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Letters to the Editor

Demonstration of bacterial capsular polysaccharide in CSF by counter immunoelectrophoresis

The demonstration of bacterial capsular polysaccharide in cerebrospinal fluid (CSF) by counter immunoelectrophoresis (CIE) has provided a useful addition to the usual diagnostic tests (Greenwood *et al.*, 1971; Coonrod and Rytel, 1972; Higashi *et al.*, 1974; Myhre, 1974), particularly in patients who have been given antibiotics. Unfortunately, with group B meningococci the method often fails because of the poor precipitating activity of commercially available antisera (Tobin and Jones 1972). However, group B meningococci share a capsular antigen with *Escherichia coli* K1 (McCracken, 1976) which can be used as a control to validate the activity of antimeningococcal group B antisera.

Equine group B meningococcal antiserum is available on request from Dr John B. Robbins of the Department of Health and Welfare, USA¹, but, according to the accompanying instructions, the antiserum is unsatisfactory when CIE is carried out using agarose as supporting media. Also, it has been found with rabbit antimeningococcal group B antisera that reaction with *E. coli* K1 antigen may be apparent only when using modified electrolyte systems in CIE (Fallon and McIlmurray, 1976).

I should like to draw the attention of those who use the method that the equine globulin kindly supplied by Dr. Robbins does give precipitation (to titre) with

purified K1 polysaccharide, also provided, when CIE is performed using ready prepared agarose plates (Millipore, UK) in barbitone buffer, pH 8.6, at 12 mA for one hour. Three commercially available antimeningococcal antisera (Wellcome Polyvalent A-D, Wellcome group B, and Difco Polyvalent A-D) did not form a precipitate with the K1 antigen or with a specimen of CSF from a patient with established group B meningococcal meningitis. However, dilutions of up to 1 in 8 of this CSF sample did precipitate with neat horse antiserum provided by Dr Robbins. A sample of neat CSF (but not in dilution) from a patient with established group C meningococcal meningitis cross-reacted with the equine globulin, a finding also observed by Dr Robbins. Therefore, long-term usage will be required to determine the absolute specificity of this equine globulin.

These preliminary findings suggest that the sensitivity of CIE for the detection of group B meningococcal antigen would be improved by using the antiserum provided by Dr. Robbins. However, in neonates CIE alone will not differentiate between *E. coli* K1 and group B meningococcal meningitis.

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Starch serum agar—a differential medium for the isolation of *Corynebacterium vaginale* ('*Haemophilus*' vaginalis)

Recently, many reports have been published on the bacteriological and clinical aspects of infection with *Corynebacterium vaginale*, a Gram-variable rod first isolated from the genitourinary tract by Leopold (1953). There is also increasing evidence that the organism may cause vaginitis and cervicitis in women and urethritis in men (Dunkelberg and Woolvin, 1963; Lewis *et al.*, 1972; Åkerlund and Mårdh, 1974).

Casman's blood agar (Casman, 1947; Dukes and Gardner, 1961) is generally used for its isolation, but colonial distinction from other genitourinary flora is often difficult. Dunkelberg's peptone starch dextrose (PSD) agar (Dunkelberg *et al.*, 1970) gives better results but colony-differentiation on the primary culture plates remains a problem.

A new differential medium, starch serum agar (SS), has been devised and found to give consistent results. Its

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composition is as follows (g/litre): special peptone (Oxoid L72) 23.0; soluble starch 10.0; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.873; Na_2HPO_4 2.044; bromocresol purple 0.030; phenol red 0.024; agar (Oxoid No. 1) 10.0; distilled water to 1 litre. The medium (which is buffered with 0.04 mol/l phosphate buffer pH 7.2) is autoclaved at 15 lb pressure (121°C) for 15 minutes, cooled down to 55°C; 100 ml of sterile inactivated horse serum is added and plates are poured. The combined pH indicator (bromocresol purple and phenol red) distinguishes starch-fermenting *C. vaginale* from non-starch-fermenting genitourinary bacteria and is more satisfactory than bromothymol blue, phenol red or bromocresol purple alone. The cultural characteristics of a type strain of *C. vaginale* (NCTC 10287) on starch serum agar have been compared with those on Dunkelberg's PSD agar. Cultures were incubated at 37°C under three atmospheric conditions—aerobically, in 5% of CO_2 , and anaerobically—and the rate of growth, colonial morphology (under a stereomicroscope), and the colour change (in the SS agar) were noted after 16–20, 48, and 72 h incubation. The anaerobic condition proved to be optimum for growth and after 48 h incubation *C. vaginale* appeared as distinctive yellow, round, convex, 1.2–1.8 mm diameter colonies on starch serum agar, as compared with 0.5–1.5 mm whitish colonies on PSD agar.

Other genitourinary organisms, eg, diphtheroids, Döderlein's bacilli, *Candida*, *Haemophilus* spp, and coliforms, produced pale purple to bluish purple colonies; most of the group B streptococci produced characteristic orange-red colonies; staphylococci and group D streptococci appear to be starch-fermenting. (A detailed report will be published later.)

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- ## Immunoglobulin-bearing cells in giardiasis
- In their paper 'Serum antibodies and jejunal histology in giardiasis associated with malabsorption', Ridley and Ridley (1976) reported their findings on the jejunal lamina propria plasma-cell response to *Giardia* infection. The authors found increased IgM cell fluorescence in the lamina propria, but no increase in IgA or IgG fluorescence, in 10 giardiasis patients with a history of illness of about one month's duration. They concluded that in the early stage of *Giardia* infection, at least, immunoglobulin production by plasma cells was predominantly IgM. We should like to submit our results from a group of giardiasis cases as a corollary to their observations on the plasma-cell response.
- In the present study, biopsies were taken from 125 adult patients referred to the Gastroenterology Unit of the Royal Adelaide Hospital with varying clinical problems including diarrhoea, weight loss, nausea, and vomiting. The tissues were taken from the jejunum just distal to the ligament of Treitz, with a Crosby-Kugler capsule. A diagnosis of giardiasis was made if organisms were identified either in the jejunal aspirate, in a smear made from a fragment of the biopsy, or in paraffin-embedded haematoxylin and eosin preparations of the biopsy (Rowland *et al.*, 1976). Seventeen such cases were diagnosed. The mucosal abnormality was classified from conventionally stained paraffin-embedded tissue, as outlined by Perera *et al.* (1975), into normal, mild villous abnormality, moderate mucosal lesion or severe mucosal lesion.
- Direct immunofluorescence (IF) on the giardiasis patients was done when the biopsies were large enough to allow tissue to be taken without interfering with light microscopic examination. This was possible in 13 cases. In addition to these cases, nine biopsies from patients with functional dyspepsia were chosen at random for counting. Tissues were prepared for IF by immediate fixation in formaldehyde (Savilahti, 1972), except in three instances when the specimens were snap-frozen in liquid nitrogen and sectioned in the cryostat, after which the sections were fixed with 95% acetone. In our experience the latter method was satisfactory for preservation and counting IgA and IgM cells, but it did not permit sufficiently clear definition for reliable counting of IgG cells. Fluorescein isothiocyanate-labelled antisera, as well as unconjugated antisera for appropriate blocking controls, specific for the heavy chain component of IgA, IgM or IgG, were from Hyland Laboratories. Counts for IgA, IgM on IgG cells were made on fluorescein-stained 5–6 μ cryostat sections in a Leitz Ortholux microscope fitted with a Tiyo cardiod condenser, an HB 200 mercury vapour lamp as the light source, and UGI exciting and K430 barrier filters. In each section the total number of specifically stained plasma cells present in those villi which were orientated at right angles to the muscularis mucosae was counted. This included that part of the lamina between the crypts as well as the areas of the cores of the villi. The area of the lamina propria to which the counts were related was measured by projection of adjacent haematoxylin and eosin-stained sections onto a screen of calibrated tracing paper. The projected image was outlined on the paper and the areas were cut out and weighed. Although the minimum area surveyed was 0.2 mm², the area of lamina propria suitable for enumeration was usually much greater; the average of the areas used for these estimates was 0.7 mm².
- The results are summarised in the Table.
- In this survey there was no true 'control' group since it was not thought desirable to perform jejunal biopsy on persons without clinical indication. All of the functional dyspeptic patients had symptoms which had initiated the request for biopsy; their light microscopy was normal, although detailed cell quantitation studies of the lamina propria and