Discussion

There are many practical aspects to be considered in the application of this method.

Only vessels which are in true cross section are measured. This is judged by looking for widening of the media at the major axis of elliptical vessels which would suggest an oblique section. Contracted vessels which are non-circular in profile may be measured provided that they are in transverse section. The contour of the elastic lamina must be traced precisely. The degree of resolution of the folding of the elastica depends on magnification and for this reason a high magnification is used for the assessment of small vessels, typically ×200 for vessels of 500 μm external diameter and ×1000 for vessels of 100 μm external diameter (magnifications are final image magnifications). Small muscular vessels with a high area of media in relation to their cross sectional area show the most severe degree of contraction artefact and it is therefore important to resolve the elastica as precisely as possible in order to avoid these errors. Another factor in dealing with small vessels at high magnification is that the width of the elastic lamina becomes significant in comparison to the width of the measuring cursor. Tracing along the outer contour of the elastic lamina gives the most reproducible results on repeated measurements of the same series of vessels. For vessels with no significant intimal thickening the inner contour of the internal elastic lamina is traced as being the intimal layer.

In dealing with vessels showing a diseased intima there is often reduplication of the internal elastic lamina with fragmented elastic lamellae. This is a problem in the application of this technique. Often, however, the original elastic lamina remains as an obviously denser structure with thin lamellae incorporated into the intimal thickening. In these cases the observed dense outer lamella is measured as the original internal elastic lamina. With severe disease of the vessel wall and particularly with the onset of collagenous replacement of intima and media, the dense original elastic lamina may be lost. In this case it is not possible to use this method as there is no reliable index to assess contraction.

In practice the above method has been used to assess the patterns of small vessel disease in limbs amputated for critical ischaemia. It allows the assessment of between 60 and 90 vessels an hour.

This technique is most suitable for the assessment of small muscular arteries and relies on the adequate demonstration of an internal elastic lamina. For large vessels, a sufficiently low magnification which will allow the tracing of the entire vessel will entail poor resolution of the contour of the elastic lamina. The error consequently generated by this poor resolution is offset by the fact that larger vessels have a lower media to lumen ratio and hence do not tend to show large degrees of elastica contraction in relation to the size of their lumen.

Further work is underway to compare results derived by this method with those obtained from direct measurements on vessels fixed in a perfused state at physiological pressures.

References


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Letters to the Editor

Further evaluation of a new filter for leucocyte depletion of blood

Since the evaluation of the leucocyte depleting filter Sepacell R-500 (Asahi Medical Co Ltd, Tokyo, Japan) was reported, the manufacturer has modified the design of the filter by incorporating into the filtration chamber a prefilter for the removal of microaggregates. The purpose of this modification was to reduce the prolonged filtration time seen in some of the original filters. We report the results obtained using the modified Sepacell R-500 filter.

Units of blood were collected into CPDA-1 anticoagulant in single or triple packs (Fenwral FKR 0844 or FKR 1369, Travenol Laboratories Ltd, Thetford, England). Whole blood and plasma reduced blood (11 units of each) were stored at +4°C for two or three days before filtration. The priming of the filter with 0.9% sodium chloride and filtration of the blood was done in accordance with the manufacturer’s instructions. The mean (±SD) filtration time was 8.5 ± 1.8 min and the range from 5 to 12 min. Red cell, leucocyte, and platelet counts were determined on samples taken before and after filtration using a Technicon H-6000 automated flow cytometry cell counter. The efficiency of the filter in removing leucocytes and platelets and the associated red cell loss were expressed as a percentage of absolute numbers of leucocytes, platelets and red cells in the blood before filtration: the mean values (±SD) were 99.1 ± 1.3%, 83.1 ± 7.6%, and 8.9 ± 7.2%, respectively. Non-haemolytic febrile transfusion reactions have not been reported in any of the seven recipients of filtered blood, all of whom had a history of such reactions in the past.

Our tests have shown that the modified Sepacell R-500 filter is highly efficient in removing leucocytes and that it is possible to filter a unit of whole blood or plasma
reduced blood in less than 12 min.

We thank Miss Joan Henthorn in the Department of Haematology, Central Middlesex Hospital, for the automated cell counts and Mr TG Proger of Kimal Scientific Products Ltd for supplying the filters.

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Reference


Acute bacterial conjunctivitis and maltose negative meningococci

Acute bacterial conjunctivitis is usually caused by the pneumococcus or Haemophilus influenzae, but other organisms are sometimes involved and it is important to identify these exactly. Among the less common pathogens are the neisseria, and both Neisseria gonorrhoeae and N meningitidis have been implicated in ophthalmic infections.

N gonorrhoeae is particularly associated with severe destructive ophthalmia in the newborn but may also cause purulent conjunctivitis in adults in whom infections by N meningitidis also occur from time to time.

These two organisms are very similar, but for obvious epidemiological reasons it is important to distinguish between them. Coagglutination tests are available to identify N gonorrhoeae but not for N meningitidis. The differentiation of these two organisms therefore still relies mainly on classic methods of glucose fermentation. In these tests N gonorrhoeae produces acid from glucose only, while N meningitidis produces acid from glucose and maltose; in many laboratories this is still the only way of identifying them. Difficulty comes about because there are some strains of N meningitidis which do not ferment maltose promptly and are therefore likely to be wrongly identified.

Case report

A 12 year old schoolboy attended the casualty department complaining of pain, redness, and stickiness of the right eye for two days. Apart from a recent sore throat he had been well. Examination confirmed purulent conjunctivitis of the right eye and a swab was taken for bacterial culture. He was treated with topical chloramphenicol ointment applied initially to the right eye and later to both eyes as the condition became bilateral the following day. The condition subsided over the next five days and the patient was well at follow up.

 Cultures produced a heavy pure growth of a capneic neisseria, which when tested for sugar reactions (Difco GC medium base) produced acid from glucose only and not from maltose, sucrose, or lactose. These are the characteristic reactions of N gonorrhoeae, but the isolate gave a negative result with the gonococcal coagglutination test (Phadebact). There was therefore some doubt as to the identity of the organism; it was further subcultured and the identification tests repeated. By the third subculture the organism produced acid from maltose, and serotyping confirmed it as a group C strain of N meningitidis which was fully sensitive to penicillin, chloramphenicol, and sulphonamide.

Discussion

N meningitidis is not a common cause of bacterial conjunctivitis. At the Manchester Royal Eye Hospital during the seven year period 1977–83, there have been five patients infected by this organism, compared with 536 pneumococcal and 451 H influenzae infections. The meningococcal infections were all in adult or adolescent patients, in contrast to the other bacterial infections for which the highest incidence was in preschool children. During the same period there have been six cases of neonatal gonococcal infection and one in an adult.

The interpretation of results from conventional tests for identifying pathogenic neisseria must be treated cautiously. There are a number of reports of maltose negative strains of N meningitidis causing meningitis4 5 but not previously from ophthalmic infection. A negative result in the gonococcal coagglutination test and repeated testing of sugar reactions on repeated subculture may be needed to establish the true identity of atypical strains of N meningitidis isolated from unusual sources.

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Antigenic variation in Latin American human pararotaviruses (atypical rotaviruses)

Recently, virus particles morphologically indistinguishable from rotaviruses but which lack the typical group antigen have been described in man and animals. Such viruses have been variously termed pararotavirus1 2 or atypical rotavirus.2 The characterisation of a pararotavirus has recently been described from a child in Mexico City,3 and a further isolate has been found in a child with diarrhoea in Chile (unpublished observations).

We have compared by electron microscopy the antigenic relation of both these human pararotaviruses using the protein A solid phase antibody capture technique. Paired serum samples were available from the child in Mexico City, which have been shown to be free from antibody to rotaviruses. These sera were used in the protein A antibody capture technique against both the Mexican and Chilean pararotaviruses. In addition, human immune globulin prepared in the United Kingdom was tested in a similar fashion. Briefly, carbon-formvar coated grids were floated on a solution of staphylococcal protein A before transferring to a solution of the appropriate antibody.4 The grids were then floated on a suspension of clarified faecal emulsion containing the antigen before staining with 1:5% phosphotungstic acid. The Table shows the results obtained after blind examination of the electron microscope grids.

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Further evaluation of a new filter for leucocyte depletion of blood.

E J Johnson, V Mijovic and B Brozovic

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