Assessment of Deoxyuridine Suppression Test in Diagnosis of Vitamin B_{12} or Folate Deficiency

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Summary

Deoxyuridine (dU) suppression tests have been performed on virtually all marrow samples aspirated at this hospital over the past 12 months. Of the 110 samples studied 26 gave abnormal results, and these 26 samples came from patients deficient in either vitamin B_{12} or folate. The dU suppression test was found to be of particular value in the diagnosis of vitamin B_{12} or folate deficiency in non-anaemic patients with macrocytosis and equivocal changes in marrow morphology and in patients in whom the serum vitamin B_{12} or red cell folate levels were within the normal range.

Introduction

There is as yet no completely objective way of defining megaloblastic change. Consequently the diagnosis of mild megaloblastic changes in the bone marrow is sometimes difficult and controversial. With the availability of fully automated blood counting equipment such as the Coulter counter (model S) an increasing number of non-anaemic patients are being found to have a high mean corpuscular volume and as a result the frequency with which the above dilemma is encountered has increased. We have therefore investigated the possibility that the deoxyuridine (dU) suppression test (Metz *et al.*, 1968; Herbert *et al.*, 1973) might provide a rapid, reliable, and objective biochemical test for the detection of megaloblastosis due to vitamin B_{12} or folate deficiency. This test is based on the fact that the preincubation of normal bone marrow with an appropriate concentration of dU severely suppresses the subsequent incorporation of tritiated thymidine (^{3}H-TdR) into DNA and that this suppression is subnormal in patients with vitamin B_{12} or folate deficiency (Killmann, 1964; Metz *et al.*, 1968; Herbert *et al.*, 1973). The biochemical basis of this phenomenon was discussed by Metz *et al.* (1968). Though dU suppression tests have been performed on small groups of patients with a few selected abnormalities (Waxman and Herbert 1969 a, b; Corcino *et al.*, 1970; Van der Weyden *et al.*, 1972; Koutts *et al.*, 1973) there has been no study of the specificity and usefulness of this test in the diagnosis of vitamin B_{12} or folate deficiency when performed as a routine procedure.

We report the results of dU suppression tests performed on 110 unselected marrow samples aspirated over the past 12 months. In addition we report preliminary results of studies undertaken to determine whether the test could be modified to distinguish between folate and vitamin B_{12} deficiency in ways other than those suggested previously Metz *et al.*, 1968; Herbert *et al.*, 1973; Van der Weyden *et al.*, 1973).

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Materials and Methods

A total of 110 marrow aspirates from patients with a wide variety of diseases were studied. The essential features of these patients are summarized in tables I and II. Of the 17 patients with malignant disease (table I) three had acute myeloblastic leukaemia, two subacute erythraemic myelosis, one chronic myeloid leukaemia, seven carcinoma, and four lymphoma. The 15 patients with drug-induced megaloblastosis (table I) consisted of two patients on mercaptopurine, one on thioguanine, one on cyclophosphamide, three on a combination of cyclophosphamide and azathioprine, and eight on azathioprine. Apart from those with drug-induced megaloblastic change and subacute erythraemic myelosis the patients listed in table I were considered to show normoblastic erythropoiesis. All the patients with high mean corpuscular volumes included in table I were subsequently found to have normal serum vitamin B_{12} and red cell folate levels. Except for one patient with a combination of vitamin B_{12} and iron deficiency and two others with a combination of folate and iron deficiency, all three of whom had normoblasts and giant metamyelocytes in their bone marrow, the patients in table II showed typical megaloblastic erythropoiesis. The final diagnosis in the patients listed in table II was arrived at on the basis of their clinical features and the results of standard laboratory investigations such as the microbiological assay of serum vitamin B_{12} and red cell folate levels and Schilling tests. A patient was considered to be iron deficient if there was no stainable iron in his marrow fragments.

TABLE I—*Clinical Conditions in which Marrow Aspirates gave Normal Results with dU Suppression Test*

Clinical State	No. of Cases Studied	Haemoglobin (g/100 ml)	Mean Corpuscular Volume (fl*)	dU Suppression† (%)	
				Range	Mean
Haematologically normal	10	11·5-18·0	82-96	2·1-9·0	6·6
Iron deficiency	12	6·4-10·5	54-80	2·2-8·6	6·2
Macrocytosis due to alcoholism or chronic liver disease	16	9·0-18·1	99-127	3·5-9·8	5·9
Malignant disease	17	7·5-13·7	79-97	4·4-10·2	7·1
Drug-induced megaloblastosis	15	9·4-15·4	100-129	3·5-9·2	5·8
Other‡	14	7·1-19·0	78-112	2·0-10·1	6·1

*Femtolitres.
†Uptake of ^{3}H-TdR after preincubation with dU expressed as percentage of uptake without preincubation with dU.
‡Includes 2 patients with rheumatoid arthritis, 3 with infection, 2 with autoimmune haemolytic anaemia, and 1 patient each with uraemia, systemic lupus erythematosus, idiopathic thrombocytopenic purpura, hypothyroidism, Sheehan's syndrome, polycythaemia vera, and congenital thrombocytopenia.

TABLE II—*Clinical Conditions in which Marrow Aspirates gave Abnormal Results with dU Suppression Test*

Clinical State	No. of Cases Studied	Haemoglobin (g/100 ml)	Mean Corpuscular Volume (fl*)	dU Suppression (%)	
				Range	Mean
B_{12} deficiency	11	5·2-15·2	104-133	17·7-54·0	29·8
Folate deficiency	3	4·2-11·0	107-130	21·1-50·5	41·6
Iron and B_{12} or folate deficiency:	6				
Untreated		3·9-13·5	74-113	14·0-36·1	26·0
After oral iron therapy		5·6-13·7	85-110	24·6-48·8	36·0

*Femtolitres.

Suppression tests with dU were performed using a modification of the method described by Metz *et al.* (1968). Freshly aspirated bone marrow was mixed with Hanks's solution containing preservative-free heparin. The marrow aspirate was forced through a 21-gauge needle once and a 25-gauge needle twice and the resulting cell suspension was centrifuged at 1,100 g for five minutes. The buffy coat was separated and resuspended in enough Hanks's solution to produce a cell suspension containing between 5,000 and 10,000 nucleated cells/mm^3. To 1 ml of the marrow cell suspension was added 0·5 ml autologous serum, 0·2 ml Hanks's solution, and 0·2 ml Hanks's solution either alone or containing 1 μmol 2-deoxyuridine/ml. In this way pairs of marrow cultures, one with and one without dU, were set up either in duplicate or in triplicate depending on the number of marrow cells aspirated. These cultures were incubated in a shaking water bath at 37°C for one hour before the addition of 0·1 ml of a solution containing 5 μCi ^{3}H-TdR/ml (specific activity 20,000-30,000 mCi/mmol). The cultures were then incubated for a further period of one hour, after which the cells were washed three times in ice-cold 0·9% NaCl and resuspended in about 2 ml 0·9% NaCl.

The concentration of nucleated cells in each washed cell suspension was determined with a Coulter counter (model S), and two 0·1-ml aliquots of each suspension were delivered on to individual Whatman filter discs (1·9-cm diameter, 3-mm grade). The discs were dried overnight, immersed in ice-cold 10% trichloroacetic acid for 20 minutes, and then washed in two changes of absolute methanol (10 minutes each). After a brief rinse in acetone the discs were dried and counted in 4 ml of standard scintillation fluid using a Packard Tricarb scintillation counter. The amount of ^{3}H-TdR incorporated into the DNA of 10^3 nucleated marrow cells was calculated from the average counts per minute per disc and the total number of nucleated cells on each disc. The uptake of ^{3}H-TdR after preincubation with dU was expressed as a percentage of the uptake without preincubation with dU, and this percentage is called the "dU-suppressed value."

The effect of adding small concentrations of pteroylglutamic acid, together with the dU, on the dU-induced suppression of ^{3}H-TdR uptake was studied in three vitamin-B_{12}-deficient and two folate-deficient patients. The concentrations of pteroylglutamic acid used were 1 μg, 100 ng, and 50 ng/ml of marrow culture.

Results

The effect of preincubating marrow cultures with dU on the subsequent incorporation of ^{3}H-TdR is shown in tables I and II. In the case of marrow samples from haematologically normal subjects dU suppressed the uptake of ^{3}H-TdR to less than 10% of the uptake by control cultures which were not incubated with dU. A subnormal suppression of ^{3}H-TdR uptake was recorded only in patients with vitamin B_{12} or folate deficiency (table II). All six patients with a combination of iron deficiency and vitamin B_{12} or folate deficiency also showed abnormal dU suppression, and this abnormality was worsened when the test was repeated after iron therapy for one to four weeks.

Whereas the abnormality in the dU suppression shown by marrow cultures prepared from two folate-deficient patients was partially corrected by the addition of 50 ng to 1 μg pteroylglutamic acid per ml of marrow culture, these concentrations of pteroylglutamic acid had no effect on the subnormal dU suppression shown by cultures prepared from three vitamin-B_{12}-deficient patients. In the two folate-deficient patients the average dU-suppressed value was corrected from 40·6% to 20·6%, 26·9%, and 30·0% by the addition of 1 μg, 100 ng, and 50 ng respectively of pteroylglutamic per ml of marrow culture.

Discussion

In this study abnormal dU suppression was recorded only in patients with vitamin B_{12} or folate deficiency. Though the coexistence of iron deficiency partially corrected the abnormality of the dU suppression test, the result was clearly abnormal in the six patients with a combination of iron and folate or vitamin B_{12} deficiency. Nevertheless, there appears to be at least a theoretical possibility that the dU suppression test may give a normal result in some iron-deficient patients with a mild degree of vitamin B_{12} or folate deficiency.

Previous workers have reported abnormal dU suppression in patients treated with 5-fluorouracil (Waxman and Herbert, 1969 a) and with the dihydrofolate reductase inhibitors methotrexate (Metz *et al.*, 1968), pyrimethamine (Waxman and Herbert, 1969 a), triamterene (Corcino *et al.*, 1970), and trimethoprim (Herbert *et al.*, 1973; Koutts *et al.*, 1973). Apart from these few situations an abnormal dU suppression test result appears to be specific for vitamin B_{12} or folate deficiency. Our study indicates that patients with megaloblastic change induced by cyclophosphamide and a variety of antipurine drugs do not show abnormal dU suppression and it also confirms a previous report (Waxman and Herbert, 1969 b) that patients with megaloblastic change due to subacute erythraemic myelosis give normal dU suppression test results.

The dU suppression test was of most value in the rapid diagnosis of vitamin B_{12} or folate deficiency in patients with a macrocytosis, a normal haemoglobin level, and equivocal changes in marrow morphology. Furthermore, in two of the patients with a combined folate and iron deficiency and normoblastic erythropoiesis the abnormal dU suppression test result was initially responsible for drawing attention to the coexistence of the folate deficiency.

The fact that vitamin B_{12} or folate deficiency may occasionally occur when the serum vitamin B_{12} and red cell folate levels are within the normal range is now well documented (Chanarin, 1969). In the present investigation one patient with pernicious anaemia had a normal serum vitamin B_{12} level of 170 pg/ml and another patient with folate deficiency due to malabsorption had a normal red cell folate level of 140 ng/ml. The dU-suppressed values, however, were abnormal in both these patients, being 27·4% and 50·5% respectively. In the vitamin-B_{12}-deficient patients who were not iron deficient there was no correlation between serum vitamin B_{12} levels and either the result of the dU suppression test ($r=0·31$, $P>0·4$) or the circulating red cell count ($r=-0·2$, $P>0·6$). By contrast a statistically significant inverse relationship was observed between the dU-suppressed values and the circulating red cell count in the entire group of vitamin B_{12}- or folate-deficient patients who were not deficient in iron ($r=-0·65$, $P<0·02$).

It has already been shown that the abnormality in dU suppression shown by vitamin-B_{12}-deficient marrows can be partly or completely corrected if vitamin B_{12} (1 μg/ml) is added together with the dU during the performance of the dU suppression test. The abnormality is completely corrected by the addition of methylcobalamin and is partially corrected by the addition of deoxyadenosylcobalamin, cyanocobalamin, or hydroxycobalamin (Van der Weyden *et al.*, 1973). By contrast these forms of vitamin B_{12} do not correct the abnormal dU suppression shown by folate-deficient marrows. It is therefore evident that this difference in the behaviour of vitamin-B_{12}-deficient and folate-deficient marrows can be employed to make the dU suppression test distinguish between these two deficiency states. The preliminary data reported in this paper indicate that the addition of 50 ng to 1 μg pteroylglutamic acid per ml of marrow culture partially corrects the abnormal dU suppression shown by folate-deficient marrows but does not correct the abnormal suppression shown by vitamin-B_{12}-deficient marrows. If this observation is confirmed in a larger group of patients it may provide an alternative method of using the dU suppression test to distinguish

between these two deficiency states. The concentration of pteroylglutamic acid used is critical, as it is known that the addition of 50 μg per ml of marrow culture completely corrects the abnormality in both deficiency states (Metz *et al.*, 1968).

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Iatrogenic Osteomalacia and Myopathy due to Phosphate Depletion

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Summary

In a patient receiving regular dialysis prolonged hypophosphataemia due to aluminium hydroxide therapy resulted in osteomalacia and severe proximal myopathy. Both osteomalacia and myopathy responded to correction of hypophosphataemia without vitamin D therapy.

Introduction

Both oesteomalacia and the proximal myopathy with which it is regularly associated respond to treatment with vitamin D, and the myopathy is presumed to be a manifestation of vitamin D deficiency or of resistance to its action. We describe a patient receiving regular dialysis treatment in whom prolonged hypophosphataemia due to aluminium hydroxide therapy resulted in osteomalacia and severe proximal myopathy. It is of particular interest that both osteomalacia and myopathy responded to correction of hypophosphataemia without vitamin D therapy.

Case Report

A 26-year-old chromatin positive woman with multiple congenital defects (congenital absence of vagina and uterus, cervicothoracic spinal fusion, webbing of the neck, and a left Sprengel shoulder deformity) began regular dialysis treatment in October 1969. Six months previously she had presented with uraemia, and an intravenous pyelogram showed a very small left kidney and a small right kidney with a bifid renal pelvis. There was no obstruction and the appearances were consistent with congenital renal dysplasia. She received initially two 14-hour single pass haemodialyses a week using a Kiil dialyser and from December 1970 three 10-hour dialyses a week at home. Her diet contained about 500 mg calcium and 180 IU vitamin D daily throughout. Dialysate calcium and magnesium concentrations were 3·3 mEq/l. and 1·0 mEq/l. respectively. The dialysate contained no phosphate.

A skeletal survey in October 1969 showed no radiological evidence of osteomalacia or hyperparathyroidism. There was no clinical evidence of myopathy or neuropathy. Motor nerve conduction velocity in the right lateral popliteal nerve was normal and remained so throughout. Serum calcium was 9·6 mg/100 ml, phosphate 7·0 mg/100 ml, and alkaline phosphatase was normal at 48 IU. Because of the hyperphosphataemia, she was treated with aluminium hydroxide, two tablets three times daily (2·25 g). Vitamin D and calcium supplements were not given.

In August 1971 she developed painful feet. *X*-ray examination of the feet showed them to be normal and a full radiological skeletal survey showed no evidence of osteodystrophy (fig. 1). Shortly after she developed pain in the legs, arms, and shoulder girdle together with difficulty in climbing stairs due to weakness in the legs. When next seen in February 1972 marked proximal muscle weakness in the legs was present, and she was unable to rise unaided from the sitting position. Distal muscle power in the legs was normal. Tendon reflexes were brisk, and no sensory loss was present. Skeletal survey in March 1972 showed Looser's zones in both superior pubic rami (fig. 3). A full-thickness iliac crest biopsy specimen showed abundant bone tissue and both excess osteoid and resorption with fibrous replacement of bone. The bone surface counts showed 75% occupied by osteoid (normal < 30%) and 25% by the resorption (normal < 25%). Electromyography of the right quadriceps muscles showed no abnormal insertion activity and no spontaneous electrical activity. Individual motor units

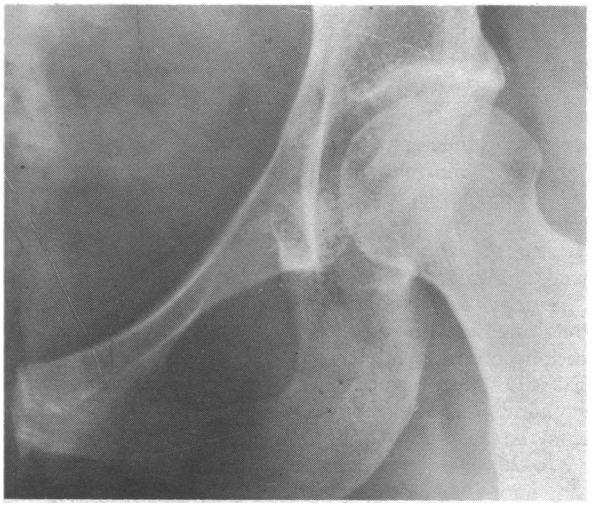

FIG. 1—*X*-ray picture of pelvis in August 1971.

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