

Letters to the Editor

Incidence of cysteine dependent *Escherichia coli* in a general practice population

Cysteine dependent isolates of *Escherichia coli* associated with urinary tract infection are now well recognised.¹ Media supplemented with cysteine have been specially formulated to detect these strains.^{2,3} We undertook a study to determine the incidence of cysteine dependent isolates from patients in a number of general practices in Cardiff. This study was also designed to evaluate the need to use a cysteine supplemented medium in preference to a bile salt medium (such as MacConkey agar) for the processing of specimens from such a population.

One hundred and twenty specimens of mid-stream urine from patients with symptoms suggestive of a urinary tract infection and an appreciable degree of pyuria (white cell count of > 10 per mm^3) were plated on to aerobic blood agar and MacConkey agar and incubated overnight. The urine samples were then refrigerated. The next day, if there was no growth on blood or MacConkey agar and no evidence of previous antibiotic treatment on the request form, the urine sample was plated on to cysteine and lactose electrolyte deficient (CLED) agar and incubated for a further 24 hours. The sample was not cultured for *Mycobacterium tuberculosis* since in this laboratory *M tuberculosis* is rarely isolated in cases of sterile pyuria.⁴ If any colonies of *E coli* appeared on the CLED agar they were subcultured to MacConkey agar to determine whether they were genuine cysteine dependent isolates. Such strains do not grow on this medium and none was obtained from any of the 120 urine samples in this study.

It appears that the incidence of auxotrophic strains of *E coli* is probably low in the population served by our laboratory. This accords well with the observation that these organisms are rarely seen in acute urinary infections, being more commonly implicated in chronic conditions, especially after use of indwelling urinary catheters.⁵ The results of this study also suggest that the use of cysteine supplemented agar media has no particular advantage over MacConkey agar for patients from a general practice population presenting with acute urinary tract infections.

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Factors II, VII, IX, and X concentrations in patients receiving long term treatment with warfarin

We read with interest the paper by Paul *et al* about concentrations of factors II, VII, IX, and X in patients receiving long term treatment with warfarin.¹ We would like to report the results of our study in which we compared activated partial thromboplastin times (APTTs) and British ratios in 50 patients receiving long term warfarin treatment.

The patients selected were in the therapeutic range² and had been so on at least three occasions in the previous six months. British ratios were measured using the Manchester comparative reagent and APTTs using the Manchester APTT reagent. Results showed a good correlation between the APTT and the British ratio ($r = 0.74$).

We then selected the 15 patients with the highest APTTs and measured factor IX concentrations in their stored plasma samples. Two had factor IX concentrations of 1.6 IU/l and 25.0 IU/l; their British ratios were 4.4 and 2.3, respectively.

Thus patients with a well controlled prothrombin time (which is not sensitive to factor IX) may have considerably reduced factor IX concentrations. We agree with Paul and her colleagues that we should not assume that patients receiving long term warfarin treatment have an equal reduction of their vitamin K dependent factors; some may be more depressed than others, despite "good control."

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Microvascular thrombosis of the bowel in paroxysmal nocturnal haemoglobinuria

Paroxysmal nocturnal haemoglobinuria is an acquired disorder in which erythrocytes, granulocytes, and platelets show increased sensitivity to complement. The clinical features of this condition are haemolysis and marrow hypoplasia. A major complication is venous thrombosis, which may affect unusual sites such as hepatic and mesenteric veins.¹ Many patients also suffer from recurrent abdominal pain, and, while intestinal thrombosis has been suspected as the cause,² there is little direct evidence for this. We report the histological findings in a patient with paroxysmal nocturnal haemoglobinuria who successfully underwent resection of thrombosed ischaemic small bowel.

The patient, a 37 year old man, had had hypoplastic anaemia since the age of 11. In 1980 ferrokinetic and chromium studies showed shortened red cell survival as well as poor erythropoiesis, but the result of a Ham test (for paroxysmal nocturnal haemoglobinuria) was negative. He first complained of abdominal pain in 1984. Ultrasound examination showed patent hepatic and portal veins, but endoscopy disclosed pronounced duodenitis. The result of a Ham test was then positive, thus confirming the diagnosis of paroxysmal nocturnal haemoglobinuria. In 1985 he developed severe acute abdominal pain, with guarding and rebound tenderness. Despite the low white cell and platelet counts, emergency laparotomy was successfully performed. The entire small bowel was inflamed, and two lengths of ileum, 30 cm and 40 cm long, were resected.

In the resected ileum there were several lengths (up to 8 cm long) in which the gut wall was thickened due to submucosal oedema and haemorrhage but with no muscular hypertrophy. There was irregular ulceration of the mucosa, varying from

superficial erosion to complete necrosis with dense infiltration of the mucosa and submucosa with polymorphonuclear leucocytes. The size of the necrotic areas varied from a few villi to 1 cm in length. The subserosa was oedematous with increased vascularity and slightly increased fibrous tissue. A striking feature of the necrotic and ulcerated areas was the presence of many thrombi in the small vessels of the mucosa and submucosa (figure). These were mainly in veins and venules and varied in age, some undergoing early reorganisation.

These pathological changes are typically those of ischaemic bowel disease,³ and are similar to those described in three previous cases of paroxysmal nocturnal haemoglobinuria, in which the patient survived resection of thrombosed bowel.^{4,5} The first two patients developed ischaemic necrosis and infarction of the ileum and caecum respectively, and both had extensive venous thrombi of varying ages in the submucosa and subserosa.⁴ The third patient had frank gangrene of several areas of bowel with widespread venous thrombosis.⁵ While it cannot be proved that paroxysmal nocturnal haemoglobinuria was the cause of our patient's bowel thromboses, his age, the exclusion of any other demonstrable vascular lesions, and the presence of paroxysmal nocturnal haemoglobinuria make the association highly probable.

Because early reports of suspected mesenteric thrombosis in paroxysmal nocturnal haemoglobinuria emphasised the high mortality associated with surgery, gut throm-

bosis has been proved during life in only a few patients. The three previous cases and the patient reported here suggest, however, that emergency abdominal surgery may be undertaken as a life saving measure without special preparation.

Unfortunately, bowel thrombosis in paroxysmal nocturnal haemoglobinuria tends to be recurrent. Our patient still experiences pain, despite treatment with warfarin, and a recent barium follow through study showed multiple small bowel strictures, thought to be ischaemic in origin.

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Method for preparing large specimens obtained by disarticulation or amputation for histological examination

Limb disarticulations and amputations for orthopaedic and other reasons produce large specimens which are difficult to manipulate and prepare for histopathological examination. The associated problems became apparent to us recently when we were presented with a left arm disarticulation containing an osteogenic sarcoma arising distally in a pagetic humerus. A pathological fracture had previously occurred proximal to the tumour. This fracture (not due to tumour deposition) had been treated by internal fixation and had healed poorly. Obviously, removal of the plate and screws would result in a frail specimen which would be difficult to dissect properly and from which it would be difficult to recover representative informative blocks of tissue for microscopical study. The particular problem was the need to bisect the limb along its long axis.

Initially, the soft tissues down to bone were removed. These, the resection margins, and the skin were sampled for histological examination. The distal portion of the limb was removed through the distal third of the forearm. The remaining specimen was examined by radiography and photographed. The periphery of the tumour was sampled for histological and electron microscopical examination. The specimen was then fixed in 10% buffered formalin for one week. Next the orthopaedic plate and screws were removed, and the specimen was embedded (lying on one side) in RALwax up to the line of proposed bisection (cocktail sticks were placed on the wax along this line to mark it); embedding was completed by surrounding the exposed surfaces with wax. After preliminary hardening the embedded specimen was placed in a chest freezer for three days. The frozen embedded specimen was bisected along the plane marked by the cocktail sticks with an AEW 250 band saw. Paraffin wax had gained access to the marrow spaces through the screw holes when it



Thrombi in submucosal vessels with oedema and early neutrophil increase: early necrosis present in the mucosa on the right (Masson trichome)