Plasma lactate dehydrogenase in megaloblastic anaemia

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SYNOPSIS Raised plasma lactate dehydrogenase (LDH) values were found in 26 patients with marked megaloblastic anaemia due either to vitamin B₁₂ or folic-acid deficiency or a combination of these factors.

Minor megaloblastic changes were not usually accompanied by plasma LDH elevation. Serial LDH estimations were as valuable as serial reticulocyte counts in assessing the response to physiological doses of folic acid and therefore in deciding whether megaloblastic anaemia is partially or completely due to folic acid deficiency.

Elevation of plasma lactate dehydrogenase concentration (LDH) is known to occur in patients with megaloblastic anaemia. The literature on this subject is reviewed by Hess (1963) and the value of LDH estimations as a screening test in the detection of megaloblastic anaemia during pregnancy is commented on by Fleming and Elliott (1964).

Our studies were carried out to provide further information on LDH levels in megaloblastic anaemia and to evaluate the usefulness of serial estimations in assessing the response to physiological amounts of folic acid, and thus as a test of folic acid deficiency.

PATIENTS AND METHODS

Three-hundred and seventy-three LDH estimations were carried out on the following groups of patients:— Twenty-seven patients whose bone marrow showed marked megaloblastic changes; 10 patients whose marrow showed minimal megaloblastic or megaloblastoid change; 27 post-gastrectomy patients with and without anaemia; 16 patients with miscellaneous conditions, mainly peptic ulcer or neurosis, who served as a control group.

In 13 of the patients with megaloblastic anaemia the haematological response to 50 µg. of folic acid daily was studied. Nine of these had predominant vitamin B₁₂ deficiency, four folic acid deficiency. The folic acid was given intramuscularly to the patients with malabsorption and orally to the others. Reticulocytes and plasma LDH were estimated daily, haemoglobin and packed cell volume on alternate days. When steady base-line values for reticulocytes were obtained the folic acid was started and continued for 10 days except where otherwise stated in the test. These patients were on a white diet containing insufficient folic acid or B₁₂ to cause a reticulocyte response during the period of folic acid administration.

Urinary formiminoglutamic acid (FIGLU) An oral loading dose of 15 g. of L-histidine was given and the urinary excretion of Figlu was measured by conventional voltage electrophoresis on cellulose acetate (Kohn, Mollin, and Rosenbach, 1961). Although the method is not quantitative a rough grading of the Figlu spot was made: +, ++, ++++. It has been shown that ++++ represents a urinary Figlu concentration greater than 500 µg./ml.

Serial folate levels were measured by microbiological assay (L. casei ATCC 746), the normal range being 5-9 to 21 mµg./ml. Serum vitamin B₁₂ levels were measured by microbiological assay (L. leichmanii), the normal range being 140 to 900 µµg./ml.

Lactate dehydrogenase was estimated as described by Berger and Breid (1964). The normal value quoted by these authors is 100 to 350 units per ml., 350 to 500 being borderline, and above this abnormal. As some of our control group had values up to 500 units per ml. we have taken this figure as the upper limit of normal.

MARROW REASSESSMENT The initial marrow films in 10 patients (Table 1) were reported to show mild megaloblastic or megaloblastoid changes. These marrow films were re-examined by one of us and a rough grading of the degree of megaloblastic change present was made. For this purpose the red and white cell precursors were separated and the grading was carried out as follows:— Megaloblastic changes present at all stages of red cell development +++; changes present in only a few intermediate and late normoblasts +, changes intermediate between + and +++ were designated ++. When numerous macropolycytes and giant metamyelocytes were present the white cell series was designated +++, when only a few macrocytops and giant metamyelocytes were present +, and intermediate changes ++.

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The term megaloblastoid (M) was used to describe the
marrow appearance when red cell precursors showed
slight asynchronism between nuclear and cytoplasmic
maturation, no Howell Jolly bodies were present, and the
white cell series was normal.

RESULTS

The LDH values of patients whose marrows showed
marked megaloblastic changes are shown in Figure 1.
All values were above the normal range.

The haematological data and the results of re-
assessment of the marrow picture, together with the
type of treatment of the 10 patients whose marrow
was initially reported as showing mild megaloblastic
or megaloblastoid change, are shown in Table I.

Two patients had LDH values greater than normal.
Case 1 had post-gastrectomy vitamin B12 deficiency
and required vitamin B12 before the haemoglobin
level rose. Case 3 was a fit 47-year-old man who had
persistently elevated levels although significant
vitamin B12 or folic acid deficiency was not present.
His haemoglobin rose rapidly when intravenous iron
was given and LDH fell to normal levels.

EFFECTS OF TREATMENT WITH PHYSIOLOGICAL AMOUNTS
OF FOLIC ACID ON LDH VALUES IN PATIENTS WITH MEGALOBLASTIC ANAEMIA. Details of patients with predo-
nominant vitamin B12 deficiency are given in Table II. In
six patients (1 to 6) there was no increase in haemo-
globin, packed cell volume, reticulocyte count, nor
was there any significant change in LDH level during
the 10-day period of treatment with physiological
doses of folic acid. One patient (no. 7) developed a

10% increase in reticulocyte count without a fall in
LDH level but she was unusual in that her reticulo-
cyte count on admission to hospital was 7% and the
serum contained large amounts of methaemalbumin.
Over a few days the reticulocyte count fell to
base line value of 3 to 4% without a change in
the methaemalbumin concentration, but with a 50% fall in haemoglobin level. The folate level and Figu-
exeration in this patient did not suggest folic acid
deficiency.

In two patients (cases 8 and 9) considerable
alteration in LDH occurred associated with reticulo-
cyte changes. In case 8 the LDH fell from a pretreatment level of 4,000 units to 2,000 and this fall

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TABLE I

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Megaloblastic Change in Marrow</th>
<th>Hb (g./100 ml.)</th>
<th>Treatment</th>
<th>LDH</th>
<th>Serum B12 (μg./ml.)</th>
<th>Serum Folate (μg./ml.)</th>
<th>Serum Iron (g./l)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red Cell Series</td>
<td>White Cell Series</td>
<td>Before Treatment</td>
<td>After Treatment</td>
<td>67</td>
<td>99</td>
<td>Vitamin B12</td>
<td>750</td>
</tr>
<tr>
<td>1</td>
<td>+ +</td>
<td>+</td>
<td>65</td>
<td>89</td>
<td>Penicillin</td>
<td>420</td>
<td>143</td>
<td>Folic acid</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>+</td>
<td>48</td>
<td>91</td>
<td>Intravenous iron</td>
<td>360</td>
<td>300</td>
<td>Value of 3 to 4%</td>
</tr>
<tr>
<td>3</td>
<td>MEGALOBLASTOID</td>
<td>0</td>
<td>66</td>
<td>78</td>
<td>Folic acid</td>
<td>67</td>
<td>67</td>
<td>Nutritional folate acid deficiency</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+ +</td>
<td>78</td>
<td>101</td>
<td>Intravenous iron</td>
<td>290</td>
<td>143</td>
<td>Post-gastrectomy</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>0</td>
<td>79</td>
<td>79</td>
<td>Iron, vitamin B12</td>
<td>260</td>
<td>280</td>
<td>2.3</td>
</tr>
<tr>
<td>6</td>
<td>MEGALOBLASTOID</td>
<td>0</td>
<td>83</td>
<td>84</td>
<td>Folic acid</td>
<td>200</td>
<td>200</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>0</td>
<td>48</td>
<td>93</td>
<td>Intravenous iron</td>
<td>290</td>
<td>269</td>
<td>4.4</td>
</tr>
<tr>
<td>8</td>
<td>MEGALOBLASTOID</td>
<td>0</td>
<td>77</td>
<td>88</td>
<td>Oral iron</td>
<td>260</td>
<td>253</td>
<td>2.0</td>
</tr>
</tbody>
</table>
was associated with a 14% increase in reticulocytes. By the twelfth day the reticulocyte count had fallen to baseline levels. Three further LDH values at this time were approximately 2,000. No alteration of haemoglobin level occurred during these 12 days. In case 9 a similar response occurred during the first 10 days; the LDH values fell by just under 5,000 units in association with a reticulocytosis. The LDH values were followed for a further 20 days during which folic acid in physiological doses was continued. During this time the LDH value rose gradually to the pre-treatment value of 10,000. The haemoglobin level did not rise during the period of observation. These two patients had strongly positive Figlu tests and were considered to have combined deficiency of vitamin B₁₂ and folic acid. All of these patients then responded promptly to vitamin B₁₂ and the LDH values fell to normal.

**TABLE II**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Haemoglobin (g./100 ml.)</th>
<th>LDH</th>
<th>Serum Vitamin B₁₂ (μg./ml.)</th>
<th>Serum Folate (μg./ml.)</th>
<th>Figlu</th>
<th>Schilling Test (%)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55 40</td>
<td>8,000 8,700 10 3-4 -ve 1-32</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>53 49</td>
<td>5,050 6,400 75 5-8 -ve 1-7</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>47 42</td>
<td>3,750 4,200 70 20-9 -ve 0-14</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40 38</td>
<td>1,650 1,700 57 2-6 + 0-14</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>48 50</td>
<td>2,250 1,850 50 3-5 ++ 0-2</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>60 55</td>
<td>2,200 2,300 94 3-5 ++ 4 0-2</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>40 32</td>
<td>12,500 17,500 110 8-5 + 0-9</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>35 37</td>
<td>4,000 2,000 95 8-2 ++ + 0-5</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>40 32</td>
<td>10,000 5,200 104 2-7 ++ + 1-2</td>
<td>Pernicious anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Ten days’ oral folic acid, 50 μg. a day.*

**TABLE III**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Haemoglobin (g./100 ml.)</th>
<th>LDH</th>
<th>Serum Vitamin B₁₂ (μg./ml.)</th>
<th>Serum Folate (μg./ml.)</th>
<th>Figlu</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42 60</td>
<td>3,900 500 95 2-7 ++ +</td>
<td>Idiopathic steatorrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>58 66</td>
<td>720 390 260 1-8 nd</td>
<td>Idiopathic steatorrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>42 61</td>
<td>1,290 520 190 1-2 nd</td>
<td>Nutritional folic acid deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>35 50</td>
<td>15,000 4,000 210 1-4 ++</td>
<td>Nutritional folic acid deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

In common with the findings of others the LDH levels in our patients with marked megaloblastic changes in their bone marrow were considerably raised above normal. Patients with slight megaloblastic changes usually had normal levels. This latter

**TABLE IV**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Serum Folate (μg./ml.)</th>
<th>Serum Vitamin B₁₂ (μg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 4</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>No. of patients</td>
<td>29 13 36 150 150</td>
<td></td>
</tr>
<tr>
<td>LDH ± ISD</td>
<td>404 ± 183 420 ± 173 404 ± 185 397 ± 170</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE V**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Serum Folate (μg./ml.)</th>
<th>Serum Vitamin B₁₂ (μg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 500</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>No. of patients</td>
<td>7 20 266 ± 131 273 ± 88</td>
<td></td>
</tr>
<tr>
<td>Serum vitamin B₁₂ (μg./ml.)</td>
<td>10 19 5-3 ± 2-7 6-1 ± 2-7</td>
<td></td>
</tr>
</tbody>
</table>
finding might be expected when it is considered that
the LDH originates in the megaloblasts of the bone
marrow and then passes to the peripheral blood
(Elliott and Fleming, 1965). The LDH produced by a
few megaloblastic cells might not be adequate to
raise the peripheral LDH levels. Studies on the bone
marrow LDH might have been more rewarding in
these patients as elevation of LDH levels would first
be detectable here. From our results it may be
called that LDH estimation does not offer a
simple means of detecting minor megaloblastic
changes but minor megaloblastic change may not be
significant, particularly in the presence of iron
deficiency.

The decision as to whether megaloblastic anaemia
is due to folic acid or vitamin B₁₂ deficiency is some-
times difficult. In such instances it may be necessary
to carry out a therapeutic trial with physiological
amounts of folic acid and to assess response by
means of daily reticulocyte counts. Daily LDH
estimations would seem to be at least as valuable as
reticulocyte counts in assessing the response to a
therapeutic trial of folic acid. In two patients, cases
8 and 9, who had combined vitamin B₁₂ and folic acid
deficiency, a reticulocyte response was accompanied
by a fall in LDH value. The LDH values in these
patients did not, however, fall to normal, as they
would if folic acid deficiency alone was present.
From the partial return towards normal of LDH
values it was possible to diagnose the combined
deficiency which could not be detected from the
reticulocyte response alone. In one patient (case 7),
who did not appear to have an associated folic acid
deficiency, a reticulocyte response occurred which
was not accompanied by a fall in LDH so perhaps
in this patient the LDH was superior to the reticulocyte
count in assessing the response. The six patients with
vitamin B₁₂ deficiency alone showed no depression of
LDH or increase in reticulocyte count during the
period of the trial whereas the patients with folic acid
deficiency showed a rapid fall towards normal.

From Tables IV and V it is clear that low serum
levels of vitamin B₁₂ or folic acid are not of them-
selves accompanied by elevated LDH values nor are
the few LDH values above 500 in normal patients
accompanied by altered B₁₂ or folic acid levels.

We wish to thank Dr. J. R. Clamp and Dr. A. B. Raper
for their help in this study and their advice with the writing
of this report.

REFERENCES