been described which responded haemopoietically to vitamin B₁₂ after failing to respond to folic acid. The experimental work of Heinle, Welch, and Pritchard (1948) is of considerable interest. They produced megaloblastic anaemia in pigs by feeding a highly purified diet free of extrinsic factor and containing succinylsulphathiazole and a crude folic acid antagonist. This diet was calculated to produce a deficiency both of vitamin B₁₂ and of pteroylglutamic acid. A pig rendered anaemic by this method first responded incompletely to liver extract containing no folic acid, but two months later failed to respond to daily injections of liver extract; folic acid then produced an excellent effect. This is a sequence of events somewhat similar to that obtained in our patient. Those authors concluded, from this and other experiments, that in these experimental conditions neither the anti-pernicious-anaemia factor (regarded as identical with or similar to vitamin B₁₂) nor folic acid can function haemopoietically in the absence of the other, and that both substances are essential for the maturation of erythrocytes.

Israëls and Sharp (1950) suggested that the megaloblastic anaemias can be divided into two groups, depending on the effect of treatment. The first is typified by Addisonian pernicious anaemia, which responds well to parenteral or oral liver preparations, vitamin B₁₂, and folic acid. The second group includes "pernicious anaemia" of pregnancy, the megaloblastic anaemia of non-tropical sprue, and probably tropical sprue as well: these conditions react satisfactorily to folic acid but not to parenteral liver extracts or vitamin B₁₂, at least in doses suitable for the first group. The present case appears to fall between these two groups, for, although by no means refractory to vitamin B₁₂, the response was suboptimal compared with that expected in Addisonian pernicious anaemia. Folic acid is unnecessary at any stage in Addisonian pernicious anaemia treated with vitamin B₁₂ and usually essential from the outset in non-tropical sprue. In our case it was required only for "topping up" after vitamin B₁₂.

Final evaluation of the respective functions of folic acid and vitamin B₁₂ must await further work. Evidence collected from various clinical experiences, such as that described in this paper, may eventually help to solve the problem.

Summary

A case of megaloblastic anaemia following total gastrectomy performed for the effects of corrosive poisoning is described.

The pathogenesis of the anaemia is discussed in the light of the classical theory of erythropoiesis.

Striking clinical improvement followed vitamin B₁₂ therapy, but the haematological response was suboptimal compared with that expected in Addisonian pernicious anaemia. The addition of folic acid brought about complete haematological remission.

The effect of treatment suggested that this type of megaloblastic anaemia and Addisonian pernicious anaemia are not identical, although morphologically similar.

The relationship of the anaemia to other megaloblastic anaemias, as regards treatment with vitamin B₁₂ and folic acid, is discussed.

We wish to thank Professor C. F. W. Illingworth for permission to publish this case and Professor J. W. McNee for advice. The blood investigations in 1944 and 1946 were performed by Dr. Peter McKenzie, and we are indebted to him for permission to quote his results.

REFERENCES

- Alsted, G. (1937). *Lancet*, **1**, 76.
 Beebe, R. T., and Meneely, J. K., jun. (1949). *N.Y. St. J. Med.*, **49**, 2437.
 Bethell, F. H., Meyers, M. C., Andrews, G. A., Swendseid, M. E., Bird, O. D., and Brown, R. A. (1947). *J. Lab. clin. Med.*, **32**, 3.
 — Sturgis, C. C., Rundles, R. W., and Meyers, M. C. (1945). *Arch. intern. Med.*, **76**, 239.
 — Swendseid, M. E., Meyers, M. C., Neligh, R. B., and Richards, H. G. (1949). *Univ. Hosp. Bull., Ann. Arbor*, **15**, 49.
 Bloomfield, A. L., and Pollard, W. S. (1935). *J. clin. Invest.*, **14**, 321.
 Castle, W. B., Townsend, W. C., and Heath, C. W. (1930). *Amer. J. med. Sci.*, **180**, 305.
 Della Vida, B. L., and Dyke, S. C. (1942). *Lancet*, **2**, 275.
 Dennig, H. (1929). *Münch. med. Wschr.*, **76**, 633.
 Dyke, W. J. C., Hind, H. G., Riding, D., and Shaw, G. E. (1950). *Lancet*, **1**, 486.
 Ellis, A. (1925). *Proc. R. Soc. Med.*, **18**, Sect. Neur., 27.
 Finney, J. M. T., and Rienhoff, W. F., jun. (1929). *Arch. Surg., Chicago*, **18**, 140.
 Fox, H. J., and Castle, W. B. (1942). *Amer. J. med. Sci.*, **203**, 18.
 Fullerton, H. W. (1945). *Edinb. med. J.*, **52**, 412.
 Girdwood, R. H. (1950). *Ibid.*, **57**, 72.
 Go'dhamer, S. M. (1937). *Amer. J. med. Sci.*, **193**, 23.
 Hall, B. E. (1950). *British Medical Journal*, **2**, 585.
 Heinle, R. W., Welch, A. D., and Pritchard, J. A. (1948). *J. Lab. clin. Med.*, **33**, 1647.
 Hochrein, M. (1929). *Münch. med. Wschr.*, **76**, 1327.
 Hurst, A. F. (1932). *Quart. J. Med.*, n.s. **1**, 157.
 Isaacs, R., and Friedman, A. (1938). *Amer. J. med. Sci.*, **196**, 718.
 Israëls, M. C. G., and Sharp, J. (1950). *Lancet*, **1**, 752.
 Landboe-Christensen, E., and Plum, C. M. (1948). *Amer. J. med. Sci.*, **215**, 17.
 MacDonald, R. M., Ingelfinger, F. J., and Belding, H. W. (1947). *New Engl. J. Med.*, **237**, 887.
 Magnus, H. A., and Ungley, C. C. (1938). *Lancet*, **1**, 420.
 Meulengracht, E. (1935). *Ibid.*, **1**, 493.
 Morgans, M. E., Rimington, C., and Whittaker, N. (1947). *Ibid.*, **2**, 128.
 Murphy, W. P. (1933). *Arch. intern. Med.*, **52**, 829.
 Penati, F., and Saita, G. (1938). *Arch. Sci. méd.*, **65**, 790.
 Schwartz, S. O., and Legere, H. (1944). *J. Amer. med. Ass.*, **124**, 637.
 Ungley, C. C. (1932). *Lancet*, **2**, 1426.
 — (1949). *British Medical Journal*, **2**, 1370.
 Weir, J. F. (1946). *Surg. clin. N. Amer.*, **26**, 934.
 Wintrobe, M. M. (1946). *Clinical Haematology*, 2nd ed., p. 397. London.

HAEMOPOIETIC ACTIVITY OF VITAMINS B_{12c} AND B_{12d} IN PERNICIOUS ANAEMIA

BY

J. N. MARSHALL CHALMERS, M.D., M.R.C.P.
D.P.H.

(From the Haematology Department, St. George's Hospital and Medical School)

After the isolation of vitamin B₁₂ from liver (Lester Smith and Parker, 1948; Rickes *et al.*, 1948a), and the subsequent discovery that the vitamin could be obtained from botanical sources (Rickes *et al.*, 1948b), physico-chemical studies showed that there are other members of the vitamin B₁₂ group of factors. Vitamin B_{12a}, the reduction product of vitamin B₁₂ (Kaczka, Wolf, and Folkers, 1949), has been shown probably to be identical with vitamin B_{12b} (Brockman *et al.*, 1950; Anslow *et al.*, 1950), and crystalline vitamins B_{12c} and B_{12d}, derived from *Streptomyces griseus*, have been described (Lester Smith, 1950, 1951; Anslow *et al.*, 1950; Buchanan *et al.*, 1950). These factors appear to be chromatographically distinct from one another; in addition they have different properties in the microbiological assay (Lester Smith, 1951).

This paper records the primary haematological responses in pernicious anaemia to vitamin B_{12c} (nine cases) and vitamin B_{12d} (five cases).

Material and Methods

The patients, in varying stages of relapse, had clinical signs and symptoms consistent with a diagnosis of pernicious anaemia. Laboratory investigations showed macrocytic anaemia associated with a megaloblastic marrow and histamine-alcohol-fast achlorhydria. Nine patients were females and five males, their ages ranging from 34 to 78 years. Five of them had received previous liver therapy with good results, but had relapsed on discontinuation of injections. No case in this series showed objective evidence of severe neurological involvement, but several of them had some degree of sensory disturbance. No recent therapy had been given before admission to hospital, and they all took "mixed British diets." When in hospital they were on "ordinary" ward diet, and were given no other therapy. Two cases had to be tested as out-patients; other variables were so introduced, but no additional haematinic was known to be taken.

When the red cell count showed a continued fall with no increase in reticulocytes over 2%—this period varying according to the severity of the case—a single dose

of 20 µg. of test material was injected intramuscularly. Regular haematological examinations were made during the 14 days after the injection. The gain in the red cell count in 15 days was taken as the measure of haematological improvement (Della Vida and Dyke, 1942).

The average weekly gain in red cells observed was compared with the so-called "average satisfactory" or "expected" figure obtained from Della Vida and Dyke's formula:

$$I = 0.93 - 0.214 E_0$$

where I = millions average weekly gain in R.B.C. per c.mm. during the first two weeks of therapy, and

E₀ = initial number of R.B.C. in millions per c.mm.

No elaborate statistical analysis is worth while for so small a series of cases (cf. Ungley, 1949): the simple recording of observed results in relation to the standard 20-µg. intramuscular dose of the two new vitamin B₁₂ factors is the object of this report.

On "day 0" and "day 15" venous blood was collected in Wintrobe's oxalate. Haemoglobin estimations were made either with a grey wedge photometer (King *et al.*, 1948), which was N.P.L. tested, or by Haldane's method. Both instruments were calibrated so that 100%

TABLE I.—Haematological Responses in 9 Cases of Pernicious Anaemia Given a Single Dose of 20 µg. Vitamin B_{12c} Intramuscularly

Case No.	Age and Sex	Day of Treatment	Hb g. per 100 ml.	R.B.C. (mill. per c.mm.)	Maximal Reticulocyte Response	P.C.V. (%)	M.C.V. (µ ³)	M.C.H.C. (%)	Bilirubin mg. per 100 ml.	Average Weekly Gain in R.B.C. (mill. per c.mm.) Compared with Della Vida and Dyke's Formula		Remarks
										"Expected"	"Observed"	
1	48 M	0 15	8.7 12.0	2.30 2.93	12% (6th day)	27.5 37.0	120 123	32 32	0.9 < 0.5	0.44	0.31	
2	58 F	0 15	7.6 10.2	1.90 2.71	16.5% (4th day)	23.0 33.0	121 122	33 31	1.1 < 0.5	0.52	0.41	
3	68 F	0 18	8.3 11.1	2.37 3.52	—	26.5	122	31	1.2 —	—	—	Treated as out-patient
4	34 M	0 15	7.8 10.8	1.86 3.05	25% (7th day)	25.0 35.0	134 115	31 31	1.3 —	0.54	0.60	
5	49 M	0 15	14.1 15.1	3.37 3.85	7% (4th day)	42.0 43.0	128 112	34 35	< 0.5 —	0.21	0.24	
6	65 M	0 15	5.8 9.6	1.30 2.22	30.5% (5th day)	16.5 28.0	126 126	35 34	1.1 0.6	0.65	0.46	
7	43 M	0 15	11.5 13.0	2.81 3.49	—	34.5 38.0	123 108	33 34	1.0 < 0.5	0.34	0.34	Treated as out-patient
8	64 F	0 15	5.3 8.6	1.2 2.3	26.5% (5th day)	16.5 29.0	138 126	32 30	1.8 < 0.5	0.67	0.56	Developed iron deficiency
9	37 F	0 15	8.4 9.5	2.2 2.8	16.5% (5th day)	28.0 31.5	128 112	30 30	0.7 < 0.5	0.46	0.30	Associated iron deficiency (see text)

TABLE II.—Haematological Responses in 5 Cases of Pernicious Anaemia Given a Single Dose of 20 µg. Vitamin B_{12d} Intramuscularly

Case No.	Age and Sex	Day of Treatment	Hb g. per 100 ml.	R.B.C. (mill. per c.mm.)	Maximal Reticulocyte Response	P.C.V. (%)	M.C.V. (µ ³)	M.C.H.C. (%)	Bilirubin mg. per 100 ml.	Average Weekly Gain in R.B.C. (mill. per c.mm.) Compared with Della Vida and Dyke's Formula		Remarks
										"Expected"	"Observed"	
10	78 F	0 9 15	5.8 6.7 8.4	1.46 1.86 2.45	19.5% (7th day)	19.5 30.0	133 122	30 28	1.0 < 0.5	— —	—	Intercurrent pulmonary collapse. Second injection given 9th day (see text)
11	76 F	0 15	6.5 10.3	1.47 3.0	28.5% (7th day)	19.0 33.5	129 112	34 31	1.3 0.9	0.615	0.765	Marrow normoblastic 48 hours after injection
12	63 F	0 15	7.6 11.8	1.65 2.93	16.0% (6th day)	23.5 37.0	142 126	32 32	1.1 < 0.5	0.567	0.640	Marrow normoblastic 48 hours after injection
13	69 F	0 15	5.0* 9.0	1.03 2.75	21.5% (6th day)	13.0 30.0	126 109	38* 30	1.7 < 0.5	0.710	0.860	Marrow normoblastic 48 hours after injection
14	69 F	0 15	7.0 10.2	1.70 3.20	26.5% (5th day)	20.0 33.0	118 103	35 31	1.8 1.2	0.566	0.750	Marrow normoblastic 72 hours after injection

* Probably too high readings due to bilirubinaemia.

Hb was equivalent to 14.8 g. Hb per 100 ml. The same instrument was used for all determinations on any one case. When performing red cell counts in an improved Neubauer chamber at least 500 cells were counted. Packed cell volumes were calculated by Wintrobe's method and reticulocytes of capillary blood were estimated by a "dry" cresyl-blue method—counterstaining with Leishman's stain.

Results.—The haematological responses over a 15-day period to a single dose of 20 μ g. of material are recorded in Tables I and II. The response in Case 11, treated with vitamin B_{12d}, is shown in the Chart.

Discussion

The recorded results must be considered in the light of the known errors associated with haematological methods (Biggs and Macmillan, 1948; Dacie, 1950). In the present series of cases no attempt at statistical analysis is deemed permissible, but further cases will be studied and those of the present group available for follow-up will be observed over a further period of time.

Vitamin B_{12c} given in a single dose of 20 μ g. caused a haematological response in the nine cases studied—reticulocytes reaching a peak on the fourth to the seventh day and the formula of Della Vida and Dyke for the 15-day increase being satisfied in three of those eight cases to which it was applicable. In Case 3 the criterion was satisfied on the 18th day; as the patient could not come into hospital she was tested as an out-patient. Case 9, that of a married woman aged 37, had a severe menstrual period during the time of test and showed signs of an associated iron deficiency. After the 15-day test, fortnightly injections of 40 μ g. of vitamin B_{12c} together with a course of oral ferrus sulphate caused rapid and progressive clinical improvement with lessen-

ing of neurological symptoms and glossitis. The blood values were within a so-called "normal range" in two months. Normal range is notoriously difficult to define (see Dacie, 1950), but is here taken as being at least 13.8 g. of Hb per 100 ml., over 4.5 million R.B.C. per c.mm., and a P.C.V. of over 40%. Other cases reached so-called "normal range" within six to ten weeks with a dose of 20 to 40 μ g. of vitamin B_{12c} intramuscularly every fortnight after the test period. All showed a striking improvement, with gain in strength, appetite, and weight.

Of the five patients treated with vitamin B_{12d}, four have fully satisfied the criteria of Della Vida and Dyke for the 15-day period, reticulocyte peaks occurring on the fifth to the seventh day. The remaining patient (Case 10) also had a good response, but she was a frail old lady with intercurrent pulmonary collapse. A second injection of 20 μ g. was given on the ninth day and the formula was not applicable. She was one of the first cases to be treated with this factor, and only two doses of it were at that time available for test. Subsequently there was further recovery on another vitamin B₁₂ preparation. Vitamin B_{12d} on primary clinical trial seems very active. It is too early for conclusions to be drawn from a follow-up, but those cases being investigated show progressive clinical and haematological improvement at the time of writing on doses of 20 μ g. of vitamin B_{12d} at fortnightly intervals.

Summary

Nine cases of pernicious anaemia in relapse have been treated with crystalline vitamin B_{12c} and five cases with crystalline vitamin B_{12d}. Both of these factors are clinically active. Marked clinical improvement, with considerable relief of glossitis and lessening or disappearance of subjective nervous symptoms, occurred. It is too soon to

Haematological Response to Intramuscular Vitamin B_{12d} in Pernicious Anaemia. Case 11.

draw further conclusions, but a more detailed report on the value of these and other fractions and concentrates of the vitamin B₁₂ group will be published in due course.

My thanks are due to Dr. E. Lester Smith and his associates, of Glaxo Laboratories Ltd., Greenford, for supplies of differentiated members of the vitamin B₁₂ group; to those physicians, pathologists, and general practitioners who referred relapsed cases of pernicious anaemia for investigation; and to my technical staff, particularly Mr. S. E. Louland, for their assistance.

REFERENCES

- Anslow, W. K., Ball, S., Emery, W. B., Fantes, K. H., Smith, E. Lester, and Walker, A. D. (1950). *Chem. & Ind.*, p. 574.
 Biggs, R., and Macmillan, R. L. (1948). *J. clin. Path.*, 1, 269.
 Brockman, J. A., jun., Pierce, J. V., Stokstad, E. L. R., Broquist, H. P., and Jukes, T. H. (1950). *J. Amer. chem. Soc.*, 72, 1042.
 Buchanan, J. G., Johnson, A. W., Mills, J. A., and Todd, A. R. (1950). *Chem. & Ind.*, p. 426.
 Dacie, J. V. (1950). *Practical Haematology*. Churchill, London.
 Della Vida, B. L., and Dyke, S. C. (1942). *Lancet*, 2, 275.
 Kaczka, E., Wolf, D. E., and Folkers, K. (1949). *J. Amer. chem. Soc.*, 71, 1514.
 King, E. J., Wootton, I. D. P., Donaldson, R., Sisson, R. B., and MacFarlane, R. G. (1948). *Lancet*, 2, 971.
 Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., and Folkers, K. (1948a). *Science*, 107, 396.
 ——— (1948b). *Ibid.*, 108, 634.
 Smith, E. Lester (1950). *Proc. R. Soc. Med.*, 43, 535.
 ——— (1951). *British Medical Journal*, 1, 151.
 ——— and Parker, L. F. J. (1948). *Biochem. J.*, 43, Proc. viii.
 Ungley, C. C. (1949). *British Medical Journal*, 2, 1370.

TWO CASES OF PERNICIOUS ANAEMIA TREATED WITH VITAMIN B_{12d}

BY

G. C. K. REID, M.B., Ch.B.

Pathologist, Walsall General Hospital

As described by Lester Smith on page 151, vitamin B₁₂ concentrates produced during the fermentation of *Streptomyces griseus* contain a number of factors having biological properties like those of vitamin B₁₂. It is clearly of importance to know whether a concentrate standardized microbiologically against pure vitamin B₁₂ is of the same therapeutic activity as if it consisted entirely of that substance. To establish this it is necessary to compare all the individual factors with vitamin B₁₂ and to discover whether or not their ratios of human to bacteriological activity are constant.

To further this object two cases of pernicious anaemia were treated with crystalline vitamin B_{12d} at the Walsall General Hospital. One of these (Case 1) also afforded an opportunity of comparing the potency of this substance with that of a liver extract that had been used on the same patient during a previous admission to hospital.

Case 1

A woman aged 67 was admitted to hospital on October 30, 1948, with the symptoms and physical signs of pernicious anaemia. Laboratory findings were: Hb, 56%; R.B.C., 2,240,000 per c.mm.; C.I., 1.25; M.D.R.C., 8.4 μ (approx.); C.V., 35%; M.C.V., 156 μ^3 ; reticulocytes, 0.8%; W.B.C., 4,800 per c.mm. The blood film showed marked anisocytosis and poikilocytosis. The van den Bergh test gave a positive indirect reaction of 10 mg. per litre. A test meal revealed that no free HCl was present.

On November 3, 1 ml. of "examen" B260A was given intramuscularly: the response obtained completely fulfilled Della Vida's criterion for satisfactory treatment.

The patient made an excellent recovery, and on return home attended the out-patient department regularly until April 7, 1949, when she elected to receive further treatment from her own doctor. However, she did not see him until the middle of July, 1950, when he was called in for the first time and found her extremely ill, being very pale, dyspnoeic, and oedematous. Only then was it discovered that she had had no anti-anaemic treatment for roughly 14 months.

On her readmission to hospital on July 20 she was in a very much worse condition than before, her blood picture being: Hb, 40%; R.B.C., 1,600,000 per c.mm.; C.I., 1.25; reticulocytes 1%. She said that she felt better than she had done a few days previously, and her own doctor, who saw her on the day of admission, confirmed this. With the 1% reticulocyte count and these observations by the patient and her doctor, it was thought that she was in mild remission.

On July 22, 1 ml. (10 μ g.) of vitamin B_{12d} was given intramuscularly, and she again showed an excellent response to the treatment, although the reticulocytes did not quite reach the expected 20%. This was thought to be due to the fact that she was in remission when the treatment was begun. The increase in red cells slightly exceeded the expected response according to Della Vida's formula (Fig. 1). She recovered well and was discharged from hospital on August 12.

FIG. 1.—Chart of Case 1.

Case 2

A man aged 60 was seen at the out-patient department on June 20, 1950, in an obviously very anaemic condition and with early signs of subacute combined degeneration. The laboratory findings were: Hb, 33%; R.B.C., 1,300,000 per c.mm.; C.I., 1.27; M.D.R.C., 7.8 μ (approx.); C.V., 14%; M.C.V., 108 μ^3 ; W.B.C., 3,800 per c.mm. The van den Bergh test showed a faint positive indirect reaction of 8 mg. per litre. The test meal revealed no free HCl.

FIG. 2.—Chart of Case 2.