LETTERS TO THE EDITOR

Campylobacter-like organisms in heterotopic gastric mucosa of the upper oesophagus

Campylobacter-like organisms (CLO) are strongly associated in the stomach with active type B chronic gastritis, and several different studies have suggested a role for CLO in the natural history of the disease. CLO are closely apposed to gastric mucin secreting cells in the stomach, and they can also be found in areas of gastric metaplasia in the duodenum where they are supposed to be a key factor in the tendency of duodenal ulcers to relapse.[1] CLO can also be observed in Barrett's oesophagus, but their incidence must probably be low, with a minor role in the natural history of the disease.[2] We wondered, therefore, whether CLO could be detected in heterotopic gastric mucosa of the upper oesophagus in adults, and what relation, if any, CLO might show to active inflammation in heterotopic gastric mucosa. Heterotopic gastric mucosa is a benign and usually asymptomatic condition, the congenital nature of which has recently been questioned.[3]

We reviewed 56 cases of heterotopic gastric mucosa from our files; the histological and histochemical aspects of 24 of these cases have already been published.[3] The heterotopic gastric mucosa was classified as antral or body type, and signs of inflammation were scored on sections stained with haematoxylin and eosin according to the criteria of Marshall[4]: grades 0 to 1, normal histological features; grade 2, increase in mononuclear cells; grade 3, increase of mononuclear and polymorphonuclear cells with intraepithelial invasion of polymorphonuclear cells. Modified Giemsa stained slides were used for the detection of CLO.

Six heterotopic gastric mucosa consisted of antral type mucosa, the remaining 50 had body-type glands; 23 showed signs of inflammation—18 cases with grade 2 and three cases with grade 3. CLO were observed in three out of the 56 (5·3%) heterotopic gastric mucosa, all three with body-type mucosa, the latter being normal in one case and of grade 3 inflammation in two cases. In addition to heterotopic gastric mucosa, gastric biopsy specimens obtained in 18 of the 56 patients during the same endoscopy, were also examined for inflammation and CLO. Two of the three patients with CLO in their heterotopic gastric mucosa had concomitant gastric biopsy specimens which also showed active chronic gastritis with CLO. Among 16 patients with CLO negative heterotopic gastric mucosa, 13 had normal concomitant gastric biopsy specimens without CLO, and three had concomitant CLO associated active chronic gastritis.

Our results show that CLO can be rarely observed in heterotopic gastric mucosa. As *Helicobacter pylori* has never been cultured from the oropharynx,[5] it is likely to be transmitted from the stomach up into the cervical oesophagus by gastro-oesophageal reflux of contaminated gastric juice. Our results in patients with heterotopic gastric mucosa from whom gastric biopsy specimens were taken would support this hypothesis. Some of our data also suggest that CLO could be responsible for the inflammatory changes which are rarely observed in heterotopic gastric mucosa. CLO could therefore have a role in the rare complications of heterotopic gastric mucosa such as inflammation, ulceration, or stenosis, in the same way as it has been shown in duodenal ulcer,[1] and suggested in Barrett's oesophagus.[2]

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Superior stain for Helicobacter pylori using toluidine O

In any histology laboratory that receives gastric biopsy specimens, staining for *Helicobacter pylori* should be part of the routine procedure. In our laboratory we are now using a toluidine blue stain (GURR) and consider this to be very effective, simple, and fast with excellent histological contrast.

As *H pylori* infection is described as producing a superficial active chronic gastritis, toluidine blue is a most satisfactory stain for the demonstration of neutrophilic infiltration between mucosa producing cells and in the lamina propria. The nuclear lobes of the neutrophils stand out against the clear unstained space of the neutrophil cytoplasm, and with proper dehydration, the mucus producing cells stain a very light, clean blue, allowing for easy examination and identification of *H pylori*.[1-4]

A maximum of 10–15 minutes in buffered toluidine blue is all that is required for very positive results. We found that the best buffer for toluidine blue was a veronal acetate buffer at pH 4·5.[5] This buffer contains a barbiturate and because of the complications involved with acquiring such we changed to Sorensen's phosphate buffer at pH 6·8[5] and found that it also works quite effectively. The unique quality of the toluidine blue O stain is its ability to give good staining results at varying pH ranges and with different buffer solutions.

Staining method

TOLUIDINE BLUE USING VERONAL ACETATE BUFFER

Veronal acetate solution:
Sodium acetate 1·943 g
Sodium barbiturate 2·943 g
Distilled water to 100 ml

For pH 4·5, make up in the proportions of 10 ml of stock veronal acetate solution, 22 ml of M/10 hydrochloric acid, and 18 ml of distilled water. To this add 1 ml of 1% toluidine blue made up in distilled water.

1 Sections are cut at 6 μm.
2 Bring sections to water.
3 Stain in toluidine blue buffered solution for 10–15 minutes.
4 Wash well in water.
5 Dehydrate, clear, and mount.

TOLUIDINE BLUE USING SORENSEN'S PHOSPHATE BUFFER

Buffer solution:
M/15 sodium phosphate dibasic; Na2HPO4. Dissolve 9·465 g in distilled water and make up to one litre.
M/15 potassium acid phosphate; KH2PO4. Dissolve 9·08 g in distilled water and make up to one litre.

For pH 6·8, add 25 ml of each solution together and to this add 1 ml of 1% stock toluidine blue solution. The staining technique for this method is the same as above.

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Thrombotic thrombocytopenic purpura (TTP) complicating leptospirosis: a previously undescribed association

Thrombotic thrombocytopenic purpura is a rare condition which can occur without an identifiable precipitant,[1] although it has been described in association with a variety of infective and therapeutic agents.[2] It is characterised by a combination of thrombocytopenic purpura, microangiopathic haemolytic anaemia, renal impairment, a neurological deficit and fever. Unlike disseminated intravascular coagulation, there is a minimal disturbance of coagulation, and histology shows characteristic granular hyaline thrombi within small vessels, an appearance not seen in disseminated intravascular coagulation.[3]

We recently encountered an example of this condition in a 50 year old Caucasian man who presented, via casualty, to the local infectious diseases unit. He gave a five day history of fever followed by non-pruritic jaundice, malaise, and both intermittent chest and abdominal pain. He was also anorectic, and had lost one stone in weight. Hepatitis was diagnosed. Despite initial treatment at home, with a combination of amoxycillin and clavulanic acid, from the third day of his illness, he had continued to deteriorate and at the time of presentation was drowsy with an expressive dysphasia.

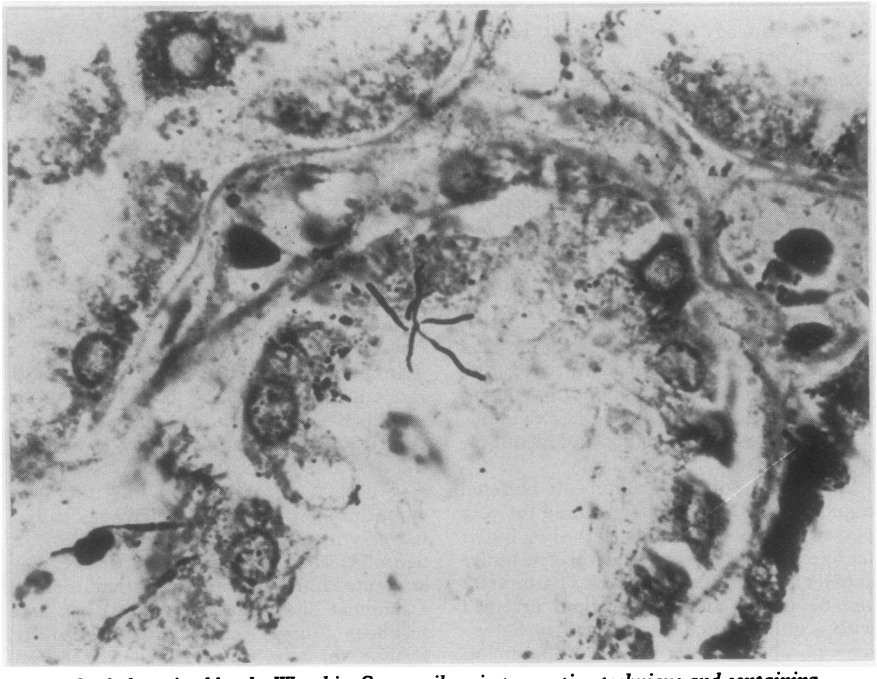
His blood count showed a haemoglobin concentration of 7·7 g/dl, a white cell count of $6{\cdot}5 \times 10^9$/l, and a platelet count of 20×10^9/l. The blood film contained nucleated red cells and microspherocytes. The working diagnosis at this time was of leptospirosis: according to the patient's relatives, he had been working in a drain a week before the onset of symptoms. Other investigations showed increased creatinine and urea concentrations with an abnormal coagulation screen, suggesting disseminated intravascular coagulation (prothrombin time 16·5 seconds; normal range 10–13 seconds: kaolin-cephalin clotting time 50 seconds; normal range 27–37 seconds: thrombin time 12 seconds; control 14 seconds, normal range control $\pm 2{\cdot}5$: fibrinogen concentration 1·8 g/dl; normal range 1·6–3·9: and D-dimers of 8 μg/ml; normal range $< 0{\cdot}25$ μg/ml), although the possibility of TTP was considered in view of the persisting neurological signs. The bilirubin was raised at 101 mmol/l and liver enzymes were raised. Blood cultures and hepatitis serology were negative, in particular the leptospira serology was reported as being negative at a titre of less than 10. A chest *x*-ray picture and electrocardiogram were normal, but an electroencephalogram did show a diffuse abnormality affecting the cerebral hemispheres.

Treatment was in the form of vitamin K, platelet concentrate, intravenous fluids and blood, fresh frozen plasma, and antibiotics (benzyl penicillin 4 MU every six hours, and gentamicin 120 mg every eight hours). The patient continued to deteriorate over the next 48 hours, despite the addition of high dose prednisolone for possible TTP. He was therefore transferred for plasma exchange at another hospital. At this time his coagulation profile was becoming more normal (prothrombin time 18 seconds, kaolin-cephalin clotting time 37 seconds, fibrinogen concentration 2·9 g/l, and D-dimers of 1 μg/ml), but there was evidence of a microangiopathic haemolytic anaemia with gross red cell fragmentation. The haemoglobin concentration was 10·3 g/dl and the platelet count 30×10^9/l. This combination of features was now suggestive of a diagnosis of TTP. A computed tomogram showed no intracerebral pathology.

Despite a two litre plasma exchange[4] along with continued antibiotics, steroids, and the addition of high dose intravenous immunoglobulin there was continued deterioration and he died 48 hours after admission: a second serum sample had not been sent for leptospira serology.

The important findings at necropsy were prominent petechial haemorrhages present over the whole skin surface and on the endocardial surface of the heart. Multiple renal infarcts were present and there was severe gastrointestinal bleeding. Histological examination showed leptospira in the liver, heart, and kidney (figure) when tissue sections were examined using the Warthin-Starry silver impregnation technique. Although no organisms were identified in the brain, as this technique also heavily stained nerve cells and their processes, a mild leptomeningitis was identified on routinely stained sections. Granular hyaline thrombi were present in the small vessels of the brain, heart, lungs and kidneys. Electron microscopical examination confirmed the platelet composition of these thrombi, while a Martius Scarlet Blue stain showed only minimal amounts of fibrin.

These findings were thought to be indicative of thrombotic thrombocytopenic purpura complicating leptospirosis. The association, which has not been described before, may explain the poor response to treatment. The precipitant for this condition is probably the extensive vessel wall damage caused by leptospirosis,[5] and it therefore seems likely that TTP is a more common complication of leptospirosis than previously supposed. Coagulation disorders are well recognised in leptospirosis, but have until now been described as disseminated intravascular coagulation, rather than TTP.



A renal tubule stained by the Warthin-Starry silver impregnation technique and containing several spirochaetes.

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Implications of delay in culturing for Campylobacter

This district has two general hospitals separated by 30 miles of winding country roads. Most of the microbiology is done at one, and the other has a small laboratory performing a limited range of tests.

We compared the effectiveness of culturing faeces for *Campylobacter* species at the small laboratory or after transport to the larger one. The logistics of specimen transport are such that most specimens are not received until mid-afternoon, too late for transport to the large laboratory. During the study period specimens were processed in the small laboratory, refrigerated overnight (at 4°C), then transported to the large laboratory at ambient temperature. In both laboratories specimens were plated directly on to selective medium (blood agar base (Oxoid) with 10% lysed blood, colistin (8 mg/l), vancomycin (3 mg/l), trimethoprim (5 mg/l)) and incubated

Culture for Campylobacter

Numbers of specimens processed at:	*Small laboratory*		
Large laboratory	*Positive*	*Negative*	*Total*
Positive	93	6	99
Negative	41	3147	3188
Total	134	3153	3287

$\chi^2 = 2084{\cdot}26$ (with Yates' correction).
$p \leqslant 0{\cdot}001$.

squamous lesions, unusual tumours and tumour-like conditions, thyroid lesions in unusual places, cytology and needle biopsy and special techniques. The major emphasis is on histopathological diagnosis, supported by over 160 figures, mostly photomicrographs, over 50 tables and an enormous bibliography: there are 504 references to the chapter on medullary carcinoma alone.

The style is clear and readable. The author points out problems, cites opposing views and gently gives her own interpretation or viewpoint. The text is up to date, comprehensive, and satisfactorily illustrated. The recently described types of clear celled tumour, signet-ring carcinoma, insular carcinoma, hyalinising trabecular adenoma, grooved nuclei in papillary carcinoma, are all there. A photomicrograph not included that would have been helpful is an example of capsular invasion which the author considers diagnostic of malignancy in follicular tumours, even in the absence of vascular invasion or complete capsular transgression. Dr LiVolsi is to be congratulated on this excellent monograph. It should be on the bookshelf in every laboratory responsible for service histopathology.

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Principles of Drug Action. The Basis of Pharmacology. 3rd ed. Ed WB Pratt, P Taylor. (Pp 816; £37.50.) Churchill Livingstone. 1990. ISBN 0-443-08676-1.

The organisation of most pharmacology texts is based on organ systems, classes of drugs, or disease states. In this easily readable but detailed book a novel approach is taken. The biological, chemical, and molecular concepts which are the basis of pharmacology and thus underlie the principles of drug action are presented.

The opening chapters cover the molecular basis of drug specificity, and this is one of the few general pharmacology books to include information on the influence of chirality and stereoselectivity on the interaction between the drug molecule and its site of action. Many examples in which an unusual response to a drug may be precipitated by hereditary factors are given in the chapter on pharmacogenetics. This book also covers the areas of carcinogenesis, mutagenesis, and teratogenesis in addition to drug metabolism, allergy, resistance, tolerance, and physical dependence.

There have been tremendous advances in the understanding of drug action in the 16 years since the last edition of this textbook and, with the advent of new technology that knowledge is still evolving. My major criticism is that textbooks which include state-of-the-art research in a rapidly advancing area will quickly become out of date. It is to be hoped that we do not have to wait another 16 years for the next revision. There is a definite need for a frequently updated text of this kind available at a reasonable price.

In short, this is a book which will help not only the pharmacologist, but also the biological scientist, chemist, and clinician to understand the factors which regulate and determine drug action.

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Advanced Histopathology. GWH Stamp, NA Wright. (Pp 350; soft cover DM60.) Springer. 1990. ISBN 3-540-19589-0.

The arrival of an "Aids to MRCPath" book has been long awaited by histopathology trainees such as myself for whom the final exam looms large on the horizon. *Advanced Histopathology* admirably fills this gap in the market. Contrary to what its title may suggest, this is not a conventional textbook of pathology; rather, it is a trainee's vademecum aimed specifically at how to pass the MRCPath.

The book is divided into sections covering all aspects of the examination including the written paper, post mortem, practical, and viva voce. The largest section of the book (242 pages) is devoted to the written exam. Papers have been reviewed back to 1969 and specimen answers are illustrated. About one quarter of the answers are in the form of essay plans, the remainder as explanatory paragraphs. The post mortem and practical sections are reviewed in slightly less detail, although this is inevitable given the variability of the exam from centre to centre.

The style of the book is informal rather than didactic and I found it very readable. One minor criticism is that for many candidates at this stage of their career, much of the information is superfluous; how many of us need to be reminded to take an extra pen to the exam in case the one we're using runs out? This aside, the book is helpful and informative and will, I believe, help most candidates optimise their approach to this formidable exam.

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NOTICES

Association of Clinical Pathologists

Junior Membership

Junior membership of the Association is available to medical practitioners who have been engaged in the practice of pathology for a period of less than four years. Junior members are able to remain in this category for a maximum of six years or on the attainment of consultant status. The annual subscription is £24 for those resident in the United Kingdom and £55 for those overseas. The annual subscription may be claimed against tax.

Junior members receive the *Journal of Clinical Pathology* each month. Other benefits are reduced registration fees to attend ACP scientific meetings, all the documents regularly sent to full members of the Association including *ACP News*, which has a regular column for juniors, and the twice yearly summary of pathology courses included in the ACP programme of postgraduate education. Junior members have their own representative body, the Junior Members' Group, which has a direct input to Council.

For Junior Membership apply to: The Honorary Secretary, Association of Clinical Pathologists, School of Biological Sciences, Falmer, Brighton, BN1 9QG. (0273) 678435.

ACP Locum Bureau

The Association of Clinical Pathologists runs a locum bureau for consultant pathologists.

Applicants with the MRCPath who would like to do locums and anyone requiring a locum should contact The General Secretary, School of Biological Sciences, Falmer, Brighton, BN1 9QC. Tel and Fax: 0273 678435.

Corrections

We are indebted to Dr Hatchérian of Fresnes for pointing out some errors in units given in the article "Thrombotic thrombocytopenic purpura (TTP) complicating leptospirosis" (1990;**43**:961). The fibrinogen concentration should have been expressed as g/l throughout (not g/dl as it was in one instance), and the bilirubin should have been indicated to be 101 μmol not 101 mmol. The editors try to be vigilant about such things, but slip-ups occur occasionally and we are grateful to the readers who keep us on our toes.

An error appeared in the paper, Importance of sampling method in DNA analysis of lung cancer (1990;**43**:820–3): in the second to last line of the abstract tumour selection should have been printed rather than turnover selection.