cut off to aid orientation (fig 1) and the first biopsy specimen is placed on the grid nearest the cut corner. The multiple biopsy specimens are placed serially on the grid with the most distal furthest from the marked corner. The filter serves the dual purpose of preventing the specimens from curling and allowing their individual sites to be localised. The specimens, which should be carefully placed along a straight line by the endoscopist, remain on the filter throughout processing. The filter strip, with attached biopsy specimens, is embedded on edge to achieve correct orientation. The macroscopic description of the specimens should confirm the number and the size of the pieces of tissue submitted for histological examination. It is also useful to note if any of

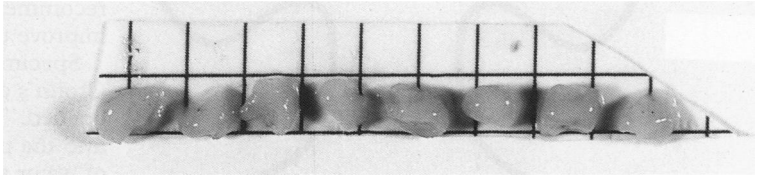

Figure 1 Colonoscopic biopsy specimens mounted on Millipore filter. A maximum of eight can be laid serially on the filter along a straight line, so that histological sections pass through the equator of each biopsy. By convention, the most proximal biopsy specimen is laid at the pointed end. Scale: 3·5 grid squares = 1 cm.

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the specimens appear to consist of mucus or pus as this can often explain the "loss" of specimens during processing.

The filter sections easily, does not blunt microtome blades, and does not take up haematoxylin and eosin. The result of this method is that up to eight well orientated biopsy specimens can be viewed on one slide with good localisation of individual lesions.

POLYPS

Polyps are often small and sessile and are treated in the same way as mucosal biopsy specimens. With a larger polyp stalk, any stalk usually retracts rapidly into the body of the polyp and becomes inconspicuous. Care is therefore required in the laboratory, both at this stage when the pathologist examines the polyp and when the processed polyp is embedded in wax. Our practice is to process a polyp whole when it is small enough to fit a Tissue-Tec cassette. A larger polyp should be trimmed to form one block which includes the stalk and other blocks of the residual tangenital slices (fig 2).

FROZEN SECTIONS

Perioperative frozen sections can have orientation improved by the use of the previously described filters. For the diagnosis of Hirschsprung's disease, appropriate full thickness specimens should be provided by the operating surgeon. Use of a rapid non-specific esterase stain will aid visualisation of nerve fibres and can provide a diagnostic answer within 15 minutes. More detailed information on the handling of such cases is adequately covered in the specialised textbooks.[1 2]

Operative resection specimens

GENERAL COMMENTS

All laboratories should adopt a policy of receiving specimens fresh. If surgery is performed out of laboratory hours the specimen can be kept fresh in a refrigerator until the laboratory opens. Only if the specimen is going to be left unfixed for over 24 hours is there any justification for theatre staff placing it into fixative. The main benefit of this fresh specimen policy is that anatomical orientation of any specimen is easier when fresh and the correct position can be maintained during fixation. Subsequent reporting is much more satisfactory. An additional advantage is that suitable tissue can be taken for those departments where active research is being carried out. To facilitate the handling of large bowel specimens it is important to have a well ventilated area containing a sluice or large bore waste disposal unit. These are essential for the adequate disposal of faeces. Large specimen tanks filled with formalin should be kept for the flotation of suitably prepared specimens. A tank of fresh formalin, with a hose attachment, above the preparation area is useful for the gentle inflation of specimens.

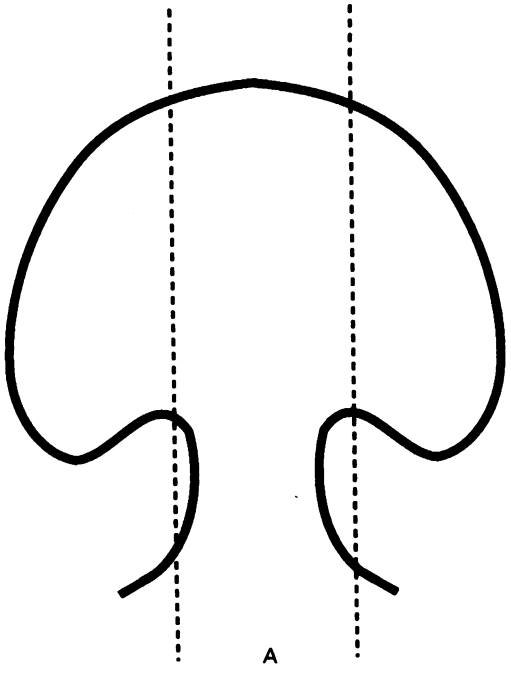

Figure 2 Blocking of a stalked polyp. The main block (A) should be embedded separately so that the stalk can be included to assess for the presence of invasion.

FIXATION

On arrival in the laboratory the request form should make clear the nature of the disease process affecting the bowel. In general, rectal specimens should be opened anteriorly and colonic specimens opened on the anti-mesenteric border. Once the specimen is open faecal material can be washed away from the mucosa with cold, fresh water. Surgeons should be asked not to open specimens in theatre. If they insist on doing so they should be taught to do so properly as a badly opened specimen can be both confusing and fix poorly. The pathologist should attempt to look at all specimens fresh and at this time should take unfixed tissue if required. For ideal fixation a fresh, opened specimen should be pinned out on to cork board under a moderate degree of tension. At St Mark's we use 0·8 × 40 mm (green) syringe needles, as these are both sharp and rustless. Most ordinary pins rust so badly that they spoil the specimen. In a colectomy specimen it is best to start pinning on the outer border of the caecum and work around the outer border to the rectum, returning back to the caecum. This allows the specimen to be stretched to an ideal tension. The suitably labelled specimen can then be floated in a tank containing fixative. The mesentery should be raised from the board prior to submersion, to allow better penetration of formalin and quicker fixation. Twenty four hours after flotation the specimen can be unpinned from the board and allowed to fix for a further day while floating free in the tank. This extra period is recommended for cancer specimens, to improve the fixation of the mesocolic fat.

Specimens that are strictured, such as in Crohn's disease or diverticulitis, should not be opened. The introduction of a flexible hose into the proximal cut end with a gentle trickle of water should be sufficient to flush away the faecal material. Once the water is running clear through the bowel, the specimen is tied off at the distal end and inflated with formalin. The

inflated specimen is then floated in the fixative tank.

PHOTOGRAPHY

Black and white photographs of all major resection specimens are taken at St Mark's. This is an invaluable permanent record of the macroscopic appearances which conveys more information than even the most eloquent of prose. Many laboratories do not photograph specimens because of the time and effort required, but a photographic record of a large specimen can frequently be of great value if problems of orientation arise after the dissection. It is also an asset when reviewing cases for research. The expense of black and white photography is minimal when compared with newer histological diagnostic techniques.

DESCRIPTION

It is traditional to describe both the length and circumference of all specimens. The description should include the appearances of diseased and normal areas. Attention should be paid to serosal changes as well as the mucosal abnormalities. Long convoluted descriptions should be avoided as often they serve only to confuse. Brief, interpretive, descriptions are often more helpful than long catalogues of measurements and minutiae.

DISSECTION AND BLOCKS

Non-malignant disease

In inflammatory bowel disease the surgeon has two questions. "What is the nature of the disease process?" and "how far does the disease extend?" Blocks taken for processing should provide answers to both questions. In colectomy specimens the proximal and the distal resection limits should be sampled. Throughout the rest of the specimen, blocks should be taken at regular intervals, at a maximum spacing of every 10 cm to assess the extent of disease. Too few blocks often fail to reveal the pattern of disease. It is most important that a permanent record is kept of the site from which every block is taken so that all sections are given a site specific identification. Only by doing this can the sections be adequately interpreted in terms of extent and distribution of disease. In addition to sampling the colon at regular intervals, further blocks should be taken from lesions of specific interest.

Similar principles should be applied to other non-malignant specimens.

Malignant disease

Almost all malignant tumours in the colon and rectum are adenocarcinomas. The pathologist should approach the dissection to answer the following questions: "what type of tumour is it?", "Is it all out?" and "what is the stage?" These questons can only be answered if the pathologist conducts a proper macroscopic examination and dissection. A systematic approach to all tumours will ensure a good standard of reporting. First, blocks are taken of the resection margins—the proximal and distal cut ends of bowel. These upper and lower ends are often unimportant as it is extremely rare to see tumour extending submucosally more than 2·5 cm in a fixed specimen. Some pathologists use this distance as their guide to whether taking these blocks is necessary. In our institute proximal and distal resection limits are always taken as they provide some normal mucosa which can be used, if needed, for future research. Blocks should then be taken of any other mucosal lesions, such as polyps. We then cut out the segment of bowel containing the tumour (fig 3). This is put aside while the search for lymph nodes is performed. The mesocolic lymph nodes are sampled in a systematic way, from the high tie vessel towards the tumour. This allows the separation of the "high tie" node from the other lymph nodes if it is not the practice to identify separately every node on a gland chart. Although there is only one "high tie" in rectal resections, in colonic resections there is frequently more than one vessel supplying and draining the tumour. Therefore, it is feasible to find more than one "high tie" node. Fat clearance has been recommended to increase the yield of lymph nodes. This method is time consuming and messy (xylene dissolves rubber gloves) and can be difficult as the reagents used are highly toxic. Provided that the mesocolon is well fixed and a sharp knife is used the yield of lymph nodes can be very high using a "toast racking" series of cuts into the mesentery. Orientation of the specimen can be preserved if the cuts are not completed through the bowel wall. At St Mark's the average number of lymph nodes found using this technique in anterior resection specimens of the rectum is 13. During dissection of the mesocolon, attention should be paid to any possible isolated mesocolic deposits and extramural venous invasion. It should also be appreciated that lymph nodes can be close to the deep resection plane and should be sampled to display the plane if it lies nearby.

After all visible and palpable nodes have

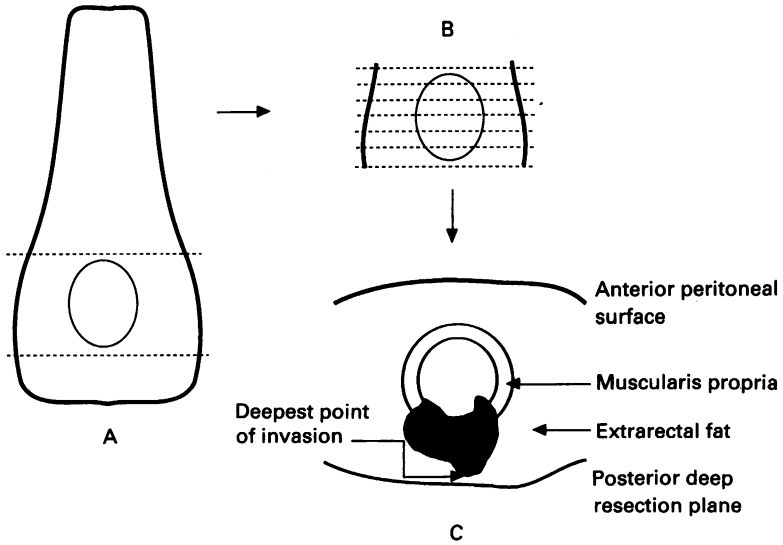

Figure 3 Dissection of cancer specimens; tumour blocks. (A) Cut out the segment of the bowel wall containing the tumour. (B) Make serial transverse slices through the tumour. (C) Visually assess the tumour slices to select the blocks where the tumour appears nearest to the resection margin.

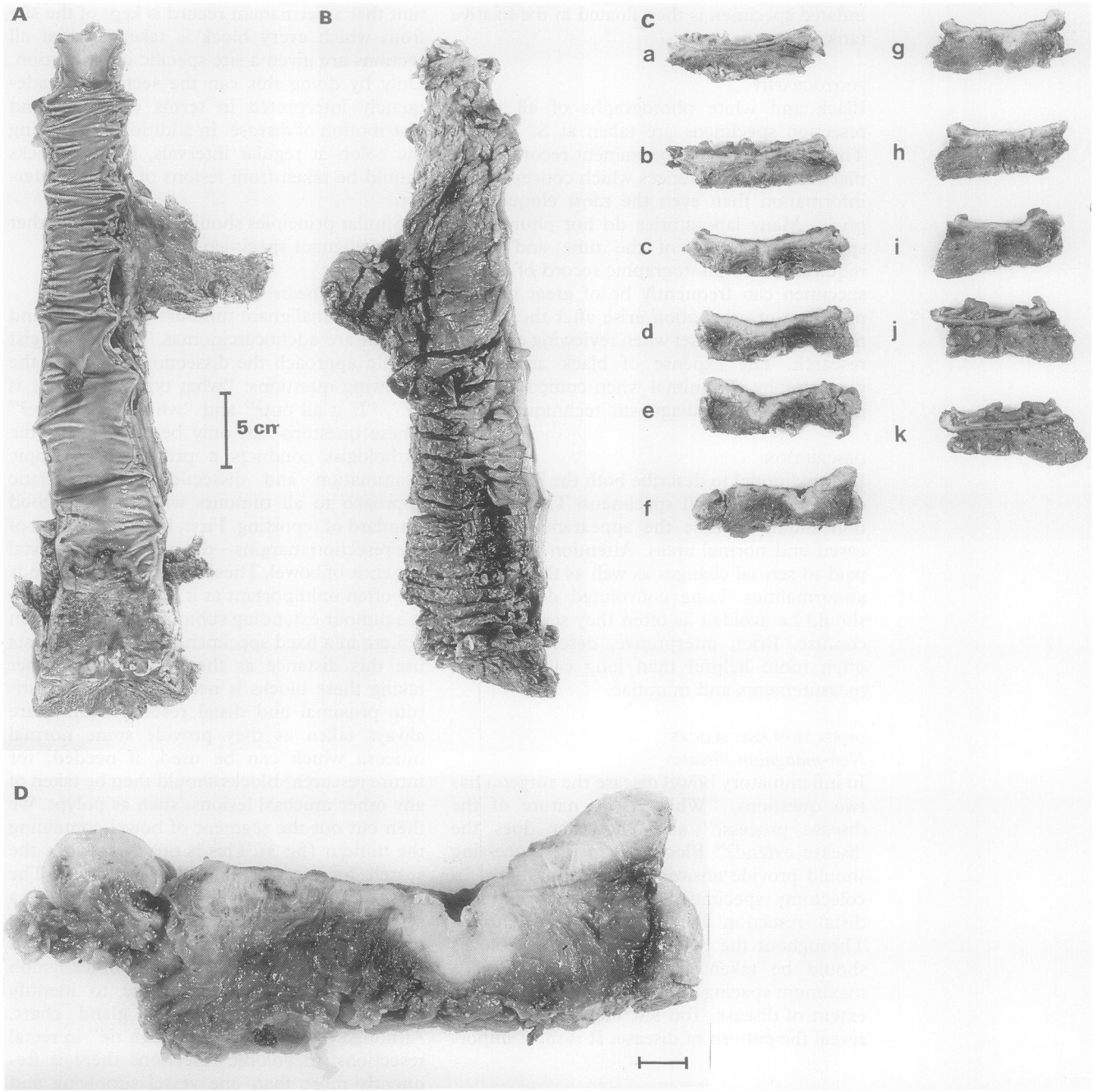

Figure 4 (A) Low rectal adenocarcinoma following fixation. (B) "Toast-racking" of the mesentery to search for lymph nodes after removal of the tumour containing bowel segment. (C) Serial transverse slices of the tumour revealing blocks f and e as the segments where the tumour is nearest to the deep resection plane. (D) Close-up view of transverse slice f.

been sampled we then return to the segment of bowel containing the tumour. This includes the most important resection margin: the deep plane of excision. This is particularly important in tumours from the extraperitoneal lower half of the rectum. The presence of tumour at or close to the deep resection margin has been associated with local recurrence.[3] For this part of the examination we take serial transverse slices through the whole segment of bowel affected by tumour and then select blocks from where the tumour appears closest to the deep margin or serosal surface (fig 4). This method also makes the dissector appreciate other features such as extramural venous pathology and affected lymph nodes deep to the primary tumour. Marking the deep resection plane with India ink or other marker materials has been advocated, but this can be unhelpful as the marker material often spreads to other edges, making microscopic interpretation difficult. It is much better to take the blocks with a really sharp knife such as a disposable microtome blade and use the straight lines created at the sides of the block by the knife as a guide. In these blocks we include any lymph nodes situated at the base of the tumour. Further blocks of the superficial parts of the tumour can be taken to look for any residual adenoma and to assess differentiation. Only if a consistent method of dissection is used can the results of the subsequent pathological staging give reliable prognostic data.[4]

Vascular disease

We have emphasised so far that the methods

used are applicable in any histopathology laboratory. For the investigation of angiodysplasia the cooperation of the radiology department may also be needed. If angiodysplasia is suspected before surgery the laboratory should be notified in advance so that suitable preparations can be made. It is impossible usefully to examine such a specimen unless it is received fresh immediately after resection and the vessels are clearly marked.[5]

Anticoagulated physiological saline is first infused in the regional artery to prevent both lysis and intravascular clotting of the blood in the specimen. A mixture of barium and gelatin can then be introduced into the mesenteric arteries to distend the vessels and help localise lesions, using dissecting microscopy and radiography, so that appropriate blocks can then be selected. If the time and the trouble to do this are not taken the demonstration of angiodysplasia is rarely successful.

Conclusion

This is only a guide to pathologists in the development of their own individual techniques and preferences. In recent years much emphasis has been placed on the newer methods to aid diagnosis and the macroscopic examination has often been neglected. In our opinion, if greater emphasis is placed on the dissection diagnosis and all other assessments can be achieved rapidly and accurately in most cases with simple histopathological staining.

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