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Incidence and pathogenesis of megaloblastic erythropoiesis in multiple myeloma

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SYNOPSIS Intermediate megaloblastic changes occurred in six (19%) of 32 patients with multiple myeloma and trivial megaloblastic changes in a further ten (31%). Folate deficiency was the predominant cause of these changes and in at least two patients was sufficiently severe to contribute to anaemia. Folate deficiency appeared to be due to excess folate utilization by the tumour and was related to the amount of paraprotein produced daily.

Five of the 32 patients had subnormal serum B_{12} levels. Reduction in the serum B_{12} level was related to the reduction in the normal circulating immunoglobulins and occurred despite normal B_{12} absorption. Possible explanations for this finding are discussed.

Subnormal serum vitamin B_{12} concentrations have been reported in patients with multiple myeloma by a number of authors (Mollin and Ross, 1952; Mandema, 1956; Mandema, Faber, de Vries, and Nieweg, 1956; Killander and Larsson, 1962; Larsson, 1962; Forshaw, 1963; Hansen, 1964). In some of these patients megaloblastic anaemia was also observed and in the patients of Mandema (1956), in two patients of Larsson (1962), and in the patient of Forshaw (1963) the anaemia responded to large doses of vitamin B_{12}. The anaemia in another patient of Larsson (1962) and the patient of Bichel (1964) responded to liver therapy.

Nonetheless, true Addisonian pernicious anaemia appears to be rare in myeloma and it has only been established by the demonstration of histamine-fast achlorhydria associated with malabsorption of vitamin B_{12} corrected by intrinsic factor in three patients (cases 2, 3, and 5 of Larsson, 1962). The cause of subnormal serum B_{12} concentrations in patients with myeloma without pernicious anaemia is uncertain. It has been suggested that they are due to malabsorption of vitamin B_{12} associated with bacterial contamination of the small intestine (Larsson, 1962) or to excess B_{12} utilization by the tumour (Mandema *et al.*, 1956; Killander and Larsson, 1962; van Dommelen, Olie, and Slagboom, 1964).

The present study was undertaken to determine the incidence, severity, and cause of B_{12} deficiency in patients with multiple myeloma. Since megaloblastic anaemia in other conditions in which there is proliferation of primitive tissue is almost invariably due to folate deficiency (Swendseid, Swanson, Meyers, and Bethell, 1952; Girdwood, 1953), the incidence and pathogenesis of folate deficiency was investigated in the same group of patients and this paper reports the results of both studies.

PATIENTS STUDIED

Thirty-two randomly selected patients with myeloma were studied. Their ages ranged from 42 to 81 (mean 67 years) and they included 17 males and 15 females. All the patients were investigated while in hospital for investigation or treatment.

In each patient, the diagnosis was made because the patient showed clinical and/or radiological evidence of bone lesions of the myeloma type, abnormal and increased numbers of plasma cells in the bone marrow, a characteristic narrow 'monoclonal' band on electrophoresis of the serum proteins and/or Bence-Jones proteinuria. Ten patients were studied at the time myeloma was first diagnosed, and in the remaining 22 patients myeloma was known to have been present from two months to nine years previously. Fifteen patients were receiving no specific therapy, seven patients were receiving melphelan (from

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0·25 to 4 mg. daily), one patient urethane (3 mg. daily), one patient prednisone (5 mg. b.d.), and eight were having deep *x*-ray therapy for bone lesions. In the patients on cytotoxic drugs, serum for microbiological assay was taken at least 24 hours after the last dose of the drug.

METHODS

SERUM VITAMIN B_{12} CONCENTRATIONS These were determined by microbiological assay using the z strains of *Euglena gracilis* as test organism as described by Anderson (1964) with a normal range from 160 to 925 $\mu\mu$g. per ml.

VITAMIN B_{12} ABSORPTION This was measured by the urinary excretion method of Schilling (1953) using an oral dose of 1 μg. radioactive ^{58}Co-B_{12} and an intramuscular flushing dose of 1,000 μg. non-radioactive B_{12}. Normal subjects excrete more than 10% of the oral dose in 24 hours and patients with Addisonian pernicious anaemia excrete less than 5% and usually less than 2·5 % in 24 hours.

SERUM FOLATE CONCENTRATIONS These were estimated by microbiological assay using *Lactobacillus casei* as test organism as described by Waters and Mollins (1961). The normal range is from 6·0 to 21·0 mμg. per ml.

SERUM MYELOMA PARAPROTEINS These were recognized by their narrow 'monoclonal' electrophoretic mobility, or so-called 'M' band. They were identified by immunoelectrophoresis as γ_G, γ_A, or Bence-Jones protein. Their light chains were shown to be of one type only and identified as K (Kappa) or L (Lambda) (Nomenclature of Human Immunoglobulins, *Bull. Wld Hlth Org.*, 1964).

TOTAL SERUM PROTEIN CONCENTRATIONS These were estimated by a Biuret method calibrated against Armour standard bovine albumin. Using this method, γ_G globulin yields 3% less colour, and γ_A globulin 8% less colour than the same weight of albumin but no correction has been made, the error being slight, since, in all but one patient, the concentration of myeloma (M) protein in the serum was less than half the total serum protein concentration. The proportion of M protein in the serum proteins was estimated after electrophoresis on cellulose acetate using the dye amidoschwarz 10B as colouring agent. This dye yields near uniform uptakes for the same weights of albumin, γ_G, and γ_A globulins and recoveries of pure added fractions are reliable to within 0·2 g. per 100 ml. (Hobbs, 1965). The concentration of M protein was then calculated from the known total serum protein concentration.

URINARY PROTEIN was estimated by a Biuret method after overnight precipitation in 10% trichloracetic acid and redissolving the precipitate in N sodium hydroxide. The content of M protein in urine was estimated after electrophoresis of concentrated urine by the method used to estimate serum M protein.

DAILY PRODUCTION OF M PROTEIN An estimate of the daily output of M protein was made using the following assumptions. It has been shown that γ_A myeloma globulins have a shorter half life (mean 6·4 days) than γ_G myeloma globulins (mean 11·6 days) (Drivsholm, 1964). For the same serum concentration, therefore, γ_A protein has a faster turnover (estimated as 10·8% of the total pool) than γ_G protein (estimated as 6% of the total pool). In order to compare our patients, we have arbitrarily considered them all to be 71 kg. in weight, with a plasma volume of 50·7 ml. per kg., with the plasma containing 71% of the total pool of myeloma globulin (averages of available data in myeloma patients, Gabuzda, 1962). Thus, for a given serum concentration of myeloma globulin, M g. per 100 ml., the total pool would be $\frac{M}{100} \times 71 \times 50{\cdot}7 \times \frac{100}{71} = 50{\cdot}7$ M g., and for patients in equilibrium, the average daily productions of γ_A would be 10·8% of 50·7 M = 5·5 M g., and of γ_G 6% of 50·7 M = 3·0 M g.

In order to obtain the total daily M protein production, the daily Bence-Jones protein loss in the urine was measured, corrected to 71 kg. from the patient's body weight, and added to the production of M protein, calculated above.

It is recognized that this calculation only gives an approximate value since plasma and total body pools and the half life of the plasma proteins all show individual variation. Nevertheless, this variation is mostly less than $\pm$ 25% of the taken means.

LOSS OF NORMAL HUMORAL IMMUNITY This was estimated by measuring the concentration of the serum immunoglobulins (γ_G, γ_A, and γ_M) and taking their average, each expressed as a percentage of the normal mean, *e.g.*, case FB had serum concentrations, γ_G 75%, γ_A 70%, and γ_M 70% of normal, giving an average 72% of the normal immunoglobulin concentration.

HAEMATOLOGICAL FINDINGS

INCIDENCE OF MEGALOBLASTIC HAEMOPOIESIS In Table I, patients are divided according to whether or not they show megaloblastic change in the bone marrow. Six (19%) of the 32 patients (group I) showed intermediate megaloblastic changes (Dacie and White, 1949) and a further 10 (31%) patients (group II) showed trivial megaloblastic changes. The remaining 16 patients (group III) had entirely normoblastic bone marrows. None of the patients showed the florid megaloblastic changes seen in severely anaemic patients with uncomplicated megaloblastic anaemia.

The appearances of the stained peripheral blood films were largely, but not invariably, consistent with these bone marrow findings. Macrocytes and hypersegmented polymorphs (polymorphs with more than five nuclear lobes) were more frequent in the stained peripheral blood films of group I, less frequent in those of group II, and rarely present in those of group III.

RELATION OF MEGALOBLASTIC HAEMOPOIESIS TO OTHER HAEMATOLOGICAL FINDINGS Nearly all (30) of the 32 patients were anaemic at the time of the present studies (Table I). There was no direct correlation

TABLE I

HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN THE 32 PATIENTS

Group	*Case No.*	*Age/ Sex*	*Hb (g./100 ml.)*	*Serum B_{12} (μμg./ml.)*	*Serum Folate (mμg./ml.)*	*Serum M Protein (g./100 ml.)*	*Immunological Type of M*	*Urine Bence-Jones (g./day)*	*Calculated Total Paraprotein Production (g./day)*	*Remaining Immunoglobulins (mean % normal mean)*
I	1	53/F	8·3	110	3·5	6·2	L	0	18·6	8
(intermediate	2	66/M	7·5	120	2·5	7·0	K	0	21·0	3
megaloblastic	3	74/F	7·2	170	3·5	3·1	K	11·6	20·9	6
changes)	4	54/M	5·1	370	1·7	4·4	AL	0	24·2	32
	5	81/F	6·4	560	1·5	4·7	L	24·8	24·8	75
	6	66/M	7·0	610	4·4	2·1	L	12·3	12·3	40
II	7	63/M	7·2	135	3·5	5·7	K	0·4	17·5	20
(trivial	8	75/F	10·0	145	11·2	1·6	K	1·2	6·0	8
megaloblastic	9	59/M	5·5	150	3·0	6·2	AK	0·5	35·2	22
changes)	10	73/F	10·5	210	3·9	1·4	K	1·5	6·9	19
	11	62/M	9·6	230	3·6	3·0	K	0	9·0	7
	12	60/M	12·0	255	2·9	1·8	AK	0·9	11·1	68
	13	75/M	11·8	280	4·5	3·3	K	0	9·9	16
	14	51/F	10·6	350	4·7	1·3	AL	0	7·2	95
	15	71/M	13·4	480	2·4	4·1	AL	3·6	26·2	50
	16	42/M	6·1	480	5·5	3·7	K	0	11·1	12
III	17	75/F	10·2	175	3·6	4·2	AK	0	23·1	37
(normoblastic)	18	70/M	14·4	255	4·8	4·4	L	0	13·2	25
	19	66/M	13·0	270	3·0	3·6	AK	0	19·9	28
	20	59/M	14·8	310	7·3	0·9	AK	1·4	5·1	18
	21	75/F	9·6	320	5·2	3·7	K	4·8	17·0	5
	22	67/F	11·1	340	6·5	2·0	K	0	6·0	10
	23	53/M	12·2	385	5·3	1·9	L	0	5·7	49
	24	55/F	8·9	385	8·2	1·2	K	3·5	7·1	17
	25	68/M	12·4	440	7·7	0·8	AK	0	4·5	36
	26	61/F	9·7	455	4·1	3·2	AL	0·12	18·8	56
	27	59/F	9·9	490	4·9	2·5	AK	0	13·8	60
	28	52/M	11·5	500	2·3	5·4	AK	0	29·7	65
	29	69/F	9·1	500	4·8	0·0	K	5·5	5·5	63
	30	73/F	9·8	655	4·5	2·6	K	4·0	11·8	65
	31	66/F	11·4	750	5·0	0	K	4·8	4·8	90
	32	71/M	10·0	920	4·4	2·6	K	0	7·8	60

between the degree of anaemia and the severity of megaloblastic changes. However, the patients with intermediate megaloblastic changes (group I) were invariably severely anaemic and their mean haemoglobin concentration was lower than that of either of the other two groups. The mean platelet count was also lower in group I (94,000 per c. mm.) than in groups II and III (173,000 and 211,000 per c. mm. respectively), but the mean leucocyte counts were similar in all three groups.

SERUM B_{12} CONCENTRATIONS

Five of the 32 patients had subnormal serum B_{12} levels (Table I) and the mean serum B_{12} level of the whole group (368 μμg. per ml.) was significantly lower than that of a normal control group (472 μμg. per ml.; Anderson, 1964) ($P < 0{\cdot}001$). In none of the patients, however, was the serum B_{12} level as low as in patients with overt pernicious anaemia (*i.e.*, less than 100 μμg. per ml.; Anderson, 1964).

SERUM FOLATE CONCENTRATIONS

Twenty-six of the 32 patients had subnormal serum folate concentrations and in six these were within the range usually found in severe folate deficiency (*i.e.*, less than 3·0 mμg. per ml.; Hoffbrand, Newcombe, and Mollin, 1966).

On the basis of these serum B_{12} and folate levels, folate deficiency appeared to be the predominant cause of megaloblastic changes since 11 of the 16 patients with megaloblastic changes had subnormal serum folate but normal serum B_{12} levels; four had subnormal serum folate and B_{12} levels, and only one (case 8) had a subnormal serum B_{12} and normal serum folate level.

RESPONSES TO FOLIC ACID AND VITAMIN B_{12} THERAPY

Despite the frequency of subnormal serum folate and B_{12} levels, it is unlikely that deficiency of either vitamin contributed significantly to anaemia in more than one or two patients. The effect of folic acid and/or B_{12} therapy was studied in five of the six patients in group I and in four patients in group II. In group I, one patient (case 3) with megaloblastic changes due to folate deficiency showed a definite haematological improvement when given folic acid in large doses (5 mg. t.d.s. by mouth) (Fig. 1). In a second patient in this group, who also had folate deficiency, folic acid

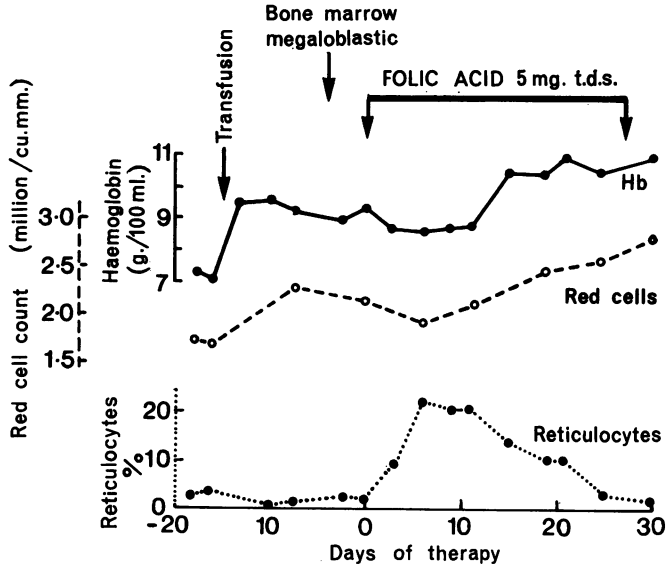

FIG. 1. *The haematological response to folic acid in case 3.*

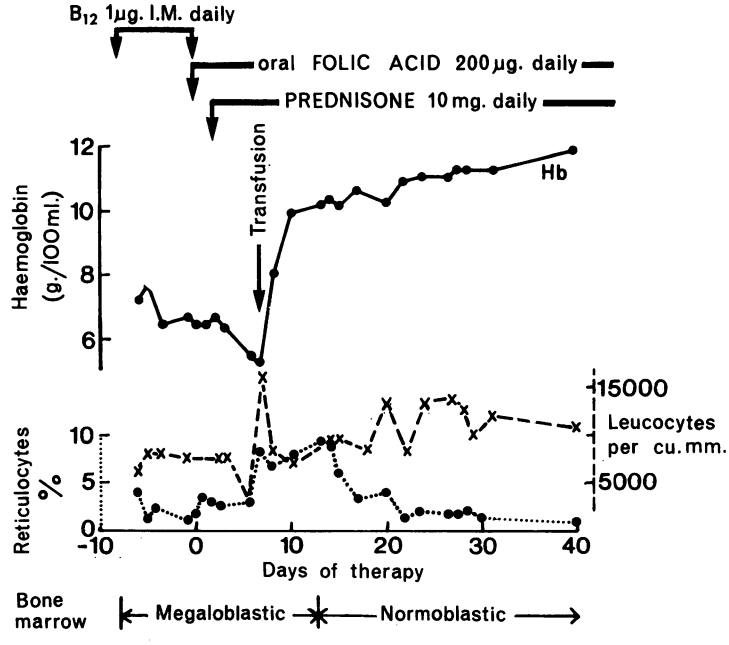

FIG. 2. *The haematological response to folic acid and steroid therapy in case 6.*

therapy probably produced a haematological response, but the issue in this patient was obscured by coincidental steroid therapy and a blood transfusion (Fig. 2). There was no significant rise in haemoglobin, leucocyte, or platelet count or other haematological benefit from folic acid in cases 4 and 5, and B_{12} therapy (100 μg. daily) in case 1, though case 4 did show successive small reticulocyte responses to oral folic acid in physiological (200 μg. daily) and then large (5 mg. t.d.s.) doses. In the remaining patient in group I (case 2) the response to folic acid and/or B_{12} therapy was not assessed.

As might be expected from the mildness of the megaloblastic changes in group II, none of the

patients in this group given folic acid (cases 12 and 15) or B_{12} (cases 7 and 8) responded.

PATHOGENESIS OF THE SUBNORMAL SERUM B_{12} LEVELS

RELATION OF SERUM B_{12} LEVELS TO CHANGES IN PLASMA PROTEINS There was no correlation between the serum B_{12} levels of the patients and their age, sex, length of history, type of treatment, degree of anaemia, serum concentration of globulin or myeloma protein or with their calculated daily paraprotein production. As shown in Fig. 3, however, the serum B_{12} levels tended to be lower in the patients with a greater degree of immuneparesis. The five patients with subnormal serum B_{12} levels had only 22% or less of the normal mean serum immunoglobulin concentration. Thirteen of the other 15 patients with serum B_{12} levels less than 400 $\mu\mu$g. per ml. had less than 50% of normal mean immunoglobulin, whereas only three of the 12 patients with serum B_{12} levels greater than 400 $\mu\mu$g. per ml. had a reduction in mean immunoglobulin level to less than 50% of normal.

B_{12} ABSORPTION STUDIES Schilling tests were performed in 12 patients including three (cases 1, 7, and 8) with subnormal serum B_{12} levels. None of these 12 patients had evidence of renal failure. The results are shown in Table II. In only one patient was B_{12} absorption reduced to the range found in pernicious anaemia, and in this patient (case 13) the increased absorption with intrinsic factor was suboptimal. In four patients with only slightly reduced B_{12} absorption, the absorption with intrinsic factor was normal. The absorption of radioactive B_{12} was within the normal range in the three patients with subnormal serum B_{12} levels and in the other four patients tested.

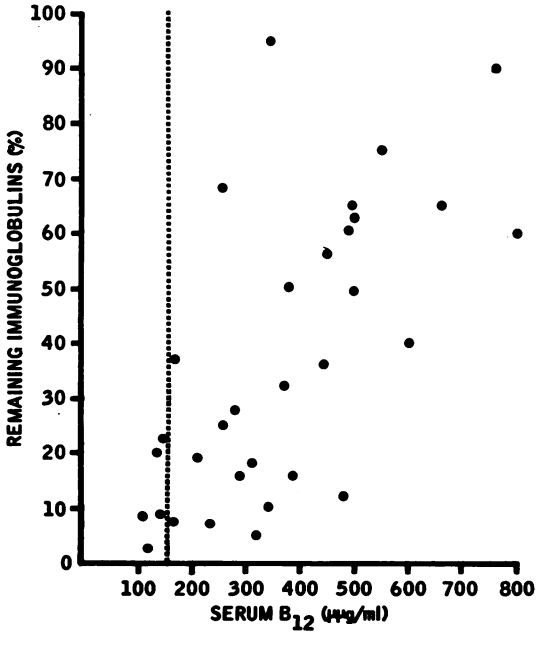

FIG. 3. *The serum B_{12} levels of the 32 patients plotted against their mean remaining immunoglobulin levels, expressed as a percentage of the normal mean. The vertical broken line is the lower limit of normal serum B_{12} levels.*

TABLE II

SERUM B_{12} LEVELS AND SCHILLING TEST RESULTS IN 12 PATIENTS

Case No.	*Serum B_{12} ($\mu\mu$g./ml.)*	*Schilling Test (24hr. urinary excretion of radioactive ^{58}Co B_{12} 1 μg.)*	
		Dose Alone	*Dose + Intrinsic Factor*
1	110	11·9	13·6
7	135	14·1	10·6
9	150	12·1	21·8
19	270	5·2	11·4
13	280	3·6	9·0
20	300	8·4	14·8
23	385	8·9	10·6
16	480	7·0	10·5
28	500	15·1	—
6	610	15·6	—
31	750	14·1	—
32	920	12·5	—

ANTIBODY STUDIES Tests for gastric parietal cell and intrinsic factor antibodies, carried out on four of the five patients with subnormal serum B_{12} levels (cases 1, 2, 7, and 8), were negative.

PATHOGENESIS OF THE SUBNORMAL SERUM FOLATE LEVELS

In contrast to the finding with serum B_{12} levels, there was a moderately good inverse correlation between the calculated daily paraprotein production of the patients and their serum folate concentrations, the patients with the greatest paraprotein production having the lowest serum folate levels (Fig. 4). On the other hand there is no relation between serum folate concentration and the degree of immuneparesis or the serum level of globulin or myeloma protein or with the age or sex of the patients, the length of history, or the type of therapy. In one patient with severe folate deficiency (case 6), the dietary folate intake was calculated (61 μg. daily) and folic acid absorption was measured. Both were normal.

DISCUSSION

The results of these studies confirm those of previous workers (Toušek and Vortel, 1947; Heilmeyer and

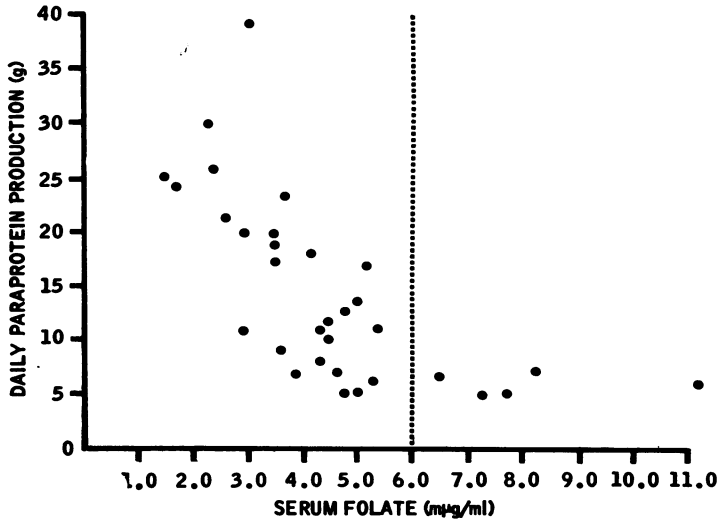

FIG. 4. *The serum folate levels of the 32 patients plotted against their calculated daily paraprotein production. The vertical broken line is the lower limit of normal serum folate levels.*

Begemann, 1951; Sandkühler, 1951; Mandema, 1956; Reisner, 1958; Forshaw, 1963) that megaloblastic changes are frequently present in patients with myeloma. In addition, they show that the predominant cause of these changes is folate deficiency. In an occasional patient studied here folate deficiency was sufficiently severe to contribute to anaemia but in the majority of the folate-deficient patients there was either no response to folic acid or the deficiency was too mild to be a cause of anaemia. Since there is a theoretical objection to giving folic acid to patients with malignant disease because it might increase the rate of growth of the tumour, it is probably advisable to reserve folic acid therapy in myeloma to patients who have clear evidence of severe deficiency and in whom anaemia or possibly thrombocytopenia are important features.

The main cause of folate deficiency in myeloma is likely to be increased folate utilization by the tumour. This is supported by the present finding of the lowest serum folate levels in the patients with the greatest paraprotein production. The latter may well be roughly related to the mass of tumour cell present (Nathans, Fahey, and Potter, 1958). Reduced dietary folate intake might also contribute to the deficiency in some of the patients since they almost invariably have anorexia.

The low serum B_{12} levels in myeloma are more unexpected and also more difficult to explain. Subnormal serum B_{12} levels are not usually found in generalized malignant diseases in which, in the absence of gastrointestinal involvement, serum B_{12} levels are normal or elevated (Mollin and Ross, 1957). A few of the patients with myeloma and B_{12} deficiency reported by other authors had frank pernicious anaemia, but, as Larsson (1962) suggests, this may be a chance association. In the majority of patients with myeloma and subnormal serum B_{12} levels, pernicious anaemia is not present, and in some, as in cases 1, 7, and 8 here, B_{12} absorption is normal.

This is an unusual finding. Subnormal serum B_{12} levels with normal B_{12} absorption occur in vegans (Wokes, Badenoch, and Sinclair, 1955), in some patients with severe megaloblastic anaemia due to folate deficiency (Mollin, Waters, and Harris, 1962), and in infants born to B_{12}-deficient mothers (Lampkin, Shore, and Chadwick, 1966). It has also been reported in a single patient with B_{12}-dependent anaemia apparently due to defective plasma binding of B_{12} (Horrigan and Heinle, 1952) and it occurs rarely after partial gastrectomy (Deller and Witts, 1962). Killander and Larsson (1962) suggested that the subnormal B_{12} levels in myeloma were mainly due to excess utilization of B_{12} by the tumour, since in their study the lower serum B_{12} levels occurred in the patients with the highest serum concentrations of myeloma protein. The present results do not confirm this. Rather they show that the reduction in serum B_{12} level is related to the reduction in the normal circulating immunoglobulins. The three patients of Killander and Larsson all had γ_G myelomata and, although the authors do not comment on this, are therefore likely to have had severe immuneparesis (Hobbs, Slot, Campbell, Clein, Scott, Crowther, and Swan, 1966). It is of interest that B_{12} deficiency is not infrequent in patients suffering from hypogamma-

globulinaemia from causes other than myeloma (Klayman and Brandborg, 1955; Larsson, Hagelquist, and Cöster, 1961; Gibbs and Pryor, 1961; Lee, Jenkins, Hughes, and Kazantzis, 1964). Although Meyer, Bertcher, Cronkite, Suarez, Miller, Mulzac, and Olivarreta (1961) could not detect any reduction in the B_{12}-binding capacity *in vitro* of serum from patients with myeloma, this does not exclude reduction in the concentration of the endogenous B_{12}-binding protein in this condition. The relation between serum B_{12} level and degree of immuneparesis found here could be explained if the low serum B_{12} levels were due to a reduction in this endogenous serum B_{12}-binding protein (an α globulin) in parallel to the reduction in immunoglobulins.

Five patients with normal serum B_{12} levels had subnormal serum B_{12} absorption but in only one of them was the absorption as low as in pernicious anaemia. In all five absorption was significantly improved by intrinsic factor. These findings suggest reduced secretion of intrinsic factor in these patients but this is not an uncommon finding in patients of a similar age group. In Forshaw's (1963) patient whose serum B_{12} level was just subnormal (120 $\mu\mu$g. per ml.), B_{12} absorption was at the lower limit of normal and improved significantly with intrinsic factor. In this patient, however, free gastric acid was present and a gastric biopsy was normal.

The present studies lend no support to the theory that B_{12} deficiency in myeloma is due to bacterial contamination of the small intestine. In none was there severe reduction in B_{12} absorption when this was tested with added intrinsic factor.

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