Effect of Folate and Cobalamin Compounds on the Deoxyuridine Suppression Test in Vitamin B₁₂ and Folate Deficiency

By Jacqueline Zittoun, Jeannine Marquet, and Robert Zittoun

In vitro, deoxyuridine (dU) failed to block the incorporation of ³H-thymidine into bone marrow from 11 cases of vitamin B₁₂ deficiency, 6 cases of folate deficiency, and 3 cases of combined deficiency, even in patients without overt anemia, macrocytosis, and/or megaloblastosis. Different folate and cobalamin compounds had different effects on the incorporation of ³H-thymidine in the two deficiencies. In folate deficiency, the suppressive effect of dU became normal with all the folate forms tested (folic acid, formyltetrahydrofolate, and methyltetrahydrofolate), while the various cobalamins, cyanocobalamin, hydroxocobalamin, and deoxyadenosyl cobalamin, failed to correct the abnormality. In vita-

min B₁₂ deficiency, methyltetrahydrofolate had little or no effect, folic acid had a partial effect, and formyltetrahydrofolate almost totally corrected the defect. The defective blocking effect of dU was very poorly corrected by deoxyadenosylcobalamin, partially corrected by cyanocobalamin, and corrected far better by hydroxocobalamin. The test became normal in vitamin B₁₂ deficiency only when both methyl- or hydroxocobalamin and methyltetrahydrofolate were added in vitro. These results provide further evidence in favor of the "methylfolate trap" hypothesis as the explanation for the defect in DNA synthesis in vitamin B₁₂ deficiency.

It is now clear that vitamin B₁₂ deficiency causes a profound disturbance of folate metabolism. The finding of high serum folate concentrations¹ and low erythrocyte folate¹ in patients with vitamin B₁₂ deficiency has led to the well-known "methylfolate trap" hypothesis proposed by Noronha and Silverman¹ and Herbert and Zalusky². Since vitamin B₁₂ is needed as the coenzyme of methyltetrahydrofolate homocysteine methyltransferase, it has been suggested that in vitamin B₁₂ deficiency there is an accumulation of N⁵-methyltetrahydrofolate (N⁵-CH₃-THF) which leads to a depletion in cells of the other forms of folate that are active in nucleic acid synthesis. The validity of the methylfolate trap has been questioned.⁵ However, many of the disturbances of folate metabolism found in vitamin B₁₂ deficiency can be explained by the trap hypothesis.⁸ The accumulation of N⁵-CH₃-THF in cobalamin deficiency¹⁰ and the effect of methionine in preventing the irreversible formation of methyltetrahydrofolate¹¹ are consistent with the theory. Other disturbances of folate metabolism in vitamin B₁₂ deficiency, such as decreased transport of N⁵-CH₃-THF into cells¹² and decreased content and synthesis of intracellular pteroylpolyglutamates,¹³ do not exclude the methylfolate trap.

Deranged DNA synthesis in vitamin B₁₂ deficiency has been shown by defective suppression of the incorporation of tritiated thymidine (³H-TdR) into
DNA thymine by preincubation with deoxyuridine (dU). This dU suppression test has been utilized by many authors for the diagnosis of vitamin B12 and folate deficiency as well as drug-induced megaloblastosis.

Metz et al. showed correction of the dU suppression abnormality in vitro by folic acid (PGA) in three of four cases of pernicious anemia, while N5-CH3-THF had no corrective effect in two cases. This finding was considered by these authors as an argument in favor of the methylfolate trap.

It seemed of interest for us to study systematically the effect of the different forms of folate and the various cobalamin derivatives on the dU suppression test in patients with untreated pernicious anemia or folate deficiency to look for further evidence for or against the methylfolate trap.

MATERIALS AND METHODS

The subjects were 20 hematologically normal individuals, 11 patients with pure vitamin B12 deficiency due to pernicious anemia (Table 1), 6 patients with pure folate deficiency (5 malnutrition and 1 coeliac disease), and 3 patients with combined B12 and folate deficiency. One of these 3 (patient 7) had a true combined deficiency with impaired vitamin B12 absorption due to a total gastrectomy. The other 2 had malnutrition: 1 with transitory B12 malabsorption induced by colchicine treatment (patient 8); the other, with alcoholic cirrhosis, also had a decreased serum vitamin B12 level without malabsorption (Table 2). The final diagnosis in the various patients tested was arrived at on the basis of their clinical features and laboratory investigations. The diagnosis of Addisonian pernicious anemia was made on the basis of gastric achlorhydria with absent or markedly reduced intrinsic factor secretion, malabsorption of vitamin B12 (Schilling test), serum and/or gastric antibodies against gastric parietal cells and intrinsic factor, and cell-mediated intrinsic factor antibody.

Vitamin B12 and serum folate content were assayed with Lactobacillus leichmannii and caseii. Free and total pteroylpolyglutamate in red blood cells were measured according to the method described by Chanarin et al.

The hematologic data and results of laboratory investigations are listed in Tables 1 and 2. Of the 11 patients with vitamin B12 deficiency, 6 were also studied after 1 or 2 mo of B12 therapy.

Suppression tests with dU were performed according to the method described by Metz et al.

### Table 1. Hematologic Data in 11 Cases of Pernicious Anemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>Bone Marrow*</th>
<th>Serum B12 (pg/ml)</th>
<th>Erythrocytes</th>
<th>dU Suppression Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum</td>
<td>Total</td>
<td>Free</td>
</tr>
<tr>
<td>1</td>
<td>8.7</td>
<td>99</td>
<td>Marked</td>
<td>90</td>
<td>16.4</td>
<td>238</td>
</tr>
<tr>
<td>2</td>
<td>9.2</td>
<td>133</td>
<td>Moderate</td>
<td>100</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>8.7</td>
<td>113</td>
<td>Mild</td>
<td>140</td>
<td>9.4</td>
<td>235</td>
</tr>
<tr>
<td>4</td>
<td>11.3</td>
<td>95</td>
<td>Marked</td>
<td>100</td>
<td>10.3</td>
<td>120</td>
</tr>
<tr>
<td>5</td>
<td>9.8</td>
<td>90</td>
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<td>50</td>
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<td>270</td>
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<tr>
<td>6</td>
<td>10.1</td>
<td>136</td>
<td>Mild</td>
<td>100</td>
<td>4.4</td>
<td>94</td>
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<td>7</td>
<td>10.7</td>
<td>133</td>
<td>Mild</td>
<td>110</td>
<td>14</td>
<td>170</td>
</tr>
<tr>
<td>8</td>
<td>14.5</td>
<td>103</td>
<td>Moderate</td>
<td>110</td>
<td>11.5</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>12.6</td>
<td>117</td>
<td>Moderate</td>
<td>120</td>
<td>10.1</td>
<td>190</td>
</tr>
<tr>
<td>10</td>
<td>6.2</td>
<td>114</td>
<td>Marked</td>
<td>80</td>
<td>7.8</td>
<td>295</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>104</td>
<td>Mild</td>
<td>115</td>
<td>16</td>
<td>170</td>
</tr>
</tbody>
</table>

*Degree of morphologic megaloblastic maturation.
†Normal: 180–440 pg/ml.
‡Normal: 5–15 ng/ml.
§Normal: >250 ng/ml.
|| Normal: >95 ng/ml.
¶Percent of 3H-TdR uptake.
Table 2. Hematologic Data on True Folate Deficiency (Cases 1-6) and Combined Folate and B₁₂ Deficiency (Cases 7-9)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>Bone Marrow*</th>
<th>Serum B₁₂ (pg/ml)</th>
<th>Erythrocytes</th>
<th>dU Suppression Value (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.8</td>
<td>122</td>
<td>Mild</td>
<td>380</td>
<td>1.6</td>
<td>80 39 20.4</td>
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<td>2</td>
<td>5.4</td>
<td>86</td>
<td>Marked</td>
<td>240</td>
<td>1.8</td>
<td>137 — 26</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
<td>125</td>
<td>Marked</td>
<td>10,000**</td>
<td>2.2</td>
<td>150 — 28.9</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>125</td>
<td>Mild</td>
<td>1,050**</td>
<td>4.8</td>
<td>175 33 27</td>
</tr>
<tr>
<td>5</td>
<td>11.2</td>
<td>119</td>
<td>Mild</td>
<td>210</td>
<td>2.2</td>
<td>80 27 48.9</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>125</td>
<td>Mild</td>
<td>340</td>
<td>1</td>
<td>40 — 19</td>
</tr>
<tr>
<td>7 (gastric)</td>
<td>8.7</td>
<td>113</td>
<td>Mild</td>
<td>80</td>
<td>3.8</td>
<td>44 — 37.9</td>
</tr>
<tr>
<td>8 (malnutrition)</td>
<td>9.8</td>
<td>121</td>
<td>Mild</td>
<td>120</td>
<td>4.4</td>
<td>66 — 17.5</td>
</tr>
</tbody>
</table>

*transitory malabsorption of B₁₂

9 (malnutrition) | 9 | 113 | Mild | 150 | 2.9 | — | 20.6 |

See footnotes to Table 1.

**After vitamin B₁₂ therapy.

Modified by Wickramasinghe and Longland. About 3 or 4 ml of bone marrow was aspirated in a syringe containing 500 units of preservative-free heparin. The buffy coat was separated after the total bone marrow stood on Plasmagel for 1 hr. The nucleated cells were centrifuged at 1100 g for 10 min, washed three times in Hanks’ balanced salt solution, and resuspended in enough Hanks’ to produce a cell suspension of 20-30 million nucleated cells/ml. To 0.1 ml of marrow cell suspension was added 0.1 ml autologous serum, 0.1 ml of 2 dU (1 μmole 2-dU/ml); in other tubes, 0.1 ml of the various forms of folate and cobalamins were also added. Two tubes were incubated with no dU folate, and B₁₂. The final volume of each of the tubes had to be adjusted to 1 ml with Hanks’ solution.

All cultures were set up in duplicate and were incubated in a shaking water bath at 37°C for 1 hr before the addition of 0.1 ml of a solution containing 10 μCi/ml of ³H-TdR (specific activity 27 Ci/m mole). The cultures were then incubated for another hour, after which the cells were washed three times in ice-cold 0.9% NaCl and resuspended in 0.25 ml of 0.9% NaCl. Two 0.1-ml aliquots of each suspension were delivered onto individual Whatman filter discs (2.3-cm diameter 3MM grade). The discs were dried overnight, immersed in ice-cold 10% trichloroacetic acid for 20 min, and then washed in two changes of absolute methanol (10 min each). After a rinse in acetone, the discs were dried and counted in 10 ml of PSC (Amersham Searle).

The amount of ³H-TdR incorporated into the DNA was calculated from the average counts per minute per disc. The uptake of ³H-TdR after preincubation with dU was expressed as a percentage of the uptake without preincubation with dU. This percentage was “the dU suppressed value.”

The various coenzymes were used at the following concentrations for the final cell suspension: PGA, 10 μg; N₅-formyltetrahydrofolate (N₅-CHO-THF), 1 μg; N₅-CH₃-THF, 10 μg. Preliminary results showed it was necessary to use higher concentrations of PGA and N₅-CH₃-THF than of N₅-CHO-THF to obtain a similar correction in folate deficiency. Cyano- (CN-B₁₂), hydroxo- (OH-B₁₂), methyl- (CH₃-B₁₂) and adenosyl- (Ad-B₁₂) cobalamins were added at 10 μg. CH₃-B₁₂ and Ad-B₁₂ were protected from light during weighing and incubation. The effect of some of the coenzymes without addition of exogenous dU was tested in normal bone marrow (four cases), pernicious anemia (two cases), and folate deficiency (two cases). The effect of the addition of 5 μg of methotrexate was tested simultaneously in all cases to control the block of the uridine-thymidine “de novo” pathway. The blocking effect of methotrexate was tentatively reversed by various coenzymes at higher concentrations than those used.

*These compounds were kindly provided by Lederle and Sigma Laboratories for folate and by Roussel and Albert Rolland for cobalamin derivatives. The specific absorbance maxima of each compound, mainly N₅-CH₃-THF and methyl- and adenosylcobalamin, were tested before using.
in the dU test (500 µg). These concentrations were chosen because in vitro the reverse effect by folinic acid was not obtained with equimolecular concentrations.

RESULTS

Uptake of $^3$H-TdR Alone

The incorporation of $^3$H-TdR in bone marrow cells without addition of any precursor or derivative was higher in $B_12$ and folate deficiency than in normal bone marrow (Table 3). In pernicious anemia, the mean incorporation returned to normal values after vitamin $B_12$ therapy.

The addition of $B_12$ coenzymes or folate derivatives did not significantly change the incorporation of $^3$H-TdR in either normal or megaloblastic bone marrow cells when no exogenous dU was added.

Uptake of $^3$H-TdR in the presence of dU

Normal. The results of the dU-suppressed value in control marrow cells and in marrow cells of patients with vitamin $B_12$ and folate deficiencies are shown in Tables 4 and 5. In controls, dU almost completely blocked the incorporation of $^3$H-TdR (mean 4.7%). The various folate and cobalamin derivatives added in vitro did not significantly change this incorporation, with the exception of Ad-$B_12$, which significantly increased $^3$H-TdR incorporation ($p < 0.02$).

Vitamin $B_12$ deficiency. All 11 $B_12$-deficient patients studied exhibited the same pattern in the dU suppression test. The incorporation of $^3$H-TdR into DNA of bone marrow nucleated cells after preincubation with 0.1 µmole dU was significantly increased compared to normal controls tested under the same conditions (range, 18.8%–47.6%). The dU test was completely normal in the 6 patients tested after treatment with vitamin $B_12$ (Fig. 1).

The various folate or cobalamin derivatives added in vitro with dU in $B_12$ deficiency gave results different from normal values and those observed after vitamin $B_12$ treatment. PGA (10 µg) caused a partial correction, but $N5$-CH$_3$-THF at the same concentration had little or no effect in the dU test. However, $N5$-CHO-THF, even at one-tenth that concentration (1 µg) almost completely reduced the $^3$H-TdR incorporation to normal. A further and total normalization was obtained after $B_12$ therapy. The response to the addition of the cobalamins (10 µg) with dU varied: Ad-$B_12$ had little effect on $^3$H-TdR incorporation; correction was a little greater with CN-$B_12$ but less than with OH- or CH$_3$-$B_12$, which provided substantial improvement but still failed to correct completely

Table 3. Incorporation of $^3$H-TdR Into DNA of Bone Marrow Cells (dpm/3 x 10$^6$ cells)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± 1 SD (Range)</th>
<th>t*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal bone marrow</td>
<td>30,920 ± 9,650 (14,080–47,800)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin $B_12$ deficiency</td>
<td>92,050 ± 57,170 (32,260–249,560)</td>
<td>4.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pure folate deficiency</td>
<td>83,330 ± 42,210 (47,740–163,190)</td>
<td>5.33</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Statistical comparisons (Student's t test) between normal and vitamin $B_12$ or folate deficiency.
### Table 4. Mean (and Range) of dU Suppression Test Values* With and Without Various Derivatives

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls (20)†</th>
<th>Pernicious Anemia (11)†</th>
<th>Pure Folate Deficiency (4)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>dU alone</td>
<td>4.7 (3.2–9)</td>
<td>34.4 (18.8–47.6)</td>
<td>28.4 (20.4–48.9)</td>
</tr>
<tr>
<td>dU + PGA</td>
<td>3.8 (1.8–7.2)</td>
<td>20 (6–33.6)</td>
<td>7.4 (5–13.6)</td>
</tr>
<tr>
<td>dU + NS-CHO-THF</td>
<td>4.2 (2.6–10.5)</td>
<td>11 (4–18.5)</td>
<td>7.5 (3.4–15.1)</td>
</tr>
<tr>
<td>dU + CN-THF</td>
<td>4.1 (2.5–9)</td>
<td>31.9 (12.3–49.5)</td>
<td>7.7 (3.6–15.2)</td>
</tr>
<tr>
<td>dU + CN-B12</td>
<td>4.9 (3.1–9.2)</td>
<td>18.1 (4.6–31.2)</td>
<td>27.3 (19.2–34)</td>
</tr>
<tr>
<td>dU + Ad-B12</td>
<td>9 (5.8–23.3)</td>
<td>26.1 (10.5–50.2)</td>
<td>29.3 (15.8–45.3)</td>
</tr>
<tr>
<td>dU + OH-B12</td>
<td>4.3 (2.2–9.4)</td>
<td>15.7 (5.5–30.2)</td>
<td>23.2 (15.6–32.6)</td>
</tr>
<tr>
<td>dU + CH3-B12</td>
<td>4.6 (2–9.5)</td>
<td>15.2 (6.1–26.9)</td>
<td>23.3 (14.5–29.8)</td>
</tr>
<tr>
<td>dU + NS-CHO-THF + OH-B12</td>
<td>3.1 (2–3.5)</td>
<td>7.2 (3.8–11.5)</td>
<td>8.3 (3.4–15.8)</td>
</tr>
<tr>
<td>dU + NS-CHO-THF + CH3-B12</td>
<td>3.4 (2–5)</td>
<td>8.7 (4.8–16.6)</td>
<td>7.3 (2.9–14.7)</td>
</tr>
</tbody>
</table>

*Percent of 3H-TdR uptake.
†Number of cases in parentheses.
‡Statistical comparisons (Student's t test) of paired data between dU alone and dU with various derivatives. NS: not significant.

### Table 5. dU Suppression Test Values* in Combined Folate and Vitamin B12 Deficiency

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Condition</th>
<th>dU</th>
<th>dU + PGA</th>
<th>dU + NS-CHO-THF</th>
<th>dU + NS-CHO-THF</th>
<th>dU + Ad-B12</th>
<th>dU + CH3-B12</th>
<th>dU + OH-B12</th>
<th>dU + CH3-B12 + NS-CHO-THF</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Gastrectomy</td>
<td>37.9</td>
<td>21.4</td>
<td>11.1</td>
<td>23.4</td>
<td>23.5</td>
<td>27.2</td>
<td>18.4</td>
<td>18.2</td>
</tr>
<tr>
<td>8</td>
<td>Folate deficiency with transitory and reversible malabsorption of vitamin B12</td>
<td>17.5</td>
<td>9.8</td>
<td>5.7</td>
<td>8.2</td>
<td>21.2</td>
<td>32.6</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>Folate and vitamin B12 deficiency (nutritional) without malabsorption of B12</td>
<td>20.6</td>
<td>6</td>
<td>6</td>
<td>5.5</td>
<td>15</td>
<td>23</td>
<td>13</td>
<td>7</td>
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</table>

*Percent of 3H-TdR uptake.
the dU suppression test. There was no difference between the last two coenzymes. However, the response to either was less than with N5-CHO-THF.

In all the deficient patients studied, the blocking effect of dU on the \(^{3}H\)-TdR uptake became normal only when both N5-CH\(_{3}\)-THF and CH\(_{3}\)-B\(_{12}\) or OH-B\(_{12}\) were added simultaneously. The combined effect was significantly better than with either coenzyme alone \((p < 0.001)\).

No correlation was found between the percentage of \(^{3}H\)-TdR incorporation and the severity of megaloblastic change. There was also no correlation with serum B\(_{12}\) levels, degree of anemia, or mean corpuscular volume (MCV).

**Folate deficiency.** The dU suppression test was abnormal in the six cases of pure folate deficiency. The correction was total whatever folate derivative was utilized in vitro, while B\(_{12}\) derivatives had no effect (Table 4).

**Combined deficiency.** The pattern observed in the three cases of combined deficiency varied. In case 7, the dU-suppressed value was not corrected by either folate or cobalamin derivatives alone, but only by both added simultaneously. In the two other patients (cases 8 and 9) with slight B\(_{12}\) deficiency, the pattern was similar to that found in pure folate deficiency (Table 5).

**Effect of methotrexate.** In all cases studied (patients and normal controls) 5 \(\mu g\) of methotrexate added in vitro showed a strong blocking effect on dU suppression. This blocking effect was reversed in normal bone marrow with 100 times higher concentrations of folinic acid, but in one case of pernicious anemia the reversion was partial with folinic acid and N5-CH\(_{3}\)-THF (500 \(\mu g\)) and complete with N5-CH\(_{3}\)-THF + CH\(_{3}\)-B\(_{12}\) (Fig. 2).
DEOXYURIDINE SUPPRESSION TEST

Effect on free and total erythrocyte folates of vitamin B\textsubscript{12} deficiency. In the five cases in which free folates (glutamate chain length 1–3) were measured in erythrocytes, no correlation was noted between their level and the response to OH- or CH\textsubscript{3}-B\textsubscript{12}. Correction of the dU test was poor in case 3 in spite of a high content of free folate, intermediate in case 8, with low level of free folate, and good in cases 4, 9, and 12, characterized by a normal or low content.

DISCUSSION

These results confirm previous studies on the value of dU suppression in the diagnosis of folate or vitamin B\textsubscript{12} deficiency.\textsuperscript{2,20,22,23,25,31}

The pattern of correction by folate and B\textsubscript{12} derivatives in cases of vitamin B\textsubscript{12} deficiency was quite different from that in folate deficiency. In folate deficiency, the abnormal dU suppression test was completely corrected by the various folates whatever the compound tested, but not by cobalamins. On the other hand, in vitamin B\textsubscript{12} deficiency, only OH-B\textsubscript{12}, CH\textsubscript{3}-B\textsubscript{12}, and N\textsubscript{5}-CHO-THF corrected the test, albeit partially. Of the three compounds, folinic acid had the greatest effect on the dU suppression test in vitamin B\textsubscript{12} deficiency.

The problem raised by the cases of combined B\textsubscript{12} and folate deficiency is more difficult to solve. Van der Weyden et al.\textsuperscript{23} have observed that the corrective effect of each vitamin added in vitro is only partial. It is well known that folate deficiency can induce a decrease in serum vitamin B\textsubscript{12} level.\textsuperscript{12} In our patients, the dU suppression test allowed a clearcut distinction between abnormalities corrected only by both folate and B\textsubscript{12} derivatives and those corrected by folate derivatives alone. The latter pattern could correspond to pure folate deficiency with a secondary fall in serum B\textsubscript{12} level.

The interest of the dU suppression test in clinical practice is emphasized by the fact that it is abnormal in cases without marked anemia and/or megaloblastosis. Herbert et al.\textsuperscript{21} have shown the superiority of this test for the diagnosis of B\textsubscript{12} or folate deficiency and for the prediction of the response to vitamin therapy, while bone marrow morphology and serum vitamin levels may be misleading. Moreover the dU suppression test may be abnormal when megaloblastosis is absent in cases with associated iron deficiency.\textsuperscript{25} Wickramasinghe and Saunders\textsuperscript{33} found that the dU-suppressed value in megaloblastic anemia correlated with the MCV and inversely with hemoglobin level, but not with
either vitamin B\textsubscript{12} or serum folate level. However, we did not find any correlation with MCV or with hemoglobin. Indeed, in the present study, the dU suppression test was frankly abnormal in patients presenting with only slight peripheral blood or bone marrow abnormalities.

There is still controversy about the exact basis for the dU suppression test and the rationale of this test has been extensively discussed.\textsuperscript{5} In normal bone marrow cells, the dU suppression effect has been related to a decrease in DNA synthesis and/or to a radiodilution effect. It is generally assumed that dU induces inhibition of thymidine kinase activity by an increase in the thymidylate pool (originating from the conversion of the exogenous dU following the de novo pathway).\textsuperscript{20,34,35} A decrease in the incorporation of either labeled TdR or dU is obtained by previous incubation with cold TdR or dU, although cold TdR even at high concentrations cannot completely suppress the incorporation of labeled dU (Zittoun et al., unpublished data). In megaloblastic anemia, our results confirm that there is increased incorporation of \textsuperscript{3}H-TdR compared to normal values when no dU is added.\textsuperscript{36,37} This increased incorporation may be due to an increased activity of thymidine kinase.\textsuperscript{38,39} However, according to Wickramasinghe and Longland, the incorporation may in fact be subnormal when the uptake of the label is related to the increased number of DNA-synthesizing cells.\textsuperscript{37}

One hypothesis proposed\textsuperscript{19} is that incomplete dU suppression in cases of B\textsubscript{12} or folate deficiency could be due to reduced depression of the higher thymidine kinase activity. The general assumption that there is incomplete conversion of dU and dU monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) has not been confirmed by direct evidence. Measurements of thymidylate synthetase activity have given contradictory results.\textsuperscript{40,41} Moreover, Beck did not observe an increase of dTMP and deoxythymidine triphosphate (dTTP) intracellular pools after addition of exogenous dU to normal cells.\textsuperscript{5}

Whatever the explanation of the dU suppressive effect, our data seem to support the view of a defect of N\textsubscript{5}-CH\textsubscript{3}-THF metabolism in vitamin B\textsubscript{12} deficiency and enhance the validity of the methylfolate trap hypothesis.

The original observation of Metz et al. in two cases of B\textsubscript{12} deficiency was confirmed in our 11 patients with pernicious anemia: N\textsubscript{5}-CH\textsubscript{3}-THF did not correct the abnormality in the dU suppression test, while N\textsubscript{5}-CHO-THF, even at one-tenth the concentration, almost completely corrected the test.

The dU suppression test returned entirely to normal (compared to normal controls and to posttherapeutic values) only when both N\textsubscript{5}-CH\textsubscript{3}-THF and CH\textsubscript{3}-B\textsubscript{12} were added in vitro. It is thus obvious that B\textsubscript{12} derivatives are necessary for a complete effect of exogenous N\textsubscript{5}-CH\textsubscript{3}-THF in the dU test, by allowing its cellular uptake and/or its intracellular utilization. Moreover, the blocking effect of methotrexate in this test in B\textsubscript{12} deficiency was reversed when N\textsubscript{5}-CH\textsubscript{3}-THF and CH\textsubscript{3}-B\textsubscript{12} were added simultaneously, while N\textsubscript{5}-CH\textsubscript{3}-THF alone was ineffective. Thus B\textsubscript{12} seems to be necessary to regenerate tetrahydrofolate from N\textsubscript{5}-CH\textsubscript{3}-THF after drug inhibition of dihydrofolate reductase.

The question is raised of the nonutilization of endogenous N\textsubscript{5}-CH\textsubscript{3}-THF for complete correction after addition of OH- or CH\textsubscript{3}-B\textsubscript{12} alone in vitro. A possible explanation lies in the poor N\textsubscript{5}-CH\textsubscript{3}-THF uptake by cells in B\textsubscript{12} deficiency\textsuperscript{12} and
the inability of cells to retain it, resulting in a normal rather than increased intracellular monoglutamate pool. However, we failed to find a correlation between the level of erythrocyte free folate, corresponding mainly to N5-CH3-THF, and the activity of OH- or CH3-B12 in vitro.

Finally, it is of interest to emphasize the very poor effect in vitro of Ad-B12, as previously observed by Van der Weyden et al. in four cases of B12 deficiency. There is strong evidence for the existence of two biochemical pathways one involved in methionine synthesis controlled by CH3-B12, and the other one based on Ad-B12 and responsible for propionyl succinyl coenzyme A conversion. Our results support the evidence for these two separate metabolic pathways without any conversion of Ad-B12 to the other active cobalamins. Moreover, it should be noted that in normal nondeficient or posttherapeutic bone marrow, Ad-B12 had a negative effect on the dU suppression test, which suggests inhibition of the function of CH3-B12 by Ad-B12.

REFERENCES

ZITTOUN ET AL.
41. Haurani FJ: Vitamin B\(_12\) and the megaloblastic development. Science 182:78–79, 1973
Effect of folate and cobalamin compounds on the deoxyuridine suppression test in vitamin B12 and folate deficiency

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