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Letters

Measurements for haemoglobin

During the meeting of the International Committee for Standardisation in Haematology (ICSH) secretariat it was noted that an increasing number of papers published in both monographs and journals are now using the World Health Organisation International Committee for Standardisation in Haematology/International Society of Haematology recommendation to express haemoglobin as gram/litre (g/l). Furthermore, haemoglobinometers, which use this scale, are being introduced.

The ICSH secretariat would like to point out the need for a rationalisation of the expression of haemoglobin results that would conform with the principles of SI units, and which should be encouraged as quickly as possible.

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(Will authors please note. Editor)

Convulsions and encephalopathy in a patient with leukaemia after treatment with metronidazole

Metronidazole is an antimicrobial agent commonly used to treat anaerobic bacterial infections. We consider that the side effect of convulsions resulting from treatment with metronidazole¹ may often be unrecognised, particularly as a consequence of cumulative exposure^{2,3} in a patient receiving many other drugs and suffering many complications of treatment. We describe a patient with acute myeloid leukaemia, who developed epileptiform seizures, encephalopathy, and sensory neuropathy after treatment with metronidazole.

A 43 year old woman presented with acute myeloid leukaemia (FAB classification M4) in December 1984. She was treated with Cytarabine 100 mg/m² intravenously for seven days and daunorubicin 50 mg/m² for three days.

During the course of chemotherapy she developed a fever, but no causative organism was identified. Metronidazole (a total of 18.5 g) was given intravenously over the next 12 days in combination with a penicillin and gentamicin. The induction treatment was repeated on 17 December for a further week. Ten days later she became feverish again and *Staphylococcus epidermidis* was isolated from blood cultures. Treatment with fucidin and netilmicin was started and there was an initial improvement, although the temperature never settled. Metronidazole (a total of 10 g) was added with the onset of diarrhoea because pseudomembranous colitis was suspected. A few days later the patient became acutely unwell, had rigors, and the parenteral antibiotics were changed to erythromycin, rifampicin, and ceftazidime. There was dramatic improvement and after five days all antibiotics were stopped.

The patient was clinically well, without fever, and ready for discharge when she was found on the floor having a grand mal fit. Over the next 24 hours she had several more fits despite treatment with chlor-methiazole and phenytoin. A computed tomographic scan showed no focal lesion. An electroencephalogram showed encephalopathy. The urea and electrolyte concentrations sampled 30 minutes before the first fit were normal. The platelet count had not fallen below $60 \times 10^9/l$.

The patient made a recovery to normal within 48 hours, but during this period she suffered some retention of urine. A repeat electroencephalogram four weeks later showed moderate abnormality. At this time the patient was in complete remission awaiting consolidation of treatment.

The patient received a total of 29 g metronidazole and developed epilepsy, encephalopathy, and, probably, sensory neuropathy manifested by retention of urine. Acute encephalopathy and epilepsy secondary to metronidazole probably resulted from a high cumulative dose.³ It is known that the drug crosses the blood brain barrier; in dogs it has been shown that high doses of metronidazole induce Purkinje cell lesions.⁴ It is especially important to be aware of this side effect in the leukaemic patient to avoid unnecessary invasive investigations in the search for

hidden leukaemic deposits or foci of infection.

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Enzyme markers in acute non-lymphoid leukaemia

We read with interest the recent review in the journal by Dr Drexler *et al.*¹ It is stated that "the main group AML did not show one distinct enzyme marker phenotype". While this may be true for the purine salvage enzymes, we would like to draw attention to the use of certain lysosomal enzymes in the diagnosis and classification of acute myeloid leukaemias in both adults and children.³ We have now studied 57 patients with acute non-lymphoid leukaemia and have measured the activities and isoenzyme profiles for the enzymes β hexosaminidase and α mannosidase. A summary of our results is shown in the table. Patients with AML, AMOL, and AMOL showed significant increases in these enzyme activities when compared with peripheral blood granulocytes of leukaemic cells of lymphoid origin. Moreover, in patients with AML in particular there was a reduction in β hexosaminidase B isoenzyme peak when compared with the A component.

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}

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

	No studied	Mean (SD) activities (nmol/min/mg protein)		No of patients showing low β hexosaminidase B peak
		β Hexosaminidase	α Mannosidase	
AML*	29	50.2 (19.4)	11.2 (8.9)	27/29
AMMOL	19	94.5 (37.9)	16.4 (14.0)	13/19
AMOL	9	157 (36.5)	15.1 (4.88)	3/9
Granulocytes	10	25.4 (5.8)	4.6 (1.8)	
ALL	12	25.4 (15.7)	1.66 (2.20)	

*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 μ l. 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 practical 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 close 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 \times 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 iced 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 resharpened needle. We dissected the epidermis from the dermis and put the dermis in a homogenator with 0.1 ml of 0.2M 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