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W.H.O. Sponsored Collaborative Studies on Nutritional Anaemia in India

1. THE EFFECTS OF SUPPLEMENTAL ORAL IRON ADMINISTRATION TO PREGNANT WOMEN

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SUMMARY

A W.H.O. sponsored collaborative study of the effects of iron supplementation to pregnant women was carried out in Delhi (northern India) and Vellore (southern India). Supplementation was given under supervision from the 26th to the 36th or 38th week of pregnancy. A control group received only placebo; one group received vitamin B₁₂ and folic acid alone; four groups received vitamin B₁₂, folate and a daily iron supplement ranging from 30 to 240 mg of elemental iron as ferrous fumarate, and one further group received 120 mg of iron without B₁₂ or folate. Groups receiving no iron showed a fall in mean haemoglobin concentration. Those receiving iron showed a rise in haemoglobin, the best results being in the groups receiving 120 and 240 mg of iron together with vitamin B₁₂ and folate. Even in these groups however, there was still a high prevalence of anaemia and iron deficiency at the end of the trial period. Iron alone did not produce as good results as iron plus vitamin B₁₂ and folate. The supplementation had no detectable effect on the birth weight of the children, nor on the haemoglobin concentration of the infants at three months of age.

The daily absorption of iron in the pregnant women, as judged from the increase in haemoglobin mass, was not as satisfactory as expected. Possible reasons for this are discussed.

It is concluded that to provide these women with adequate iron a daily oral supplement of 120 mg of elemental iron or more is needed. This can only be achieved by medicinal means. Before supplementation can be recommended on a public health scale, further information regarding the cost and expected benefits of such measures must be obtained.

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INTRODUCTION
In previous studies sponsored by the World Health Organization, the prevalence of anaemia in pregnant women was found to range in different countries from 21 to 80 per cent. The anaemia was due mainly to iron deficiency, which was present in 40 to 99 per cent of the women, with folate deficiency as a subsidiary factor in some subjects (World Health Organization, 1968). Of the countries studied, the prevalence of anaemia and iron deficiency was highest in India. It was therefore decided to undertake a collaborative trial of the effects of iron supplementation to pregnant women in two regions of India. This communication reports the results of this study.

MATERIALS AND METHODS
The trial was carried out in New Delhi (northern India) and in Vellore, Tamilnadu (southern India). In New Delhi it was conducted in two maternal and child welfare centres within three miles of the laboratory. In Vellore the trial was carried out in a Red Cross antenatal clinic in Vellore town and in a group of villages, twelve miles from the town, where a special antenatal clinic was set up for the purpose. In both Delhi and Vellore most of the women studied belonged to the lower socio-economic strata.

![Flowchart](Fig. 1. Design of the trial. To try and ensure uniformity in the composition of the groups, at the 26th week the two streams were divided into 3 strata according to the initial haemoglobin concentration, and each stratum divided randomly into groups. Only one stratum is depicted here.

The design of the trial is illustrated in Fig. 1. Women with heart disease, chronic diseases such as pyelonephritis, tuberculosis and leprosy, women with a history of chronic diarrhoea and women with a haemoglobin concentration of less than 5 g per 100 ml were excluded. All other consenting women who had not received any medication during the previous six months were admitted to the trial at 22 ± 2 weeks gestation. At this time the 'preliminary' blood specimen was obtained—in
Delhi this was a venous sample and in Vellore a capillary one. By reference to previously prepared random tables the women were allocated to one of two streams—‘A’ and ‘B’. For the next four weeks all subjects in stream ‘A’ were given placebo tablets six days a week and placebo injections once every two weeks, and all subjects in stream ‘B’ were given one 5-mg tablet of folic acid daily, six days a week, and an injection of 100 μg of cyanocobalamin once every two weeks. At the end of the preliminary period (26 ± 2 weeks of gestation) the ‘initial’ blood sample was obtained by venepuncture. The subjects were then divided into one of three strata (not shown in Fig. 1) according to their haemoglobin concentration (5.0 to 7.9; 8.0 to 11.9 and 11 g per 100 ml and above). Within each stratum subjects were allotted to final treatment groups according to a set of random numbers. Subjects from stream ‘A’ were allocated to either group 0 and continued to receive placebo tablets and injections, or to group 6 and received tablets containing 120 mg of elemental iron and placebo injections but no folic acid or vitamin B₁₂. Subjects from stream ‘B’ were allotted to one of five groups numbered 1 to 5. All received oral folic acid 5 mg daily, and an injection of vitamin B₁₂, 100 μg every two weeks. Subjects in group 1 received no iron supplement; those in group 2 received 30 mg of iron; those in group 3, 60 mg of iron; those in group 4, 120 mg and those in group 5, 240 mg of iron. In each case the iron salt was ferrous fumarate. All the tablets had the same appearance and had the daily folic acid and iron dose divided into two tablets. Two tablets were given daily six days a week throughout the trial period, by a public health nurse, who watched the tablets being swallowed. No attempt was made to regulate the time of administration of the tablets with respect to food, it being assumed that any adverse effect of food on iron absorption would even out over the various groups.

Every care was taken to ensure that subjects did not receive any extraneous iron or vitamin supplements. All subjects were questioned at regular intervals regarding the presence of any side effects of the supplementation and all were seen regularly for antenatal care by a medical officer engaged in the study.

Supplementation was carried on for 10 to 12 weeks. At the conclusion of this period (36 to 38 weeks of gestation) and at least 24 hours after the last medication, the ‘final’ sample of venous blood was obtained and the various tests repeated. Where possible, samples of capillary blood were also obtained from mother and baby three months after delivery.

Standard haematological techniques were employed (Daicie, 1956). Haemoglobin was estimated by the cyanmethemoglobin method using a photo-electronic colorimeter, checked periodically against international reference standards.¹ Haematocrit values were determined using the microhaematocrit method. In Vellore serum iron was measured by the method recommended by the International Committee for Standardization in Haematology (1971) checked periodically against reference standards.² The serum iron binding capacity was determined by the isotopic method of Herbert, Gottlieb, Lau, Fisher, Gervirtz, and Wasserman (1966).

¹ Kindly provided by Prof. W. Crosby and Dr. S. M. Lewis.
² Kindly supplied by Dr. J. B. Cook.
A stool examination was carried out in each patient at the beginning of the trial by the zinc sulphate centrifugal-flotation method of Faust, Sawitz, Tobie, Odom, Peres, and Lincicome (1939). Hookworm egg loads were quantitated by Stoll's dilution technique (Stoll and Hausheer, 1926) but no attempt was made toworm the patients.

The iron content of a random selection of each set of the tablets was checked at the beginning and end of the trial by chemical analysis and found to be within 6 per cent of the expected values. To test the availability of the iron in the trial tablets they were administered to four subjects with iron deficiency anaemia, who were not bleeding and did not have intestinal malabsorption and who had normal serum and red cell folate and serum vitamin B₁₂ concentrations.

**CASE 1**

**CASE 2**

**CASE 3**

**CASE 4**

![Graphs showing response to iron tablets](image)

**Fig. 2.** Response of four non-pregnant subjects with iron deficiency anaemia to the test tablets. The daily dose of iron is indicated in the horizontal bars. ● Haemoglobin; ○ reticulocytes.

**RESULTS**

**Effect of tablets administered to patients with iron deficiency anaemia**

The haematological response of four subjects with severe iron deficiency anaemia who were given the trial tablets half to one hour after breakfast, is shown in Fig. 2. Three subjects started treatment with 30 mg iron daily. All showed a rise in haemoglobin and haematocrit, and two had a delayed reticulocyte response. Doubling the dose of iron to 60 mg daily in case 1 produced a second reticulocyte response and a slight increase in the rate of rise of haemoglobin. In case 2, increasing the dose to 60 mg daily caused no change, whereas in case 3, 60 mg/day produced a definite increase in the rate of haemoglobin formation without a second reticulocyte response. Case 4 who received 60 mg from the time of commencement of therapy,
showed a slow response similar to that seen in case 2 receiving 30 mg. If it is assumed that all the absorbed iron was used for haemoglobin production then the average daily amount of iron absorbed was 9.5 mg in case 1, 14.5 mg in case 2, 9.6 mg in case 3 and 10.6 in case 4.

The trial

Of those entering the trial 30 per cent failed to complete it, usually due either to lack of cooperation, leaving the study area, or premature delivery. The chief reason for non-cooperation was refusal to allow the drawing of blood, which traditionally meets with considerable resistance. The number of dropouts was greatest during the initial period. After allocation to one of the groups, the numbers failing to complete the trial were similar in each group, except for those who discontinued due to intolerance to the medication (see later).

Haemoglobin and haematocrit

'Preliminary' venous blood (22 weeks) was only obtained in the Delhi subjects. The frequency distribution of the haemoglobin concentration values at this time is shown in Fig. 3. Over the four-week period between the preliminary and initial

![Graph showing frequency distribution of haemoglobin concentrations](image)

**Fig. 3.** Haemoglobin concentration frequency distribution curves for preliminary blood samples (22 ± 2 weeks) in Delhi subjects and for the initial blood samples (26 ± 2 weeks) in Delhi and Vellore subjects, separately and combined.
<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of women</th>
<th>Mean g/100 ml Initial</th>
<th>Mean of individual differences</th>
<th>Standard error of mean of differences</th>
<th>P paired 't' test</th>
<th>Mean % Initial</th>
<th>Mean of individual differences</th>
<th>Standard error of mean of differences</th>
<th>P paired 't' test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>70</td>
<td>9.94</td>
<td>9.27 -0.37</td>
<td>0.086</td>
<td>&lt;0.001</td>
<td>32.44</td>
<td>32.04 -0.40</td>
<td>0.267</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>B12 + F.A.</td>
<td>91</td>
<td>9.98</td>
<td>9.36 -0.22</td>
<td>0.099</td>
<td>&lt;0.05</td>
<td>32.38</td>
<td>32.38 0</td>
<td>0.385</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>B12 + F.A. + 30 mg iron</td>
<td>89</td>
<td>9.36</td>
<td>10.19 +0.83</td>
<td>0.103</td>
<td>&lt;0.001</td>
<td>31.95</td>
<td>34.46 +2.51</td>
<td>0.314</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B12 + F.A. + 60 mg iron</td>
<td>91</td>
<td>9.34</td>
<td>10.32 +0.98</td>
<td>0.135</td>
<td>&lt;0.001</td>
<td>31.96</td>
<td>34.68 +2.72</td>
<td>0.405</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B12 + F.A. + 120 mg iron</td>
<td>115</td>
<td>9.34</td>
<td>10.84 +1.26</td>
<td>0.137</td>
<td>&lt;0.001</td>
<td>32.47</td>
<td>35.79 +3.32</td>
<td>0.365</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B12 + F.A. + 240 mg iron</td>
<td>84</td>
<td>9.43</td>
<td>10.82 +1.39</td>
<td>0.149</td>
<td>&lt;0.001</td>
<td>32.25</td>
<td>39.05 +3.80</td>
<td>0.440</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>120 mg iron only</td>
<td>107</td>
<td>9.68</td>
<td>10.40 +0.72</td>
<td>0.101</td>
<td>&lt;0.001</td>
<td>32.27</td>
<td>34.78 +2.50</td>
<td>0.267</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

**Table 1. Mean initial and final haemoglobin and haematocrit values in the different groups**
samples, as judged by the paired ‘t’ test, there was a significant fall in haemoglobin concentration ($P < 0.001$). A similar fall in haemoglobin concentration was seen in subjects in Vellore, although since the preliminary sample was capillary blood, the preliminary and initial values were not strictly comparable. This fall was the same in the subjects who received vitamin B₁₂ and folic acid (B stream) and those who received only placebo (A stream).

The frequency distributions of the ‘initial’ haemoglobin concentration values (26 weeks) in both Vellore and Delhi were virtually identical and are shown separately and combined in Fig. 3. Eighty-eight per cent of women in Delhi and 87 per cent of women in Vellore had an initial haemoglobin concentration below 11 g/100 ml.

The initial (26 weeks) and final (36 to 38 weeks) mean haemoglobin concentrations, mean haematocrit, mean of individual differences and its standard error, in each of the groups, are shown in Table 1. The majority of women in groups 0 and 1 who did not receive iron showed a fall in haemoglobin but little or no change in haematocrit.

In all groups receiving iron the majority of women showed a rise in haemoglobin and haematocrit, the best results being in group 5 (240 mg iron) where 90 per cent of subjects had a rise (Table 2). However, at the time of the final sample, 88 per cent of women in groups 0 and 1, 79.0 per cent in group 2; 73.6 per cent in group 3; 56.2 per cent in groups 4 and 5 and 58.8 per cent in group 6 still had haemoglobin concentrations below 11 g/100 ml.

**Table 2. Per cent of subjects in each group showing a fall or rise in haemoglobin concentration of 0.1 g/100 ml or more between initial and final haemoglobin estimations**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of women</th>
<th>% of group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fall</td>
</tr>
<tr>
<td>0 Placebo</td>
<td>78</td>
<td>55</td>
</tr>
<tr>
<td>1 B₁₂ + F.A.</td>
<td>91</td>
<td>87</td>
</tr>
<tr>
<td>2 B₁₂ + F.A. + 30 mg iron</td>
<td>89</td>
<td>17</td>
</tr>
<tr>
<td>3 B₁₂ + F.A. + 60 mg iron</td>
<td>91</td>
<td>22</td>
</tr>
<tr>
<td>4 B₁₂ + F.A. + 120 mg iron</td>
<td>115</td>
<td>16</td>
</tr>
<tr>
<td>5 B₁₂ + F.A. + 240 mg iron</td>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td>6 120 mg iron</td>
<td>107</td>
<td>21</td>
</tr>
</tbody>
</table>

In each of the groups receiving iron the rise in haemoglobin tended to be the greatest in those with the lowest initial haemoglobin concentration. Preliminary examination of the data revealed that regressions of final haemoglobin levels on initial levels, in the different treatment groups were likely to be heterogeneous.

To examine this, a statistical comparison of the regression lines was made and the results are presented in Table 3. The differences in the slopes of the regression lines were found to be statistically highly significant ($F = 10.33$, $P < 0.001$). Hence separate regression lines were fitted to the data from each group (Fig. 4). These regression lines, along with the predicted haemoglobin concentration after therapy, according to the initial haemoglobin concentrations, are presented in Table 4.

Within each group an analysis was made to see if there was any relationship between the serum albumin concentration and the rise in haemoglobin or the final haemoglobin concentration. No such correlation could be found.
Mean corpuscular haemoglobin concentration

The mean initial and final mean corpuscular haemoglobin concentration (MCHC), the mean of individual differences and its standard error, in each of the groups, are shown in Table 5. The final MCHC was slightly but significantly lower than the initial value in the two groups (0 and 1) not receiving iron therapy, but in all groups receiving iron the final MCHC was a little higher than the initial, though this only attained statistical significance in the case of groups 4 and 5 (120 and 240 mg iron/day together with B₁₂ and folate). However, even in group 5, only 15 per cent of women had an MCHC of 32 or more at the end of the supplementation period.

Serum iron and per cent saturation transferrin

The mean initial and final serum iron concentrations and per cent saturation of transferrin for all the groups are shown in Table 6. Seventy-one per cent of all women had an initial serum iron of less than 60 μg/100 ml, and 69 per cent of all the women had an initial per cent saturation less than 15.

The final mean serum iron concentrations and per cent saturation of transferrin were the same or slightly lower than the initial values, in groups 0 and 1 who did not receive iron, but in all groups receiving iron the final values were significantly higher than the initial, the highest being in group 5 (240 mg/day).

Foetal birth weight

Foetal birth weight was available in 47 per cent of the subjects. There was no significant difference between the mean birth weights in the various groups (Table 7).
### Table 3. Comparison of regression lines: haemoglobin values after therapy on initial haemoglobin values

<table>
<thead>
<tr>
<th>Group number</th>
<th>Source of variation number</th>
<th>Degrees of freedom</th>
<th>Σ (X - X̄)^2</th>
<th>Σ (X - X̄)(Y - Ȳ)</th>
<th>Σ (Y - Ȳ)^2</th>
<th>Regression coefficient</th>
<th>Deviations from regression</th>
<th>df</th>
<th>s.e.</th>
<th>m.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Placebo</td>
<td>60</td>
<td>90.62</td>
<td>92.96</td>
<td>130.40</td>
<td>0.96</td>
<td>88 41.41</td>
<td>0.24</td>
<td>86.00</td>
<td>3,00</td>
</tr>
<tr>
<td>1</td>
<td>B₃₁₇ + F.A.</td>
<td>90</td>
<td>144.75</td>
<td>119.53</td>
<td>132.86</td>
<td>0.78</td>
<td>89 70.82</td>
<td>0.25</td>
<td>85.00</td>
<td>3,00</td>
</tr>
<tr>
<td>2</td>
<td>B₃₁₇ + F.A. + 30 mg iron</td>
<td>88</td>
<td>177.59</td>
<td>115.05</td>
<td>145.17</td>
<td>0.86</td>
<td>87 64.99</td>
<td>0.28</td>
<td>86.00</td>
<td>3,00</td>
</tr>
<tr>
<td>3</td>
<td>B₃₁₇ + F.A. + 40 mg iron</td>
<td>88</td>
<td>250.77</td>
<td>106.54</td>
<td>165.58</td>
<td>0.95</td>
<td>91 68.20</td>
<td>0.29</td>
<td>89.00</td>
<td>3,00</td>
</tr>
<tr>
<td>4</td>
<td>B₃₁₇ + F.A. + 125 mg iron</td>
<td>114</td>
<td>234.89</td>
<td>50.67</td>
<td>122.99</td>
<td>0.84</td>
<td>90 63.08</td>
<td>0.31</td>
<td>86.00</td>
<td>3,00</td>
</tr>
<tr>
<td>5</td>
<td>B₃₁₇ + F.A. + 240 mg iron</td>
<td>88</td>
<td>105.92</td>
<td>56.32</td>
<td>115.35</td>
<td>0.81</td>
<td>90 60.35</td>
<td>0.32</td>
<td>85.00</td>
<td>3,00</td>
</tr>
<tr>
<td>6</td>
<td>120 mg iron alone</td>
<td>106</td>
<td>110.50</td>
<td>133.53</td>
<td>164.74</td>
<td>0.79</td>
<td>91 59.45</td>
<td>0.34</td>
<td>87.00</td>
<td>3,00</td>
</tr>
</tbody>
</table>

Pooled within treatment groups  640  1192.81  655.77  1020.21  0.44  650 649.45  1.91

Differences between slopes  6  5.74  9.61

Overall comparison between slopes  F = 12.31 (df = 6, 335)  P < 0.001

### Table 4. Estimated regression functions and expected mean haemoglobin concentration after ten weeks of therapy depending on initial haemoglobin concentration

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Estimated regression function</th>
<th>Expected (mean) haemoglobin concentration after 10 weeks therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial haemoglobin concentration g/100 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6  7  8  9  10  11</td>
</tr>
<tr>
<td>0 Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 B₃₁₇ + F.A.</td>
<td>Y = 0.10 + 0.96 X</td>
<td></td>
</tr>
<tr>
<td>2 B₃₁₇ + F.A. + 30 mg iron</td>
<td>Y = 4.11 + 0.65 X</td>
<td></td>
</tr>
<tr>
<td>3 B₃₁₇ + F.A. + 60 mg iron</td>
<td>Y = 5.37 + 0.58 X</td>
<td></td>
</tr>
<tr>
<td>4 B₃₁₇ + F.A. + 120 mg iron</td>
<td>Y = 8.16 + 0.28 X</td>
<td></td>
</tr>
<tr>
<td>5 B₃₁₇ + F.A. + 240 mg iron</td>
<td>Y = 8.32 + 0.28 X</td>
<td></td>
</tr>
<tr>
<td>6 120 mg iron</td>
<td>Y = 3.62 + 0.70 X</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. Mean initial and final mean corpuscular haemoglobin concentrations in the different groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of women</th>
<th>MCHC Initial</th>
<th>Mean of individual differences</th>
<th>Standard error of mean differences</th>
<th>P paired t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Placebo</td>
<td>70</td>
<td>20.06</td>
<td>20.69 - 0.708</td>
<td>0.219</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 B₃₁₇ + F.A.</td>
<td>91</td>
<td>20.49</td>
<td>20.96 - 0.338</td>
<td>0.233</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>2 B₃₁₇ + F.A. + 30 mg iron</td>
<td>89</td>
<td>20.17</td>
<td>20.65 - 0.382</td>
<td>0.241</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3 B₃₁₇ + F.A. + 60 mg iron</td>
<td>91</td>
<td>20.19</td>
<td>20.32 + 0.341</td>
<td>0.270</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>4 B₃₁₇ + F.A. + 120 mg iron</td>
<td>115</td>
<td>20.45</td>
<td>20.17 + 0.711</td>
<td>0.236</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>5 B₃₁₇ + F.A. + 240 mg iron</td>
<td>84</td>
<td>20.18</td>
<td>20.53 + 0.007</td>
<td>0.234</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6 120 mg iron alone</td>
<td>107</td>
<td>20.04</td>
<td>20.60 + 0.060</td>
<td>0.278</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Treatment group</td>
<td>Number of women</td>
<td>Mean serum iron concentration (μg/100 ml)</td>
<td>Mean of individual differences</td>
<td>Standard error of mean of differences</td>
<td>P paired t-test</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>-------------------------------------------</td>
<td>---------------------------------</td>
<td>--------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>6 Placebo</td>
<td>70</td>
<td>55.6</td>
<td>52.6 - 3.0</td>
<td>2.49</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>1 B12 + F.A.</td>
<td>30</td>
<td>47.2</td>
<td>43.6 - 3.0</td>
<td>3.89</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>2 B12 + F.A. + 30 mg iron</td>
<td>41</td>
<td>42.5</td>
<td>66.0 +25.5</td>
<td>12.12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3 B12 + F.A. + 60 mg iron</td>
<td>44</td>
<td>42.2</td>
<td>75.7 +53.3</td>
<td>9.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4 B12 + F.A. + 120 mg iron</td>
<td>71</td>
<td>55.2</td>
<td>90.2 +14.3</td>
<td>5.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5 B12 + F.A. + 240 mg iron</td>
<td>38</td>
<td>44.8</td>
<td>88.3 +43.5</td>
<td>6.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 120 mg iron</td>
<td>55</td>
<td>57.0</td>
<td>80.4 +23.4</td>
<td>4.83</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Mothers and infants at three months post-partum

Blood samples were obtained in approximately 40 per cent of the mothers and infants three months post partum. There was no significant difference between the maternal mean haemoglobin concentrations in the various groups (Table 8) except that the women in group 5 (240 mg/day) had a slightly higher haemoglobin concentration than those in groups 0, 1 and 2 who received no iron (P < 0.05) in each case.

In the infants all groups had a similar mean haemoglobin concentration (Table 9).

### Table 7. Birth weight of infants born to mothers in the different groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number</th>
<th>Birth weight in grams</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Placebo</td>
<td>42</td>
<td>2760-5</td>
<td>74-45</td>
<td></td>
</tr>
<tr>
<td>1 B12 + F.A.</td>
<td>56</td>
<td>2652-0</td>
<td>49-85</td>
<td></td>
</tr>
<tr>
<td>2 B12 + F.A. + 30 mg iron</td>
<td>43</td>
<td>2696-9</td>
<td>64-35</td>
<td></td>
</tr>
<tr>
<td>3 B12 + F.A. + 60 mg iron</td>
<td>42</td>
<td>2633-6</td>
<td>75-78</td>
<td></td>
</tr>
<tr>
<td>4 B12 + F.A. + 120 mg iron</td>
<td>41</td>
<td>2663-6</td>
<td>77-22</td>
<td></td>
</tr>
<tr>
<td>5 B12 + F.A. + 240 mg iron</td>
<td>44</td>
<td>2731-1</td>
<td>55-19</td>
<td></td>
</tr>
<tr>
<td>6 120 mg iron alone</td>
<td>33</td>
<td>2821-5</td>
<td>62-99</td>
<td></td>
</tr>
</tbody>
</table>

### Table 8. Maternal haemoglobin concentration at three months post partum

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number</th>
<th>Haemoglobin concentration Mean g/100 ml</th>
<th>Standard error</th>
<th>Comparison with placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Placebo</td>
<td>36</td>
<td>11-17</td>
<td>0.151</td>
<td>—</td>
</tr>
<tr>
<td>1 B12 + F.A.</td>
<td>24</td>
<td>11-05</td>
<td>0.239</td>
<td>N.S.</td>
</tr>
<tr>
<td>2 B12 + F.A. + 60 mg iron</td>
<td>41</td>
<td>11-09</td>
<td>0.182</td>
<td>N.S.</td>
</tr>
<tr>
<td>3 B12 + F.A. + 120 mg iron</td>
<td>29</td>
<td>11-27</td>
<td>0.218</td>
<td>N.S.</td>
</tr>
<tr>
<td>4 B12 + F.A. + 240 mg iron</td>
<td>61</td>
<td>11-33</td>
<td>0.153</td>
<td>N.S.</td>
</tr>
<tr>
<td>5 B12 + F.A. + 240 mg iron</td>
<td>20</td>
<td>11-71</td>
<td>0.183</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>6 120 mg iron</td>
<td>32</td>
<td>11-29</td>
<td>0.201</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S. = not significant.

### Table 9. Infant’s haemoglobin concentration at three months of age

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number</th>
<th>Haemoglobin concentration Mean g/100 ml</th>
<th>Standard error</th>
<th>Comparison with placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Placebo</td>
<td>45</td>
<td>9-86</td>
<td>0.193</td>
<td>—</td>
</tr>
<tr>
<td>1 B12 + F.A.</td>
<td>40</td>
<td>10-08</td>
<td>0.173</td>
<td>N.S.</td>
</tr>
<tr>
<td>2 B12 + F.A. + 60 mg iron</td>
<td>31</td>
<td>10-01</td>
<td>0.234</td>
<td>N.S.</td>
</tr>
<tr>
<td>3 B12 + F.A. + 60 mg iron</td>
<td>36</td>
<td>10-01</td>
<td>0.204</td>
<td>N.S.</td>
</tr>
<tr>
<td>4 B12 + F.A. + 120 mg iron</td>
<td>33</td>
<td>10-02</td>
<td>0.147</td>
<td>N.S.</td>
</tr>
<tr>
<td>5 B12 + F.A. + 240 mg iron</td>
<td>24</td>
<td>10-16</td>
<td>0.203</td>
<td>N.S.</td>
</tr>
<tr>
<td>6 120 mg iron</td>
<td>40</td>
<td>10-16</td>
<td>0.209</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S. = not significant.
Hookworm

Of all the women 41.5 per cent had hookworm infestation but only 4 per cent of the women in Vellore and none of those in Delhi had ova counts above 1000 eggs/gram of faeces. There was no relationship between the initial haemoglobin concentration, haematocrit, serum iron concentration or response to supplementation and the presence or absence of hookworm.

Side effects of medication

In each of the groups 0 to 5, a total of one to three individuals dropped out of the trial because of intolerance to medication. In group 6, 20 subjects dropped out because they developed gastrointestinal symptoms chiefly abdominal pain and vomiting. This difference between group 6 and the other groups was significant at the 0.1 per cent level.

DISCUSSION

This study confirms previous findings of a high prevalence of anaemia and iron deficiency in unsupplemented pregnant Indian women (Sidhu, Sood, and Ramalingaswami, 1967; Sood, Banerji, and Ramalingaswami, 1968; Yusufji, Mathan, and Baker, 1973). Rather similar prevalences have also been reported from other developing countries such as Malaysia (Lourdenadin, 1964), Trinidad (Chopra, Noe, Mathew, Dhewa, Rose, Cooperman, and Lubhy, 1967), Latin America (Cook, Alvandol, Gutinsky, Jamra, Labardini, Layrisse, Linares, Loria, Mases, Restrepo, Reynafarje, Sanchez-Medal, Velez, and Viteri, 1971), Israel (Rachmlewitz, Nitzkin, Levy, Salmonowwitz, Grossovics, and Izak, 1966), Sri Lanka (de Mel, 1974; Senewiratne, Hettiarachchi, and Senewiratne, 1974) and Thailand (Aung-Than-Batu, Hla-Pe, Thein-Than, and Nyunt, 1972) indicating that pregnancy anaemia is still a problem of considerable global magnitude.

The frequency distribution of the preliminary (22 weeks) haemoglobin concentration in the Delhi women formed an almost normal distribution (Fig. 3) indicating a fairly homogeneous anaemic population. In Vellore, only capillary blood values were obtained at the preliminary period so they are not strictly comparable. For the first four weeks the subjects in both centres were given either placebo or vitamin B₁₂ and folic acid. This was intended to eliminate the effects, if any, of vitamin B₁₂ and folic acid deficiency on the subsequent response to iron. During the four-week period, the mean haemoglobin dropped similarly both in those receiving only placebo (stream 'A'), and in those receiving vitamin B₁₂ and folate (stream 'B'). It may be concluded either that vitamin B₁₂ and folate deficiency were not an important cause of anaemia in these communities at this stage of pregnancy, or that the concomitant iron deficiency masked any response to vitamin B₁₂ and/or folate administration (Tasker, 1959).

The frequency distribution curve for the initial haemoglobin concentration at the end of the four-week preliminary period (28 ± 2 weeks) was almost identical in both centres, and again indicated a fairly homogeneous anaemic population (Fig. 3).

During the 10 to 12 weeks of the study, in groups 0 and 1 who did not receive any iron supplement, the mean haemoglobin haematocrit and MCHC fell
significantly. The fact that the two groups behaved similarly indicates that administration of folate and vitamin B₁₂ alone did not prevent or cure the anaemia in these women.

When the groups receiving folate, vitamin B₁₂, and different amounts of iron were compared, groups 4 and 5, receiving 120 and 240 mg of iron daily, had the best response, as measured by the rise in haemoglobin concentration (Table 4) and the lowest prevalence of 'anaemia' (World Health Organization, 1972) at the time of the final examination. In terms of serum iron and per cent saturation transferrin the results were slightly better with the 240 mg dose than the 120 mg. It should be noted, however, that even in groups 4 and 5, 56 per cent of the women had a final haemoglobin concentration of less than 11 g/100 ml and 27 per cent had a serum iron of less than 60 μg/100 ml and a per cent saturation of transferrin of less than 15 indicating persisting iron deficiency. Since all these women were seen to swallow their tablets regularly it would appear that the iron in the tablets was either not available, due, for example, to failure of dissolution of the tablets, or that the percentage absorption from the available intraluminal gastrointestinal iron pool was unusually low.

Assuming that all the additional iron absorbed from the given supplement was used for maternal haemoglobin production, the mean daily amount of iron absorbed from the supplement at each dose level was calculated. This ranged from 2.0 mg/day in groups 2 and 6 (6.7 per cent and 1.7 per cent of the administered dose respectively) to 3.4 mg/day in group 5 (14 per cent of the dose). These calculations take no account of extra iron that may have been transferred to the foetus and placentas. Since there was no significant difference, between the different groups, in the infants' haemoglobin concentration at three months of age, it is probable that the foetal iron stores in the different groups were similar and, therefore, the assumption that the majority of the absorbed supplemental iron was used in the manufacture of maternal haemoglobin may be justified. In Fig. 5 the mean daily iron absorption in each group is compared with that of the four non-pregnant subjects with iron deficiency anaemia and with the anticipated absorption from a single dose of iron, in iron deficient and iron replete German subjects, drawn from the data of Heinrich (1970). It will be seen that the absorption in the pregnant women is much less than in the non-pregnant subjects and is also much less than the anticipated absorption in German subjects at all dose levels, even though many of the pregnant women were still markedly iron deficient at the end of the period of supplementation. Heinrich's data is based on radioactive iron absorption studies carried out in fasting subjects. Since, in the present study, the time of administration of the tablets with respect to food intake was not controlled, it is possible that absorption may have been depressed by some inhibitory factors present in the prevailing largely vegetarian diet. (Layrisse, Martinez-Torres, and Roche, 1968; Layrisse, Cook, Martinez-Torres, Roch, Kuhn, and Fineh, 1969). However it should be noted that in the four non-pregnant subjects with iron deficiency anaemia the mean iron absorption was greater than in the pregnant women by a factor of four or more, even though the tablets were regularly given just after food.

The possible role of intestinal disease in decreasing the iron absorption in these
women must also be considered. Iron malabsorption has been documented in non-tropical sprue (Badenoch and Callender, 1954) but not so far in tropical sprue. The present subjects did not have overt tropical sprue since all subjects with chronic diarrhea were excluded from the study. Nevertheless asymptomatic enteropathy, as manifested by changes of gastrointestinal structure and function, is prevalent in India (Baker and Mathan, 1972) and it is possible that this may have been a factor in some of the women. If malabsorption of iron, due either to inhibitory substances in the food or due to intestinal disease, is a significant factor, better results might be expected with parenteral iron therapy. Studies to explore this possibility are in progress.

Group 6 was added to the study after its initiation, in order to determine whether or not vitamin B₁₂ and folate administration had any significant effect. This group was given tablets containing 120 mg of elemental iron as ferrous fumarate with no vitamin B₁₂ or folate supplement. These tablets were made in the same way as those given to the other groups but were a different batch. The rise in haemoglobin in this group was significantly less than that in group 3 who received the same dose of iron together with vitamin B₁₂ and folate. It must be concluded either that the availability of the iron in the group 6 tablets was in some way different from that
in the group 3 tablets, or that the vitamin B₁₂ and folate did have an effect on the final haemoglobin concentration, even though administration of vitamin B₁₂ and folate in the first four weeks had no detectable effect and the final result in group 1, receiving only B₁₂ and folate, was only marginally better than in group 0 receiving placebo. Previous studies in India have shown a high prevalence of low serum folate and vitamin B₁₂ concentrations in pregnant women and have suggested that folate deficiency is a contributory factor in the prevalent pregnancy anaemia (Kartagani, Ganakasundaram, and Baker, 1984; Sidhu, Sood, and Ramalingaswami, 1967; Iyengar, 1971; Yusufji, Mathan, and Baker, 1973). It seems probable, therefore, that the difference between groups 6 and 3 is attributable to the folate received by the latter, rather than the vitamin B₁₂ or possible variations in tablet composition.

It is also of interest that, in both centres, in group 6 there were a significant number of subjects who dropped out of the trial because they developed symptoms such as abdominal pain and vomiting which they attributed to the medication, whereas women receiving double this amount of iron, together with folic acid, noted no such effects. Whether the folic acid in the tablets in some unknown way prevented the occurrence of side effects is not clear.

The finding, that the best results were obtained with 120 or 240 mg iron plus vitamin B₁₂ and folate, is in marked contrast to the study in southern India of Iyengar and Apte (1970) who recommend a supplement of 20 mg of elemental iron. However these workers were dealing with a less anaemic population and they only used the one dosage level of iron. They might well have got better results with higher amounts of iron. De Leeuw, Lowenstein, and Yang-Shu (1966), in a carefully conducted study in Canada, showed that oral daily supplementation of normal pregnant women with 39 mg of elemental iron as ferrous sulphate for 24 weeks was not as effective as 78 mg of iron in maintaining an optimum haemoglobin. They therefore recommended the latter amount. If this is true for Canada, in view of both the higher prevalence and severity of iron deficiency in India, it is not surprising that the results of the present study indicate that a higher amount of iron is more suitable.

The finding in this study, that even with the administration of 120 or 240 mg of iron, folate and vitamin B₁₂, 56 per cent of the women still had a final haemoglobin concentration below 11 g/100 ml, is in marked contrast with the findings in the West (DeGruchy, 1964) which have led W.H.O. (1972) to define ‘anaemia’ in pregnancy as a haemoglobin concentration less than 11 g per 100 ml.

In a study in Israel, Levy, Rachmilewitz, Izak, Solomonowitz, and Rachmilewitz (1968) administered 160 mg of elemental iron (as ferrous gluconate) plus 5 mg of folate and 100 μg vitamin B₁₂ daily to 184 pregnant women with haemoglobin concentrations ranging from 10 to 6 g per 100 ml. Medication was given daily for an average of 4-9 months. At the end of this time 51 per cent still had a haemoglobin concentration of 10 g per 100 ml or less. Although this was a selected group, the persistence of anaemia in spite of apparently adequate, iron folate and vitamin B₁₂ administration, bears a striking resemblance to the findings reported here.

The factors which are responsible for the high prevalence of anaemia in Indian women in spite of supplementation with the three main haematinics may be multiple. As already discussed, many women still had evidence of iron deficiency even though
they had received a supplement of 240 mg of elemental iron per day for 10 to 12 weeks and this is probably the major factor. Many of the women lived on a diet which has a low protein content (Rao and Rao, 1969) and it is theoretically possible that protein deficiency may also play a role in the prevalence of anaemia. However the fact that there was no correlation between the rise in haemoglobin concentration and the concentration of serum albumin makes this possibility unlikely.

It is evident from this study that any programme of prophylactic administration of oral iron supplements to pregnant women in India must provide at least 120 mg of elemental iron daily, if not more. Such an amount cannot be provided by any programme of food fortification and can only be achieved by medicinal supplementation. However, before recommending such supplementation on a national scale, it is important to evaluate the cost and public health benefits of the programme. Although the groups of women receiving supplements all had significantly higher mean final haemoglobin values, it has not been possible in this study to demonstrate any other beneficial effect of iron therapy. Thus there was no significant difference in foetal birth weight between the control groups and those receiving iron, vitamin B₁₂ and folate, nor was there a significant difference in haemoglobin concentration of the infants three months after delivery.

Severe degrees of anaemia increase maternal morbidity and mortality and carry an increased risk to the foetus, however the deleterious effects of milder degrees of anaemia in pregnancy are not well defined. In the ideal situation all pregnant women should have a normal haemoglobin concentration and normal stores of iron, folate and vitamin B₁₂. But where resources are limited it may not be possible to aim at achieving this ideal. In such a situation priorities must be established so that available resources can be utilized to achieve the greatest benefit. The women most at risk are presumably those with the lowest haemoglobin concentration and treatment for these women must be a high priority for any public health programme. The precise haemoglobin concentration below which initiation of therapy becomes important is not clearly defined, but it is suggested that, for purposes of discussion, a concentration below 8 g/100 ml may be taken. In this study, at the time of the final haemoglobin estimation, 13 per cent of unsupplemented women had a haemoglobin concentration of less than 8 per cent. It should however, be noted, that the prevalence of severe anaemia in pregnant women is slightly higher than this (less than 1 per cent higher) because three women with an initial haemoglobin concentration of less than 5 g/100 ml were excluded from the trial. From the public health point of view it must be decided whether it is better to provide treatment for the severely anaemic women in the community by attempting to supply suitable supplementation to all pregnant women, or to identify and treat the very anaemic group. The former approach has the big advantage that it will simultaneously treat the milder degrees of anaemia, but the disadvantages that it may be ineffective in curing the severe anaemia in a small proportion of cases (in the present study 2 per cent of women in groups 4 and 6, receiving 120 and 240 mg or iron, still had a haemoglobin concentration below 8 g/100 ml) and it may be more costly. A programme aimed at identifying the severely anaemic might enable closer supervision of the results of therapy and might therefore achieve better results in this group. However the detect-
ion of the severely anemic women in the community also poses some problems. Presently, there is not sufficient information regarding technical feasibility, relative costs and expected benefits, to enable a rational choice to be made between these two alternatives.

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