Technical methods

New micro method for deoxyuridine suppression test

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The deoxyuridine suppression (dU) test measures the effect of deoxyuridine in suppressing the uptake of radioactively labelled tritiated thymidine into DNA and is specifically abnormal in vitamin B₁₂ or folate deficiency, or when the metabolism of these is disturbed. The test is based on the observation that bone marrow cells from patients deficient in vitamin B₁₂ incorporated much more thymidine into DNA after prior exposure to deoxyuridine in vitro than normal cells.¹ This is also true for folate deficient cells and can be corrected by incubation in vitro with either vitamin B₁₂ or folate, as appropriate.²

The deoxyuridine suppression test has proved valuable in the diagnosis of megaloblastic anaemia. Abnormal results are specifically found only in disorders of vitamin B₁₂ or folate metabolism, and in most cases the addition of vitamin B₁₂ and folic acid in vitro gives a clear distinction between vitamin B₁₂ and folate deficiency,³⁻⁵ and results relate well to the severity of the haematological changes and results of microbiological assays of vitamin B₁₂ and folate.⁵ Examples of its contribution as an analytical method include showing that the effect of nitrous oxide is to inactivate intracellular vitamin B₁₂.⁶

The micro method of the deoxyuridine suppression test was developed to reduce the amount of bone marrow and reagents required for each individual marrow culture by a factor of 10: to simplify the preparation of the cells for scintillation counting; and to permit more exhaustive studies with limited material.

Material and methods

Samples from 16 patients with vitamin B₁₂ deficiency and eight patients with folate deficiency were analysed. The criteria for the diagnosis were: megaloblastic bone marrows; appropriate results of vitamin B₁₂ and folate assays; response to treatment with either vitamin B₁₂ or folate alone; and for vitamin

B₁₂ deficiency, confirmation of the cause of the deficiency by radioactive vitamin B₁₂ absorption studies using a whole body counter.

Samples from five haematologically normal patients were tested. These marrow samples were taken as part of assessment for other reasons. In these patients assays of vitamin B₁₂ and folate were normal and the marrows were normoblastic.

ASSAYS

Serum B₁₂ was measured by microbiological assay using *Euglena gracilis*;⁷ serum and red cell folate were measured with *L casei*.⁸

DEOXYURIDINE SUPPRESSION TEST

For the deoxyuridine suppression test freshly aspirated bone marrow was added to Hanks's solution containing preservative free heparin. A single cell suspension was obtained by forcing the aspirated marrow through a 21 gauge needle once and a 25 gauge needle twice. The suspension was centrifuged and the buffy coat resuspended in Hanks's solution sufficient for the standard method. Two to three ml was reserved for the micro method. A sufficient quantity of cells was obtained for the study if about 2 ml of bone marrow was aspirated after the diagnostic sample into a separate syringe containing a small amount of preservative free heparin. The concentration of nucleated cells in the buffy coat for successful testing was between 1.5 and 20 × 10⁹/l in a final volume of 20-25 ml. A much smaller volume would be required for the micro method.

In the standard method, a modification of the method of Wickramasinghe and Longland,³ additions to 1.0 ml of marrow suspension were: autologous serum 0.5 ml; 0.2 ml deoxyuridine 1.0 μM/ml in 0.15 M saline (except to the control tubes); and to additional sets of tubes either 0.1 ml cyanocobalamin 40 μg/ml in 0.15 M saline or 0.1 ml pteroylglutamic acid (Sigma) 400 μg/ml in 0.15 M saline. It has been suggested⁹ that separate controls with cyanocobalamin and no deoxyuridine and pteroylglutamic acid and no deoxyuridine should be set up, but in our experience this has not proved necessary. In the micro method the volume of marrow cultures and reagents were scaled down by a factor of 10 but the concentration of solutions was identical in both procedures. For the standard method marrow culture was set up in duplicate and for the micro method in quadruplet. Equipment included the Titertek multiple cell harvester (Flow Laboratories Ltd), multiwell plates (volume 250 μl), and a Titertek plate shaker (Flow Laboratories).

Table Comparison of standard and micro methods

	Standard method			Micro method		
	+dU	+B ₁₂	+PGA	+dU	+B ₁₂	+PGA
B ₁₂ deficiency (n = 16) mean (SD)	20.9 (8.7)	14.0 (6.0)	10.7 (4.8)	18.4 (11.7)	13.0 (8.8)	10.1 (7.2)
Folate deficiency (n = 8) mean (SD)	21.1 (11.4)	18.7 (10.4)	8.2 (4.1)	24.4 (18.1)	21.0 (16.0)	11.6 (6.0)
Controls (n = 5) mean (SD)	2.7 (0.5)	2.1 (0.2)	1.7 (0.3)	2.2 (0.7)	2.6 (1.2)	2.1 (0.7)

dU = deoxyuridine; B₁₂ = cyanocobalamin; PGA = pteroylglutamic acid.

The marrow cultures were incubated at 37°C. In the micro method settling of the cells was prevented by shaking on a plate shaker. At one hour 0.1 ml (10 µl in the micro method) of 5 µCi/ml ³H-thymidine in 0.15 M saline was added to each tube or well and incubation was continued for a further hour.

After incubation the marrow cells in the standard method were resuspended in ice cold 0.15 M saline and then washed three times in 0.15 M saline. Following this 0.1 ml aliquots were transferred to filter paper discs (two for each tube). In the micro method the marrow cells were transferred to a specially made glass fibre filter mat using the cell harvester. The micro method differed from the standard method at this point in that all the marrow cells were transferred to the filter mat in contrast to the transfer of 100 µl aliquots to filter paper discs in the standard method. They were washed with a continuous flow of 0.15 M sodium chloride from a reservoir drawn by vacuum and subsequently counted.

Preparation for counting was the same for both methods. The cells were dried, immersed in 10% trichloroacetic acid for 20 minutes, and then washed in two changes of methanol for 10 minutes each time. After a final rinse in acetone the discs were dried and counted in 5 ml of scintillation fluid. Results were expressed as uptake of ³H-thymidine and as a percentage of that in the control tubes.

Results

The table compares the results of the two methods in patients with vitamin B₁₂ deficiency, folate deficiency, and in normal controls. For each patient the result for each part of the test is the mean of four separate counts. The coefficient of variation for these four counts for each value was 10% in both methods.

There was good agreement between the two methods, and the degree of correction with vitamin B₁₂ or folate in vitro was similar so that the interpretation of the test was the same in each case.

Discussion

The micro method for the deoxyuridine suppression test proved valid and convenient. It gives results that agree well with the standard method using larger quantities. Problems were encountered only with samples of marrow containing few cells. The micro method has obvious advantages in using less material

and so allowing other studies to be performed on a diagnostic bone marrow sample.

Interpretation of the results as abnormal and in distinguishing between B₁₂ and folate deficiency was as satisfactory using either method.

A miniature method for the deoxyuridine suppression test has been described before, using cultured peripheral blood lymphocytes.¹⁰ The incubation period for this method is several days, invalidating much of the value of the deoxyuridine suppression test, which is that a rapid result can be obtained.

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