

detected with the iodine stain; but inclusions (albeit smaller than usual) were detected in the coverslips stained with the cell culture confirmation stain.

As a result of these trials we have replaced a traditional cell culture method with the Syva MicroTrak direct smear test for routine screening. The costs of this test may appear prohibitive, but we have found that 10–15 μ l of reagent can be used, if carefully applied to the smear, with no apparent loss of sensitivity. This means that the cost may be reduced to less than £1.00 per test. We believe that this system offers considerable benefits to any laboratory whose technical staff are prepared to familiarise themselves with this technique. We are currently evaluating two further commercial direct smear reagents and hope to report the results in the near future.

RA FRANCIS
AMA ABBAS

Department of Medical Microbiology,
District General Hospital,
Rotherham

this may well have been due to a relative failure to culture, it does raise the general question of the extent to which false positive results might also arise, a question which is likely to have even greater force if monoclonal antibodies are used that are less specific than those of Syva. A spurious epidemic of disease attributed to chlamydiae as a result of false identification is not an idle thought and we have already expressed apprehension concerning this possibility.² Stained smears travel well and it seems to us that thought should be given to the setting up of a central referral system for quality control and for help and guidance on difficult cases. This would go a long way to avoiding a calamity of the kind we mention.

D TAYLOR-ROBINSON
DA HAWKINS
BJ THOMAS

Division of Sexually Transmitted Diseases,
MRC Clinical Research Centre, Harrow,
Middlesex, and the Praed Street Clinic,
St Mary's Hospital, Paddington, London.

Reference

- ¹ Thomas BJ, Evans RT, Hawkins DA, Taylor-Robinson D. Sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of fluorescein labelled monoclonal antibody: comparison with conventional chlamydial isolation. *J Clin Pathol* 1984;37:812–6.

Dr Taylor-Robinson and others reply as follows:

We know that there will be many investigators throughout the UK and elsewhere who find, like Dr Francis and Dr Abbas, that the use of fluorescein conjugated chlamydial monoclonal antibodies is a rapid and sensitive way of detecting chlamydial elementary bodies directly in genital or conjunctival smears. We were and still are confident that what we see in smears are chlamydial elementary bodies not just because of their distinctive appearance but because of the excellent correlation between the results of this direct test and those of culture.¹ We realise that no matter in whose hands, the sensitivity of culture, as Dr Francis and Dr Abbas mention, is not optimal from time to time. Many other factors may also lead to failure to isolate chlamydiae and we note that Dr Francis and Dr Abbas recorded 60% of their positive results by smear alone. While

References

- ¹ Thomas BJ, Evans RT, Hawkins DA, Taylor-Robinson D. Sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of fluorescein labelled monoclonal antibody: comparison with conventional chlamydial isolation. *J Clin Pathol* 1984;37:812–6.
- ² Jones BR, Taylor-Robinson D. Observations on and future trends in chlamydial research. *Br Med Bull* 1983;39:201–3.

Erythrocyte acetylcholinesterase in Hirschsprung's disease

Acetylcholinesterase activity in the aganglionic segment of bowel in Hirschsprung's disease is increased.¹ Boston² and She³ have also found increased erythrocyte acetylcholinesterase activity in Hirschsprung's disease and have suggested that this might be of value diagnostically. We have measured erythrocyte acetylcholinesterase in children with confirmed Hirschsprung's disease and in control patients of similar age.

Patients and methods

Erythrocyte acetylcholinesterase activity was measured in blood from eight children with Hirschsprung's disease (age range 18

days–27 months) and in 53 children without the disease (age range 1 day–36 months). Seven of the patients with Hirschsprung's disease had already undergone colostomy or a definitive procedure, but in no case had more than half of the aganglionic bowel been resected. Thirty two of the controls had gastrointestinal disease, mainly congenital; of these, 18 presented with small or large bowel obstruction. The remaining children had a variety of surgical and medical conditions, but patients with liver disease, haematological disorders, or a history of recent blood transfusion were excluded.

Erythrocyte acetylcholinesterase activity was assayed by Ellman's colorimetric method,⁴ as modified by Lewis.⁶ Heparinised blood (0.5 ml) was centrifuged within 2 h of collection. The red cells were resuspended in a roughly equal volume of saline (9 g/l) and the packed cell volume was measured. The red cells were stored at –20° until assay. Each sample was assayed in triplicate.

Results were analysed by Student's t test.

Results

Initial studies showed that the enzyme was stable in unseparated heparinised blood at room temperature for 6 h. Red cells stored at –20° for one month showed no loss of erythrocyte acetylcholinesterase activity. There was 3.6% and 11.8% loss of activity after two and three months' storage respectively. The between batch coefficient of variation for erythrocyte acetylcholinesterase was 5.5%.

There was no significant difference in erythrocyte acetylcholinesterase activity between controls and patients with Hirschsprung's disease (Fig. 1) ($p > 0.05$).

Erythrocyte acetylcholinesterase activity related to age in both controls and patients is shown in Fig. 2. Erythrocyte acetylcholinesterase activity is 52% of the adult value in the first month of life, and reaches the adult value by the age of 3 months.

Discussion

The diagnosis of Hirschsprung's disease, particularly in neonates, may be difficult, and an additional non-invasive diagnostic test would be of value. Measurement of erythrocyte acetylcholinesterase activity has been proposed as such a test. In this study, erythrocyte acetylcholinesterase activity was measured in controls and patients with Hirschsprung's disease between 0 and 36 months of age. All but two