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 μl 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 that £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.

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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 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.

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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] Boston[2] and She[3] 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,[5] 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) ($p > 0{\cdot}05$).

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

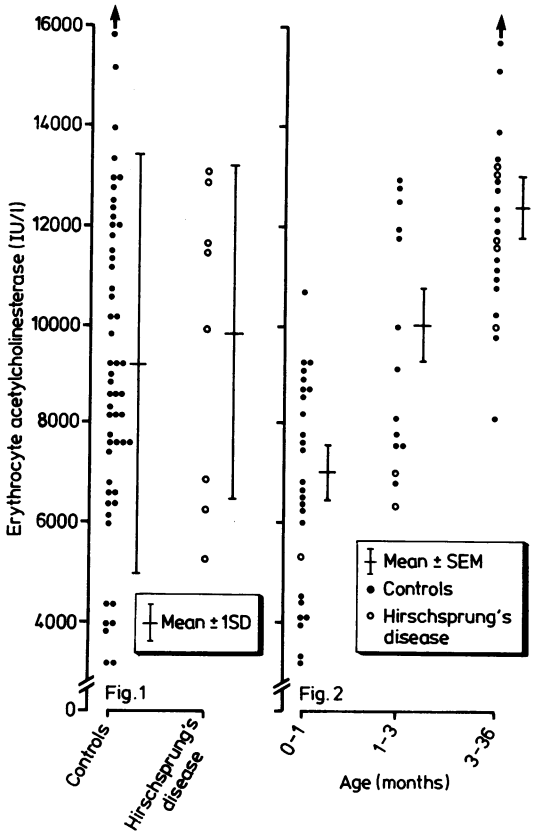

Fig. 1 *Erythrocyte acetylcholinesterase activity in controls and patients with Hirschsprung's disease.*

Fig. 2 *Erythrocyte acetylcholinesterase activity in controls and patients with Hirschsprung's disease as a function of age. Controls: 0–1 month v 3–36 months, p = <0·001: 0–1 month v 1–3 months, p = <0·01; 1–3 months v 3–36 months, p = NS.*

of the control patients under 1 month old had gastrointestinal problems, predominantly congenital in origin. Although many were receiving intravenous infusions and drug treatment at the time of blood collection, we thought that they would constitute a valid control group.

Histochemical staining of the aganglionic bowel in Hirschsprung's disease shows increased amounts of acetylcholinesterase.[1] Boston[2] showed increased activities of both serum and erythrocyte acetylcholinesterase in patients, although there was overlap with the normal range. She[3] also showed increased erythrocyte acetylcholinesterase activity in Hirschsprung's disease, but without increased serum acetylcholinesterase activity. Okasura[4] found normal erythrocyte acetylcholinesterase activity but increased serum acetylcholinesterase activity. In this study, there was no significant difference between erythrocyte acetylcholinesterase activity in controls and patients with Hirschsprung's disease.

Seven of the patients with Hirschsprung's disease had undergone a modified Duhamel procedure, in which part of the aganglionic bowel is resected, but 15–20 cm of aganglionic recto-sigmoid is left. Boston[2] found that erythrocyte acetylcholinesterase activity was still raised in three patients after resection of about half of the aganglionic bowel, although She[3] has suggested that the degree of increase of erythrocyte acetylcholinesterase activity is related to the length of aganglionic segment present. Okasura found that erythrocyte acetylcholinesterase and serum acetylcholinesterase activities were unchanged following Duhamel's procedure. It therefore seems unlikely that resection of part of the aganglionic segment would account for the normal erythrocyte acetylcholinesterase activity in Hirschsprung's disease found in this study.

We have been unable to show that measurement of erythrocyte acetylcholinesterase activity in patients with Hirschsprung's disease under 3 years old would assist in the diagnosis.

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References

[1] Boston VE, Dale G, Riley KWA. Diagnosis of Hirschsprung's disease by quantitative biochemical assay of acetylcholinesterase in rectal tissue. *Lancet* 1975;ii:951–3.
[2] Boston VE, Cywes S, Davies MRQ. Serum and erythrocyte acetylcholinesterase activity in Hirschsprung's disease. *J Paediatr Surg* 1978;**13**:407–10.
[3] She Y, Shi C, Chen J, Wu Y. Observations on erythrocyte acetylcholinesterase in infants and children with Hirschsprung's disease. *J Paediatr Surg* 1984;**19**:281–4.
[4] Okasura T, Okamoto E, Kuwata K, Toyoshima A, Ohashi S, Veko S. Serum and erythrocyte acetylcholinesterase in Hirschsprung's disease. *Z Kinderchir* 1983;**38**:298–300.
[5] Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;**7**:88–95.
[6] Lewis PJ, Lowing RK, Gompertz D. Automated discrete kinetic method for erythrocyte acetylcholinesterase and plasma cholinesterase. *Clin Chem* 1981;**27**:926–9.

Book reviews

Oral Microbiology. 2nd ed. Aspects of Microbiology 1. (Pp 120; paperback £5·50.) Van Nostrand Reinhold (UK) Co Ltd. 1984.

Since an increasing number of hospitalised patients develop severe oral problems due to new forms of treatment, it is important that bacteriologists have some knowledge of oral microbiology. This concise and inexpensive book is an excellent introduction to the subject. The six main chapters contain accurate and up to date information written in a clear style and well illustrated by figures, tables, and line diagrams which, with a few exceptions, enhance the