ERYTHROPOIESIS IN PATIENTS UNDERGOING REGULAR DIALYSIS TREATMENT (R.D.T.) WITHOUT TRANSFUSION

J. C. MACKENZIE, J. E. FORD, A. H. WATERS, N. HARDING, W. R. CATTELL and B. B. ANDERSON

St. Bartholomew's Hospital, London and National Institute for Research in Dairying, Reading, United Kingdom

Erythropoiesis in patients with chronic renal disease is predominantly normoblastic, but megaloblastic changes have been reported, the incidence being higher in patients undergoing regular dialysis treatment (Hampers et al., 1967). These megaloblastic changes have been ascribed to folate deficiency secondary to inadequate dietary intake or absorption and, in patients on R.D.T., to the loss of folate during dialysis (Hampers et al., 1967).

The requirement for folate in any individual must be related to the metabolic turnover of the vitamin and in particular to marrow activity. In terminal renal failure erythropoiesis is depressed and the requirements may be low. However, following effective R.D.T. there is a gradual improvement in erythropoietic function (Eschbach et al., 1967) which could increase the demand for essential haematins and result in latent deficiencies becoming manifest. This increase in erythropoiesis is partly due to withholding regular blood transfusions which, although maintaining arbitrary high haematocrits, serve only to suppress erythropoiesis (Crockett et al., 1967).

Folic acid and other water-soluble vitamins, with the exception of vitamin B₁₂, exist largely in a free, or loosely bound state in the plasma and may be removed by dialysis, possibly resulting in the gradual development of specific deficiencies. Recent studies have shown low plasma levels of several of the water-soluble vitamins including folic acid in patients maintained on R.D.T. (Lasker et al., 1963).

This study was designed to re-examine the evidence for folate deficiency and to assess the nutritional status of the water-soluble vitamins, vitamin B₁₂, thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid and biotin, in patients on R.D.T.

MATERIAL AND METHODS

The nutritional vitamin status of six patients established on R.D.T. was measured on the basis of the daily dietary intake, the plasma levels, and the urine and dialysate loss of these vitamins. In addition red cell folate levels were estimated and serial marrow films examined.

At the time of study the patients had been dialysed 28–30 hours per week for periods from 3–9 months on Kiil dialysers with a warm single pass automatic dialysate supply system. All patients received a high calorie 60 g protein diet, with fluid, sodium and potassium restriction. In addition they were taking oral vitamin supplements (thiamine 1 mg, riboflavin 0.5 mg, nicotinic acid 7.5 mg, and ascorbic acid 15 mm), but no iron, folic acid or vitamin B₁₂. Before starting R.D.T. the patients had been maintained on modified Giovannetti diets (18 g protein) for 3–12 months. These diets were assayed for folate content. Before dialysis treatment the patients had also required treatment with regular blood transfusions for severe anaemia.

Blood loss from dialysis and blood sampling averaged 150 ml per month per patient. No maintenance transfusions were given, except for patient 2 who had 100 ml of packed
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cells per month. However, replacement transfusions with packed red cells were necessary in patients 3 and 4.

Haematological methods were those described by Dacie and Lewis (1963). Serum and red cell folate, and vitamin B₁₂ were estimated by the methods of Waters and Mollin (1961), Hoffbrand et al. (1966) and Anderson (1964), respectively. The plasma levels of the remaining vitamins, and the urine and dialysate levels of each vitamin were measured using modified micro-biological assay techniques (Barton-Wright, 1963; Spray, 1955; Herbert, 1961; Deibel et al., 1957).

The 'free', L. casei-active folate content of the Giovannetti and R.D.T. diet was assayed using the method described by Chanarin et al. (1968). The R.D.T. diet was also assayed for the other vitamins and the values compared with those calculated from the data of McCance and Widdowson (1967).

Serum iron and iron binding capacity were estimated by the method of Brozovich (1968). Intestinal absorption of folic acid was assessed by the method of Chanarin et al. (1958) using tritiated folic acid (Anderson et al., 1960).

The plasma flow (ml/min.) was calculated from the haematocrit and the blood flow as measured by a Doppler ultra-sonic flow meter (Rushmer et al., 1966).

RESULTS

Clinical details of the six patients are shown in Table I.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Disease</th>
<th>Months on dialysis</th>
<th>Haematocrit</th>
<th>Reticulocytes</th>
<th>Maintenance transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.B.</td>
<td>F</td>
<td>32</td>
<td>G.N.</td>
<td>9</td>
<td>22</td>
<td>1.8</td>
<td>NIL</td>
</tr>
<tr>
<td>L.C.</td>
<td>M</td>
<td>26</td>
<td>P.N.</td>
<td>8</td>
<td>22</td>
<td>0.9</td>
<td>100 ml/month</td>
</tr>
<tr>
<td>E.C.</td>
<td>F</td>
<td>39</td>
<td>P.N.</td>
<td>7</td>
<td>24</td>
<td>0.2</td>
<td>NIL*</td>
</tr>
<tr>
<td>M.C.</td>
<td>F</td>
<td>33</td>
<td>G.N.</td>
<td>5</td>
<td>19</td>
<td>1.4</td>
<td>NIL*</td>
</tr>
<tr>
<td>B.C.</td>
<td>M</td>
<td>49</td>
<td>G.N.</td>
<td>4</td>
<td>19</td>
<td>0.4</td>
<td>NIL</td>
</tr>
<tr>
<td>P.Q.</td>
<td>M</td>
<td>33</td>
<td>G.N.</td>
<td>3</td>
<td>21</td>
<td>0.4</td>
<td>NIL</td>
</tr>
</tbody>
</table>

G.N. = glomerulonephritis
P.N. = pyelonephritis
* 1 unit of packed cells replacement transfusion

Haematological findings

The mean haematocrit was 23%. The blood films were normochromic and showed anisocytosis with occasional fragmented ('burr') cells. Hypersegmented neutrophils were not a feature of the films. The reticulocyte count was less than 2% in all patients.

The bone marrow was examined in all cases in the present study. Cases 2 and 5 had also had marrow examinations before commencing R.D.T. These latter bone marrows were hypoplastic with normoblastic erythropoiesis and increased iron stores. In contrast, after R.D.T. for 3 to 9 months, the marrow cellularity was normal in all of the patients except patient 5, in whom it remained slightly hypocellular. Erythropoiesis was active and mainly

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normoblastic. However, many of the polychromatic normoblasts were larger than normal, but did not have the nuclear characteristics to justify their classification as intermediate megaloblasts. Granulopoiesis was increased and giant metamyelocytes were present. In contrast to the patients studied by Hampers et al. (1967) none of our patients had classical megaloblasts in the bone marrow, and they correspond to Hampers’ patients with ‘minimal’ changes.

_Iron, vitamin B\textsubscript{12} and folate_

The serum iron concentration ranged from 49–117 \textmu g/100 ml, the total iron binding capacity 251–441 \textmu g/100 ml, and the saturation 12–34%. The two patients with low serum iron levels had depleted bone marrow iron stores; of the other four patients two had increased and two had normal iron stores.

The serum B\textsubscript{12} levels were normal and remained unchanged throughout the present study (Fig. 1).

![Graph showing serum folate, red blood cell folate, and serum vitamin B\textsubscript{12} levels over time](image)

_Fig. 1_

_Serum and red cell folate_ concentrations are shown in Figure 1. The average serum folate level pre-dialysis was 3.9 ng/ml, post-dialysis 2.9 ng/ml and three months later 2.1 ng/ml. The red cell folate was normal in four and subnormal in two patients at the beginning of the study. All but one patient had low serum folate levels when first investigated. Over the period of the study the serum folate level fell in all patients, but despite this the red cell folates remained virtually unchanged.

_Dietary intake and absorption of folate_

The Giovannetti diet contained 94 \mu g and the R.D.T. diet 72 \mu g of free, \textit{L. casei}-active folate. Intestinal absorption tests in three patients showed no defect in the absorption of crystalline folic acid.
Dialysance of folate

Serial measurements of the plasma folate content of arterial and venous blood and the dialysate were made on the patients during dialysis, allowing the plasma and dialysate dialysance to be calculated from the usual dialysance equation (Sweeney and Galletti, 1964). During dialysis the red cell folates remained unchanged. Table II shows the arterio-venous differences in plasma folate and the folate concentration in the dialysate during the course of dialysis in one patient.

<table>
<thead>
<tr>
<th>Dialysis time</th>
<th>( C_{P \text{ in}} ) (ng/ml)</th>
<th>( C_{P \text{ out}} ) (ng/ml)</th>
<th>( D_P ) (ml/min.)</th>
<th>( C_{D \text{ out}} ) (ng/ml)</th>
<th>( D_D ) (ml/min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.6</td>
<td>4.4</td>
<td>27</td>
<td>0.22</td>
<td>16.5</td>
</tr>
<tr>
<td>65</td>
<td>6.5</td>
<td>3.5</td>
<td>35</td>
<td>0.28</td>
<td>22</td>
</tr>
<tr>
<td>125</td>
<td>6.4</td>
<td>4.3</td>
<td>21</td>
<td>0.22</td>
<td>17</td>
</tr>
<tr>
<td>480</td>
<td>6.0</td>
<td>3.8</td>
<td>29</td>
<td>0.17</td>
<td>14.5</td>
</tr>
</tbody>
</table>

\( C_{P \text{ in}} \) = Concentration in plasma entering dialyser
\( C_{P \text{ out}} \) = Concentration in plasma leaving dialyser
\( C_{D \text{ out}} \) = Concentration in dialysate leaving dialyser
\( D_P \) = Plasma dialysance
\( D_D \) = Dialysate dialysance

The plasma dialysance in this case was 28 ml/min. and in the collected series the mean plasma dialysance was 25.5 ml/min. The corresponding dialysate dialysance gave figures of 17.5 ml/min. and 12.4 ml/min. These lower values probably represent loss of folate from oxidation during the collection of the dialysate samples, although mercaptoethanol was used to reduce this to a minimum. Very little folate was adsorbed onto the membrane. These data indicate that over the subnormal plasma folate range studied the smallest loss during dialysis would be about 30 \( \mu \)g per dialysis, applying the dialysate dialysance, and up to 150 \( \mu \)g calculated from the plasma dialysance. The 24 hr. urinary loss of folate is less than 1 \( \mu \)g in R.D.T. patients.

<table>
<thead>
<tr>
<th>Vitamin content of R.D.T. diet (60 g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid (( \mu )g)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>R.D.T. diet estimate</td>
</tr>
<tr>
<td>R.D.T. diet assay</td>
</tr>
<tr>
<td>Multivitamin supplement</td>
</tr>
<tr>
<td>Total intake</td>
</tr>
<tr>
<td>Recommended daily</td>
</tr>
<tr>
<td>allowance*</td>
</tr>
</tbody>
</table>

Folate therapy

All patients received ‘Folvite’ 15 mg i.m. for three days to saturate the tissues prior to the absorption tests. There was a small reticulocytosis (up to 5%) within 5–7 days in all patients, but in only three was there a sustained rise in haematocrit over a follow-up period of one month (Fig. 2).

Re-examination of the marrow when serum folate levels were normal, however, showed that there had been little change.

Other vitamins

Table III shows the dietary vitamin intake compared with the recommended daily allowance of each of the vitamins.

In most cases the intake fell below the recommended daily allowance, particularly in the case of pantothenic acid, pyridoxine and biotin which were not added as supplements to the diet.

![Graph showing reticulocytes and haematocrit percentages before and after folate therapy](image_url)

Fig. 2

<table>
<thead>
<tr>
<th>Vitamin levels in plasma</th>
<th>R.D.T. patients</th>
<th>Normal</th>
<th>Approx. plasma dialysance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid (ng/ml)</td>
<td>3.9</td>
<td>9.9</td>
<td>25 ml/min.</td>
</tr>
<tr>
<td>Vit. B₁₂ (pg/ml)</td>
<td>509</td>
<td>488</td>
<td>not measurable</td>
</tr>
<tr>
<td>Thiamine (µg/ml)</td>
<td>0.028</td>
<td>0.025</td>
<td>13 ml/min.</td>
</tr>
<tr>
<td>Riboflavin (µg/ml)</td>
<td>0.02</td>
<td>0.04</td>
<td>52 ml/min.</td>
</tr>
<tr>
<td>Pyridoxine (µg/ml)</td>
<td>0.005</td>
<td>0.01</td>
<td>not measurable</td>
</tr>
<tr>
<td>Nicotinic acid (µg/ml)</td>
<td>0.34</td>
<td>0.1</td>
<td>4 ml/min.</td>
</tr>
<tr>
<td>Pantothentic acid (µg/ml)</td>
<td>0.08</td>
<td>0.14</td>
<td>30 ml/min.</td>
</tr>
<tr>
<td>Biotin (ng/ml)</td>
<td>0.58</td>
<td>1.18</td>
<td>45 ml/min.</td>
</tr>
</tbody>
</table>
Table IV shows the mean plasma levels of these vitamins in the six patients compared with a normal group. There was a wide range, but three patients had subnormal plasma levels of pantothenic acid and two had subnormal plasma levels of biotin. All six patients exhibited low plasma pyridoxine levels. The total calculated dialysate loss was approximately equal to the amount excreted in normal 24 hr. urine, except for folate and riboflavin, which were lost in excess.

Urinary losses of the vitamins studied were negligible in R.D.T. patients.

**DISCUSSION**

In our study the cooked Giovannetti and R.D.T. diets contained 94 \( \mu g \) and 72 \( \mu g \) of free, L. casei-active folate, respectively, which was approximately equal to the minimum adult requirement of 50 to 100 \( \mu g \) of crystalline folic acid, for a normal person with normal requirements (Herbert, 1962a) but less than the average adult intake of 150 to 225 \( \mu g \) per day (Hurdle, 1967). The low folate content of the R.D.T. diet probably reflects the much greater cooking time, and the leaching effect of the large volume of water used to rid the diet of excess sodium and potassium. The estimated average daily folate lost from dialysis would range from 10–40 \( \mu g \) depending on the plasma folate level.

It has been shown that the dialysate loss is proportional to the plasma folate level, and it would seem therefore that at the upper plasma levels the dialysate loss may not be offset by the diet, but that as the plasma folate level falls on R.D.T. a new steady state is established at a lower plasma folate level, in patients on the reference diet.

Hampers et al. (1967) measured 3 to 4 ng per ml in their dialysate, but gave no details of the clearance or total loss during dialysis.

Whitehead et al. (1967) found that the fall in plasma folate during dialysis was proportional to the starting level and averaged 51%. Our data would confirm that there is greater loss of folate at higher plasma levels. However, in contrast to these authors we found that the serum folate levels did not return to normal with diet alone, and the red cell folate in four patients was maintained at normal levels in spite of very low or falling levels in the serum. If the red cell folate concentration is used as a measure of tissue folate stores there was no measurable depletion of the tissues. Nevertheless, some degree of folate deficiency cannot be excluded completely as there was a small reticulocytosis, and in three cases a rise in haematocrit.

Herbert (1962b) has shown that a patient may pass through this stage of minimal marrow changes with normal red cell folate in the development of severe folate deficiency. However, the interesting feature in these patients is that these changes persist after folate therapy. It seemed likely, therefore, that they were not due to folate deficiency, but possibly represented slow DNA synthesis due to some other factor; in this respect it has been reported that hypersegmented neutrophils may be seen in patients with uraemia who have normal serum folate levels (Herbert, 1965). It would appear, therefore, that the reference diet will maintain a folate balance in most patients. Nevertheless, this balance is precarious and a true folate deficiency may develop if folate requirements increase, for example, as a result of further improvement in erythropoiesis or with an acute intercurrent infection. We cannot say from our present study whether a tissue folate deficiency as measured by red cell folate develops on R.D.T. This point is under further investigation.

Although losses of the other vitamins from the plasma on dialysis were not great, the poor intake in the diet may lead to progressive depletion of the vitamin stores.

The nutritional status of R.D.T. patients is of fundamental importance and if we are looking for marginal improvements we should ensure that adequate supplements of all the vitamins are given, and not only the restricted range commonly provided in multivitamin preparations; in particular the spectrum of replacement should be extended to folic acid, biotin, pyridoxine, and perhaps also pantothenic acid. Further studies are necessary to evaluate the action of some of the vitamins on marrow function, in patients on R.D.T., particularly ascorbic acid and the tocopherols which were not included in this investigation.
REFERENCES


