Letters

In our experience the study of these enzyme activities and their isoenzyme expression is of great value in the assessment of leukaemic patients with both lymphoid or non-lymphoid involvement, particularly when equivocal results are obtained from other marker studies.

Our observations are supported by similar findings reported from other laboratories.4,5

References


Activities and profiles for β hexosaminidase and α mannosidase in 57 patients with acute non-lymphoid leukaemia

<table>
<thead>
<tr>
<th>No studied</th>
<th>Mean (SD) activities (nmol/min/mg protein)</th>
<th>No of patients showing low β hexosaminidase B peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>β Hexosaminidase</td>
<td>α Mannosidase</td>
<td></td>
</tr>
<tr>
<td>AML*</td>
<td>50.2 (19.4)</td>
<td>11.2 (8.9)</td>
</tr>
<tr>
<td>AMML</td>
<td>94.5 (37.9)</td>
<td>16.4 (14.0)</td>
</tr>
<tr>
<td>AMOL</td>
<td>157 (36.5)</td>
<td>15.1 (4.88)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>25.4 (5.8)</td>
<td>4.0 (1.8)</td>
</tr>
<tr>
<td>ALL</td>
<td>25.4 (15.7)</td>
<td>1.66 (2.20)</td>
</tr>
</tbody>
</table>

*Diagnoses were established by independent cytochemical and immunological markers.
AML—Acute myeloid leukaemia; AMMOL—Acute myelomonocytic leukaemia; AMOL—Acute monocytic leukaemia; ALL—Acute lymphoblastic leukaemia (non-T non-B cell).

Device to collect fibrils of collagen from biopsies of human skin

Pathologists and biologists are often faced with the problem of obtaining cellular or extracellular fractions from small volumes of tissue samples. A percutaneous biopsy obtained with a Vim-Silverman needle has a volume of about 20–40 μl. Moreover, if this sample is shared by two or more laboratories the electron microscopist obtains a microhomogenate of 0.1–0.2 ml.

This scanty volume has to be centrifuged in a narrow lumen tube to separate clearly the fractions; these procedures, however, have several limitations.1,2

Apart from factors such as hydrodynamic effects and friction against the wall of the tube, the principal problem is collection of the fractions. In the standard procedure a suction needle or Pasteur pipette is used from the top to remove the gradient layers. Other procedures include passing a needle through the fractions to the bottom of the tube, where a denser solution is allowed to settle. Clearly, however, in a narrow tube the introduction of a needle will disturb the phases already formed. Another method is to make a hole in the bottom of the centrifuge tube,3 but this does not work at all for microsamples because of the effects of capillarity. We have developed a device that allows the separation and collection of fractions from homogenates of 0.1 ml or less.

We used disposable calibrated glass pipettes graduated from 1 ml to 5 ml. From these pipettes we cut a piece 28 cm in length, which was bent by heating to make a tight U (Fig. 1). We also used plastic tubing of suitable diameter. One arm of the U device was kept 2 cm longer than the other, but the total length of the device should be less than 15 cm to fit into a standard centrifuge tube. The bottom of the device was filled with mercury but 2–5 cm of both arms remained empty. The homogenate was put in the short arm and the U device put into a standard 17 × 150 mm centrifuge tube. Another U device may be used to counterbalance the centrifuge.

After centrifugation one of two methods can be adopted for collecting the fractions according to the degree of accuracy required. In one method the column of mercury in the short arm is raised by gently increasing the pressure in the other arm, using a syringe connected by a short piece of rubber tube. While the piston of the syringe is slowly moved the fractions reach the end of the short arm from where they are picked up with a 400 mesh grid coated with Formvar. If the level of purity required for the fractions is high the entire device should be frozen, so that a solid ice column is formed. This technique prevents the fractions being mixed by friction against the walls of the tube. The iced column can be cut into sections with a razor blade and the iced pieces allowed to thaw. It is advisable to use an ice and salt bath for freezing to prevent the temperature dropping below −4 °C, which is the point at which mercury freezes.

We obtained biopsies of skin from the anterolateral portion of the leg of healthy patients, using a 12–14 gauge sharpened needle. We dissected the epidermis from the dermis and put the dermis in a homogenator with 0.1 ml of 0.2 M sucrose. After four strokes in the homogenator we let the suspension stand for 2–3 minutes to allow the thick portions of the homogenate, which may clog the device after centrifugation, to form a sediment. We put the supernatant in the short arm of the device, which was then centrifuged at 2500 g for 10 minutes. Negative staining was achieved by putting a drop of 1% phosphotungstic acid at pH 8.5 on the grid for 2–5 minutes. Excess liquid was removed with a piece of filter paper, and the grid was allowed to dry before observation with an electron microscope.

Fibrils of collagen from human dermis
were collected in a coherent fraction immediately before the nuclear fraction. Figure 2 shows a negatively stained fibril of collagen displaying clear and dark bands. A neat fraction of collagen fibril was obtained despite the reduced volume: no special device was required to collect it on to the grids of the electron microscope.\(^4\) The slow freezing of the homogenate did not cause distortion of the ultrastructures of the collagen fibrils but produced an undisturbed and distinct layer. Undoubtedly, this device will be useful for isolating cellular fractions whenever the volumes are reduced, and great precision is not required. The main advantage is the cost of the device because it can be made in the laboratory with standard pipettes or plastic tubing without using any special equipment.

Fig. 1 U device comprising disposable 1-0ml glass pipette.

Fig. 2 Isolated fibril of collagen from human dermis with phosphotungstic acid negative staining. × 24 000.

References


Rapid detection of bacteraemia

We read with interest the paper on the rapid detection of bacteraemia by Mr Corkill.\(^1\) His extremely low detection rate of 9-5% at 24 hours using a manual system differs considerably from the average of 71% reported by us,\(^2\) and this rate has subsequently risen to 78% using the same technique. This striking difference may be ascribed to three main aspects of our methods: the consistent use of a biphasic medium containing Columbia agar in parallel with fastidious anaerobe broth; incubation without delay of blood cultures taken after laboratory hours; and blind subculture of both media at 10 pm on the day the specimen was received.

Mr Corkill's detection rate rose from 9-5% to 76-0% with a semiautomated radiometric system (Bactec 460). Our overall rate of 83% for the first 24 hours with Bactec is not significantly higher than our manual rate. With the manual system, however, the isolates were available on solid media 24 hours earlier than with the radiometric system. The radiometric system has some important drawbacks; a further study showed negative radiometric growth indices in the first 24 hours when blind subcultures were positive. The cost is also considerably greater than that of a manual system.\(^3\)

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Mr Corkill replies as follows:

Our detection rate of bacteraemia at 9-5% with traditional broth systems (preincubated for up to 24 hours and blindly subcultured once a day) highlights the delay occasioned by standard techniques for blood culture.\(^1\) Routine application of a biphasic system over 12 months more than doubled our detection rate at 24 hours, even with a single daily inspection. We decided to discontinue this system, however, because of recurrent contamination related to the manufacturer and a lack of support for pneumococci. If we had inspected the biphasic bottles more frequently in an extended working day our detection rate could probably have reached the level subsequently obtained using Bac-
Device to collect fibrils of collagen from biopsies of human skin.

H Nunez-Duran and U Gelos

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