detected with the iodine stain; but include
sions (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 ml 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

Dr Taylor-Robinson and others reply as fol-
lows:
We know that there will be many inves-
tigators 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 appear-
ance but because of the excellent correla-
tion 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 men-
tion, 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 posi-
tive 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 gui-
dance 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.

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Erythrocyte acetylcholinesterase in
Hirschsprung's disease

Acetylcholinesterase activity in the agan-
glionic segment of bowel in Hirschsprung's
disease is increased.1 Boston2 and She3
have also found increased erythrocyte
acetylcholinesterase activity in Hirsch-
sprung's disease and have suggested that
this might be of value diagnostically. We
have measured erythrocyte acetylcholin-
esterase 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 with-
out the disease (age range 1 day–36
months). Seven of the patients with
Hirschsprung's disease had already under-
gone 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 pre-
sented with small or large bowel obstruc-
tion. 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.5 Heparin-
ised blood (0.5 ml) was centrifuged within 2
h of collection. The red cells were resus-
pered 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
erthrocyte acetylcholinesterase activity.
There was 3.6% and 11.8% loss of activity
after two and three months' storage respec-
tively. The between batch coefficient of
variation for erythrocyte acetylcholinester-
ase 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 acetyl-
cholinesterase 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 be-
tween 0 and 36 months of age. All but two
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. Boston showed increased activities of both serum and erythrocyte acetylcholinesterase in patients, although there was overlap with the normal range. She also showed increased erythrocyte acetylcholinesterase activity in Hirschsprung's disease, but without increased serum acetylcholinesterase activity. Okasura 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 found that erythrocyte acetylcholinesterase activity was still raised in three patients after resection of about half of the aganglionic bowel, although She 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.

FJ BAMFORTH
1 KIN
DM ISHERWOOD
J LISTER
Departments of Biochemistry and Paediatric Surgery
Alder Hey Children's Hospital
Liverpool

References


Book reviews


Since an increasing number of hospitals and 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