The procedure described is inexpensive, relatively fast, and increases the isolation rate of anaerobes, most of which are found in ulcers. A study is proposed to evaluate the clinical importance of these findings.

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Unexpected expectoration

We would like to draw attention to the unusual appearance of a bronchial cast that first led us to believe a patient had expectorated a portion of lung during the course of a respiratory arrest, (figure).

A 58 year old man was admitted with acute chest pain and had known chronic obstructive airways and coronary artery diseases. Shortly after admission he had a respiratory arrest and during resuscitation a length of reddish brown tissue 14 cm x 1.5 cm x 1 cm was sucked from the back of the throat. It had the consistency and appearance of lung (fig 1a). Microscopical examination showed irregular air spaces with thin walls resembling alveoli (fig 1b). The walls comprised red cells, eosinophils, and acellular eosinophilic strands. To ensure that this was not lung tissue, a reticulin stain was necessary. This confirmed the absence of any permanent structures of the alveolar wall.

We were relieved to realise this was not lung and concluded that this was a bronchial cast “whisked” into its present “pulmonary” form by coughing. It clearly differs from the common descriptions of bronchial casts that show alternating cellular and acellular areas. The former exhibit large numbers of eosinophils, the latter are mostly mucous. Charcot-Leyden crystals and Kurschmann’s spirals are also common in the typical cast. Only one area was eventually found on the current case with laminated mucus strands that bore any resemblance to a typical bronchial cast.

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Non-polio enteroviruses and motor neurone disease

There is considerable interest in the possible viral aetiology of various neurological diseases. The recent observation by one of us (SK) of an apparent cluster of cases of motor neurone disease prompted us to examine the possible role of the group B Coxsackie viruses (CBV). The putative role of CBV in the post-viral fatigue syndrome is currently being evaluated by us using the \( \mu \)-antibody capture ELISA technique for the detection of CBV IgM antibody.\(^1\) Detection of this antibody implies recent or persisting infection. It is well established that this CBV ELISA test can cross react with other non-polio enteroviruses,\(^2\) thus extending its diagnostic scope.

We studied sera from 17 clinically defined cases of motor neurone disease (10 men, seven women, aged between 39–87 years) and 11 patients with other neurological disorders (seven men, four women, aged between 29–64 years). All serum specimens were collected between January and March 1986 (table). There was little difference in the number of patients with motor neurone disease giving a CBV IgM positive response compared with the control group. Using the more conventional CBV neutralising antibody assay, however, there was evidence of a higher proportion with raised (\( \geq 256 \)) antibody titres in patients with motor neurone disease.

In evaluating these data it should be pointed out that CBV was epidemic in the local community during 1985–86; this is reflected by higher background titres in asymptomatic adults assessed by both the CBV IgM and neutralising antibody assays. This may possibly explain the higher titres seen in our cases, but the number of patients studied was small, and the more sensitive CBV IgM assay failed to show a pronounced difference in the two groups studied.

Although the results of this preliminary investigation are inconclusive, we suggest that further epidemiological studies of CBV infection in patients with motor neurone disease are merited. Definitive evidence of the role of any virus in this and other neurological diseases is now feasible using the molecular biological techniques of in situ and Southern blot hybridisation to detect viral nucleic acid sequences in affected neurological tissues.

<table>
<thead>
<tr>
<th>Table</th>
<th>Results of Coxsackie B tests in patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>No (% tested)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>17 (100)</td>
</tr>
<tr>
<td>Controls*</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

*Neutral titres not available in one control as serum finished.

Letters to the Editor

J Clin Pathol: first published as 10.1136/jcp.41.5.596-b on 1 May 1988.
Letters to the Editor

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Cytopathic effects of Campylobacter pylori urease

It is now accepted that there is a close association between Campylobacter pylori and gastroduodenal disease,1 but the precise nature of the association remains unclear, and the potential pathogenic role of the organism requires investigation. Many possible virulence mechanisms merit consideration including direct toxic effects of bacterial products on cells. We investigated the action of bacteria free preparations derived from C pylori on cell cultures and obtained evidence that urease may play an important part in cell damage.

Three isolates of C pylori from gastric antral biopsy specimens from separate patients in this hospital were used. Organisms were grown on 10% blood agar for 48 hours, suspended in phosphate buffered saline, and centrifuged at 7000 g for 20 minutes. The supernatants were filtered using a 0·2 μm filter and applied to established Vero cell monolayers. Cytopathic effects were then observed.3

None of the three preparations produced a clearly discernable cytopathic effect after incubation with cells over 96 hours, but when urea was added to the system (30 mmol/l), the cells rounded up within 90 minutes (table) and subsequently lysed. These effects were accompanied by a pronounced rise in pH. The three C pylori preparations contained urease, and similar cytopathic effects were obtained using Jack bean and Bacillus ureases (Sigma) in the presence of urea. A two-fold dilution series of ammonia added directly to cell monolayers resulted in the same characteristic cytopathic effect at final concentrations of 1·35 mmol/l and above. If the ammonia was pre-neutralised to give a pH of 7·4 the cytopathic effect was retained at concentrations of 2·7 mmol/l and above. Raising the pH using NaOH produced an entirely different cytopathic effect. These findings support the view that the cytopathic effect produced by the C pylori preparations was related to the generation of ammonia by ureolytic activity and that this effect was largely independent of pH.

The cytopathic activity of our preparations withstood a temperature of 56°C for 15 minutes but was abolished at 80°C. Under the conditions used this activity was not affected by the addition of the competitive urease inhibitor thiourea, which, in contrast, did inhibit the cytopathic effect produced by the commercially available purified ureases. The addition of serum from a patient colonised with C pylori and with high titres of circulating antibodies against the organism, determined by ELISA,4 caused a substantial reduction in cytopathic effect titre (table). This serum had no analogous neutralising effect on the two commercially obtained ureases.

Our findings suggest that the urease activity of C pylori can cause cytopathic effects by the production of ammonia. Although other workers have suggested an important role for urease we have shown directly the cytopathic potential of this activity. As we have also shown that concentrations of ammonia as low as 2·7 mmol/l can produce clear cytopathic effect even at physiological pH, it is likely that localized ammonia production by this organism is sufficient to produce cell damage and result in inflammation. We conclude that the ureolytic activity of C pylori may be important in the pathogenesis of gastritis and peptic ulcer.

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References

Table  Titres of cytopathic effects produced by C pylori preparations after 20 hours’ incubation with Vero cells under different conditions

<table>
<thead>
<tr>
<th>Test preparation</th>
<th>Additions</th>
<th>30 mM urea</th>
<th>30 mM urea + serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C pylori 1</td>
<td>Nil</td>
<td>1:384</td>
<td>1:96</td>
</tr>
<tr>
<td>C pylori 2</td>
<td>No cytopathic effect</td>
<td>1:48</td>
<td>1:12</td>
</tr>
<tr>
<td>C pylori 3</td>
<td>Nil</td>
<td>1:384</td>
<td>1:96</td>
</tr>
</tbody>
</table>

*added at 1:32 final dilution.

Other correspondence

Brown fat and sudden death

We were interested by the report of brown fat necrosis found in post-perinatal necropsy specimens by Stephenson and Variend.1 Brown fat is a favoured substrate for various virus infections in newborn mice infected with group B Coxsackie viruses.2 Brown fat necrosis can also be produced in mice infected with some group A Coxsackie viruses.3 Particularly Coxsackie A7 virus which can do so in adult cotton rats.4 It was for this