Vitamin B₁₂ levels in erythrocytes in hypochromic anaemia

R. J. HARRISON

From St Helens Hospital, St Helens, Lancs.

SYNOPSIS Vitamin B₁₂ levels in erythrocytes were low in untreated hypochromic anaemia, rose to abnormally high levels during therapy with iron alone, and finally slowly fell to normal. These changes were similar to those previously found in pernicious anaemia in response to vitamin B₁₂ therapy and in folate-deficiency anaemia in response to folic acid, thus changes in erythrocyte B₁₂ levels are not always due directly to changes in B₁₂ metabolism but may be secondary to changes in the levels of other haematinic factors.

During the investigation of the changes in vitamin B₁₂ levels in erythrocytes in vitamin B₁₂ and in folate-deficiency anaemia, subnormal levels of vitamin B₁₂ in the erythrocytes were also found in patients suffering from untreated hypochromic anaemia, despite normal plasma levels of the vitamin. An investigation of vitamin B₁₂ levels in erythrocytes in hypochromic anaemia, before and during therapy with iron, was therefore undertaken.

Method

Blood was examined from 20 subjects suffering from uncomplicated iron-deficiency anaemia in whom the plasma vitamin B₁₂ level was within normal limits, ie, above 140 pg/ml. The diagnosis was based upon, and response to therapy assessed by, haemoglobin (Hb 100% = 14.8 g/100 ml) and packed cell volume (PCV %) readings. A Hawkesley microcentrifuge was used for the latter estimation. Plasma and washed erythrocytes were assayed for vitamin B₁₂ activity, before and during therapy, using *Lactobacillus leichmannii*, by a technique described earlier (Harrison, 1970). Iron only was used in treatment, with the exception of patient no. 9 who also received a blood transfusion (4 pints) on day 9. Patients 1 to 10 received intramuscular iron (Jectofer 2 ml on alternate days for periods ranging from eight to 20 days) and the remainder received iron by mouth (Fersolate tabs. 1, tds, or Ferrogradumet tabs. 1 daily).

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Results

These are shown in Table I and Figure 1. The initial Hb concentration in each case ranged from 4.6 g/100 ml to 8.8 g/100 ml (mean = 6.6 g/100 ml) and the MCHC from 22 to 29%; the plasma vitamin B₁₂ levels lay between 144 and 760 pg/ml and the mean was 328 pg/ml (normal = 140-800 pg/ml). The erythrocyte vitamin B₁₂ levels before treatment ranged from 60 to 130 pg/ml (mean = 99 ± SD

Fig. Concentration of vitamin B₁₂ in erythrocytes during therapy with iron, showing individual levels and the average.

Case	Age and Sex	Initial			Final			Vitamin B ₁₂ Level (pg/ml)					
		Hb (g/100ml)	PCV(%)	MCHC (%)	Hb (g/100ml)	PCV(%)	MCHC (%)	Plasma	Erythrocytes				
									Initial	Peak Observed	(Days)	Final	(Days)
1	51F	5.7	23	25	15.4	45	34	200	82	340	(10)	192	(103)
2	43F	7.3	30	24	14.8	46	33	720	82	640	(7)	170	(66)
3	68M	8.4	28	29	12.0	37	32	540	108	280	(8)	168	(32)
4	24F	8.8	29	29	13.4	43	31	152	120	308	(14)	180	(112)
5	75F	4.6	18	26	14.7	47	31	360	76	320	(14)	133	(57)
6	48F	7.7	27	28	12.9	40	31	180	100	288	(14)	120	(113)
7	40F	7.2	27	26	11.3	36	30	280	86	220	(10)	100 ¹	(68)
8	79M	6.0	20	29	11.7	35	32	200	122	440	(9)	280	(50)
9	79M	4.9	19	26	11.9	37	32	260	108	740	(8)	—	—
10	85F	5.5	20	27	10.5	34	31	240	60	380	(23)	190	(56)
11	18F	6.3	24	26	11.2	37	30	520	92	440	(7)	156	(240)
12	76F	7.9	28	28	12.8	37	34	360	76	280	(6)	160	(42)
13	46F	5.8	24	27	12.5	40	31	210	130	200	(16)	150	(32)
14	58F	5.4	22	24	14.1	41	34	240	128	720	(9)	140	(90)
15	38F	6.8	26	25	10.6	31	33	240	96	250	(8)	120	(16)
16	45F	7.2	28	25	12.3	38	32	480	96	320	(16)	142	(44)
17	17M	6.7	27	25	15.4	45	35	144	108	270	(10)	140	(87)
18	17F	7.3	30	24	13.1	43	30	760	108	188	(30)	164	(43)
19	51F	6.4	29	22	13.4	47	28	300	120	420	(22)	200	(40)
20	38F	6.4	25	26	13.0	40	32	172	86	230	(11)	160	(52)
Mean		6.6	25	26	13.0	40	32	328	99	363	(12)	161	(71)

Table I Erythrocyte B₁₂ and Hb response to iron in hypochromic anaemia¹No iron therapy for six weeks—becoming iron-deficient again.

19.7 pg/ml), which was significantly below the normal range of 155 ± 35 pg/ml ($P = < 0.00005$). During therapy with iron the observed erythrocyte vitamin B₁₂ levels rose to between 188 and 720 (mean = 344 ± 140 pg/ml, which was a highly significant rise ($P = < 0.00005$) above both the untreated and the normal levels. There was little difference between the highest levels observed in those patients treated with intramuscular iron (mean = 346 pg/ml at the 12th day) and those with oral iron (mean = 331 pg/ml at 13.5 days). The erythrocyte B₁₂ level subsequently fell gradually and ultimately ranged from 100 to 280 (mean = 161 ± 27) pg/ml which was close to the normal ($P = 0.2$). The Hb, PCV, and MCHC all improved during therapy, the mean Hb rising to 13 g/100 ml.

The high standard deviation at the peak response was due to individual variation in the degree of response (see Table I).

Discussion

The subnormal levels of assayable vitamin B₁₂ in erythrocytes in hypochromic anaemia before treatment may be due to iron playing a part in many enzyme systems in cells, particularly those involved in cellular respiration. Deficiency of iron, therefore, might be reflected in a diminution of the enzyme content of the cells and thus either prevent the uptake of vitamin B₁₂ through the cell membrane, or

prevent its utilization within the cells. Retief, Gottlieb, and Herbert (1966), however, found that the uptake of radioactive B₁₂ from the plasma by reticulocytes *in vitro* was not inhibited by metabolic poisons such as sodium cyanide. This suggests that cell respiration does not play an important part in the transfer of B₁₂ into the cell. However, changes discovered *in vitro* do not necessarily reflect those which occur *in vivo*. Thus Retief *et al* (1966) also noted that the radioactive crystalline B₁₂, which was taken up from plasma by reticulocytes *in vitro*, was located largely in the stromal fraction of the cells, whereas Kato (1958) found that naturally occurring erythrocyte B₁₂, ie, that which had been incorporated into erythroid cells via the bone marrow, was present in the non-stromal fraction. It is also possible that iron and B₁₂ might both be parts of the same cellular enzymes which are deficient in the cells. Another explanation is that vitamin B₁₂ is required in protein synthesis. In this case if, due to iron deficiency, haemoglobin itself was being formed in inadequate amounts, it is also possible that there would be a diminution in the precursors of haemoglobin synthesis, including those containing assayable vitamin B₁₂. In hypochromic anaemia the changes in erythrocyte vitamin B₁₂ levels following therapy with iron are essentially similar to those found during treatment of pernicious anaemia (Harrison, 1970) and folate-deficiency anaemia (Harrison, 1971). Thus the immediate response to therapy coincides with a

considerable increase in the assayable concentration of vitamin B₁₂ within the erythrocytes. As this rise occurs in cases of pernicious anaemia treated with vitamin B₁₂, and folate-deficiency anaemia treated with folic acid, and also in iron-deficiency anaemia treated with iron, it appears to be a function of the response itself and not necessarily of any particular haematinic. The peak level of assayable vitamin B₁₂ in the erythrocytes in most subjects occurred between the sixth and 14th days, which corresponded approximately with the reticulocyte peak.

The fact that a therapeutic response is associated with young cells suggests that the immature cells themselves contain greater than average concentrations of assayable vitamin B₁₂. After the peak has been reached the B₁₂ level falls more slowly than the reticulocyte count and does not, on average, reach normal until the 40th day. This slower fall is probably due to retention of this form of vitamin B₁₂ in the young erythrocytes after the reticulins has disappeared and indicates a gradual loss of assayable B₁₂ from the young erythroid cells. A point that should be borne in mind is that all techniques for assay of vitamin B₁₂ by microbiological methods depend ultimately upon the quantity of the vitamin available for assimilation by the test organism. Thus a certain amount is made available by simple heating to 56°C. This is usually termed 'free' vitamin B₁₂. A further amount in plasma and erythrocytes becomes available after autoclaving, and this additional fraction is conventionally called 'bound' B₁₂. The fractions reported in this communication are those obtained after autoclaving and therefore represent

the so-called 'total' B₁₂, ie, the sum of 'bound' plus 'free' fractions. However, most protein (eg, meat, casein) does not yield its true total B₁₂ content by simple physical means and a large quantity still remains bound to protein and unavailable to assay by microbiological techniques even after autoclaving at 10 lb for 30 min (Mooney and Heathcote, 1967). This raises the question, therefore, whether the 'total' B₁₂ estimated in erythrocytes is, in fact, a true total, and also whether the 'fall' in vitamin B₁₂ observed after the initial response is a true fall in the absolute concentration of the vitamin within the cell or whether the vitamin B₁₂ has gradually become firmly incorporated into the protein in a non-assayable form, ie, it can no longer be freed by physical means.

The results indicate that low levels of erythrocyte vitamin B₁₂ are found not only in pernicious and folate-deficiency anaemias but also in the iron-deficiency state. The investigation, therefore, is of little direct diagnostic value in pernicious anaemia but is a useful method of confirming a response to treatment.

References

- Harrison, R. J. (1970). Vitamin B₁₂ levels in erythrocytes in normal subjects and in pernicious anaemia. *J. clin. Path.*, **23**, 219-224.
- Harrison, R. J. (1971). Vitamin B₁₂ levels in erythrocytes in folate deficiency anaemia. *Brit. J. Haemat.*, **20**, 623-628.
- Kato, N. (1958). Location of vitamin B₁₂ in human erythrocytes. *J. Vitaminol.*, **4**, 226-234.
- Mooney, F. S., and Heathcote, J. G. (1967). Lack of proteolytic digestion in the causation of pernicious anaemia. *Lancet*, **2**, 397.
- Retief, F. P., Gottlieb, C. W., and Herbert, V. (1966). Mechanism of vitamin B₁₂ uptake by erythrocytes. *J. clin. Invest.*, **45**, 1907-1915.