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FOLIC ACID AND CYANOCOBALAMIN IN PERNICIOUS ANÆMIA

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THE observation that B.12 deficiency can occur without megaloblastic anæmia has been noted repeatedly (1, 6, 7, 8, 11, 13, 16 and 19). Thus it is increasingly difficult to explain the hæmopoietic defect of pernicious anæmia solely on the basis of the B.12 deficiency. Spray and Witts (15) and Girdwood (3) observed reduced urinary excretion of folic acid following test doses of 1 and 5 mg. of folic acid and Mollin and Ross (9, 10) have suggested that variations in body content of folic acid may account for the variable clinical picture of pernicious anæmia. Nevertheless, a deficiency of folic acid would appear to be of little therapeutic significance since Ungley (18) has pointed out that supplementary folic acid is rarely required in pernicious anæmia. The therapeutic activity of folic acid and the lack of anæmia in some patients with B.12 deficiency does, however, indicate the possibility of an important relationship between these compounds the nature of which is still apparently obscure.

The purpose of this study is to investigate the metabolism of folic acid in pernicious anæmia and observe the effect of B.12 therapy.

METHODS

Folic acid (5 mg.) was given intramuscularly to 20 normal subjects, 23 hospital patients with no evidence of neoplasm or renal disease, 10 patients with pernicious anæmia in relapse and 10 with pernicious anæmia in remission. The urine was collected for 24 hours after the test dose and an aliquot of each specimen was assayed in duplicate with *Streptococcus fæcalis* to estimate the folic acid content (5).

The patients with pernicious anæmia in relapse were given test doses of folic acid and the urinary excretion of folic acid was determined on alternate days over a period of at least 14 days. Giving the test dose on alternate days prevented any significant overlap in the increased urinary excretion of folic acid. Cyanocobalamin (250 or 1000 μg. intramuscularly daily for 10 days) was commenced in eight patients after the third folic acid injection and after the first injection in the other two. The high dosage of vitamin B.12 was given to obtain saturation as soon as possible in amounts similar to those suggested by Mollin and Ross (9) for this purpose. These experiments were controlled by performing similar folic acid excretion tests on alternate days in three normal subjects and two patients in complete remission induced by B.12.

Two patients with pernicious anæmia were studied in the same way as the other pernicious anæmia patients but, instead of folic acid, test doses of 3 mg. of leucovorin (folinic acid) were used. The urinary excretion of folic acid and folinic acid were

assayed with *Str. fæcalis* and *Leuconostoc citrovorum* (14) together with the differential assay of Swendseid, Bethell and Ackermann (17).

The criteria of a diagnosis of pernicious anæmia are the presence of a macrocytic anæmia, megaloblastic erythropoiesis, abnormally low serum B.12 level, (below 104 μμg./ml. as determined with *Lactobacillus leichmanii* by the method of Meynell, Cooke, Cox and Gaddie (8)), histamine fast achlorhydria and an absence of evidence of disease of the small bowel as indicated by a daily fæcal fat excretion of less than 6 g. a normal X-ray pattern of the small intestine, and normal folic acid excretion test (3 and 4) performed at the conclusions of the present investigation.

Results

The urinary excretion after 5 mg. folic acid intramuscularly are given in Table I. The age distribution was similar in patients with pernicious anæmia in relapse and remission and also in the hospital patients. The age was much lower in the " normal " subjects who were mainly medical staff and students.

TABLE I

Mean values of folic acid excretion following 5 mg. of folic acid intramuscularly

Group	Number of subjects	Urinary excretion of folic acid (mg.)		
			Range	S.E.
Normal	20	2·62	1·39 to 3·21	±0·11
Hospital patients	23	2·18	0·68 to 3·31	±0·13
P.A. in relapse	10	1·62	0·13 to 2·89	±0·37
P.A. in remission	10	2·61	1·23 to 4·09	±0·25

The lower limit of the normal excretion was accepted as 1·5 mg. in 24 hours as found initially by Girdwood (4). One normal subject, one patient with treated pernicious anæmia, two patients with severe ulcerative colitis and one patient who had been on a rigid gastric diet for years had lower excretions. Five patients with pernicious anæmia excreted less than 1·5 mg. and as a group, these patients excrete significantly less than normal subjects and patients with treated pernicious anæmia. Table II shows the urinary excretions of each of these patients and compares it with the degree of anæmia as assessed by the percentage hæmoglobin level (14·8 g. = 100%) and the serum B.12 level.

TABLE II

Effect of the initial injection of 5 mg. of folic acid on ten patients with pernicious anæmia

Folic acid excretion mg.	Hb. percentage	Serum B.12 μμg/ml.
0·11	27	25
0·13	35	<25
0·51	34	25
0·95	34	25
1·33	60	75
2·06	32	<25
2·21	86	85
2·76	69	25
2·89	92	<25
3·21	49	30

The urinary excretions following the administration of folic acid on alternate days for 5 or 6 injections in 3 normal subjects and two patients with pernicious anæmia in remission had a range of 2·13 to 2·90 mg. (mean 2·58, S.E. ± 0·04 mg.). Results (Table III) in patients with pernicious anæmia in relapse show that four of the 5 patients who had an initial excretion below 1·5 mg. excreted 1·97 mg. or over following the second injection. Following B.12 therapy, there was a fall in the excretion of folic acid which in only one patient occurred before the fifth day after the commencement of B.12 therapy. In 7 cases, the fall occurred after the reticulocyte peak and in another case it occurred at the peak. Reticulocyte counts were not

TABLE III

Folic acid excretion in mg./24 hours and the percentage of hæmoglobin and of reticulocytes in ten patients with pernicious anæmia

he administration of cyanocobalamin was commenced on zero day

Case No.		Day								
		−5	−3	−1	+1	+3	+5	+7	+9	+11
1	Mg.	0·125	2·9	2·35	2·66	2·36	1·92	—*	—*	2·53
	Hb.	35	—	—	38	—	47	—	49	—
	Retics	1·5	2·5	7·0	29·4	19·5	15·6	—	—	—
2	Mg.	3·21	3·47	3·27	3·58	3·49	2·68	—*	2·48	2·24
	Hb.	49	—	—	45	—	—	55	—	—
	Retics.	0·8	0·8	1·9	8·2	15·2	11·2	11·2	10·4	11·9
3	Mg.	—	—	2·89	2·86	3·61	3·27	2·43	1·90	2·26
	Hb.	91	—	92	—	—	—	—	—	—
	Retics.	0·8	0·8	0·8	—	3·6	4·2	2·2	—	—
4	Mg.	2·76	2·68	2·64	3·24	3·69	2·24	2·37	3·44	—
	Hb.	69	—	74	—	71	—	77	—	—
	Retics.	0·7	1·0	0·4	2·8	3·4	—	1·2	0·4	—
5	Mg.	2·21	2·35	2·42	2·34	2·70	2·22	2·50	—	—
	Hb.	86	—	88	—	92	—	96	—	—
	Retics.	—	—	—	—	—	—	—	—	—
6	Mg.	0·51	2·25	2·33	2·36	1·55	0·69	1·99	3·02	—
	Hb.	34	—	31	—	—	49	—	52	—
	Retics.	1·1	—	5·0	23·6	28·8	15·0	—	—	—
7	Mg.	2·06	1·96	1·97	1·93	0·57	1·80	1·92	—*	3·10
	Hb.	32	—	—	45	—	55	—	64	—
	Retics.	1·8	2·2	5·8	11·2	24·8	32·8	14·0	2·8	—
8	Mg.	0·11	0·37	1·12	2·86	3·34	2·89	1·51	1·94	—
	Hb.	27	—	—	35	—	43	—	55	—
	Retics.	0·6	3·8	4·8	19·0	29·6	29·8	24·4	17·6	—
9	Mg.	0·95	2·09	1·86	2·43	0·52	2·26	2·29	2·48	—
	Hb.	34	—	—	36	—	48	—	61	—
	Retics.	0·8	1·2	1·2	8·0	23·2	20·0	7·0	—	—
10	Mg.	—	—	1·33	1·97	2·13	1·36	0·49	2·07	—
	Hb.	—	60	—	57	—	61	—	—	—
	Retics.	—	0·4	0·2	4·4	10·8	4·6	—	—	—
	Mean folic excretion	1·49	2·13	2·22	2·62	2·40	2·13	1·94	2·26	2·53

* Folic acid given but urine lost.

performed in one patient with little anæmia. The fall in the urinary excretion was most marked in the more severely anæmic patients than in those with mild anæmia, as is seen by comparing Cases 7, 8 and 9, with Patients 3, 4 and 5. Following the fall in the folic acid excretion, a rise was observed in all but one case.

The excretion of material with only folic acid-like activity (P.G.A.) and that assayable with *L. citrovorum* (*C.F.*) in the two patients given leucovorin test doses are given in Table IV. Both patients showed increasing excretions of C.F. which persisted until after the commencement of B.12 therapy. However, significant reductions in the excretions occurred after the reticulocyte peak and was followed by an increased excretion. The urinary excretion of P.G.A. was also reduced after B.12 therapy. These low levels persisted in the two patients : in one it was zero and the other had the lowest recorded level of therapy 11 and 15 days respectively after the commencement with cyanocobalamin. The P.G.A. disappeared abruptly in one patient and gradually in the other.

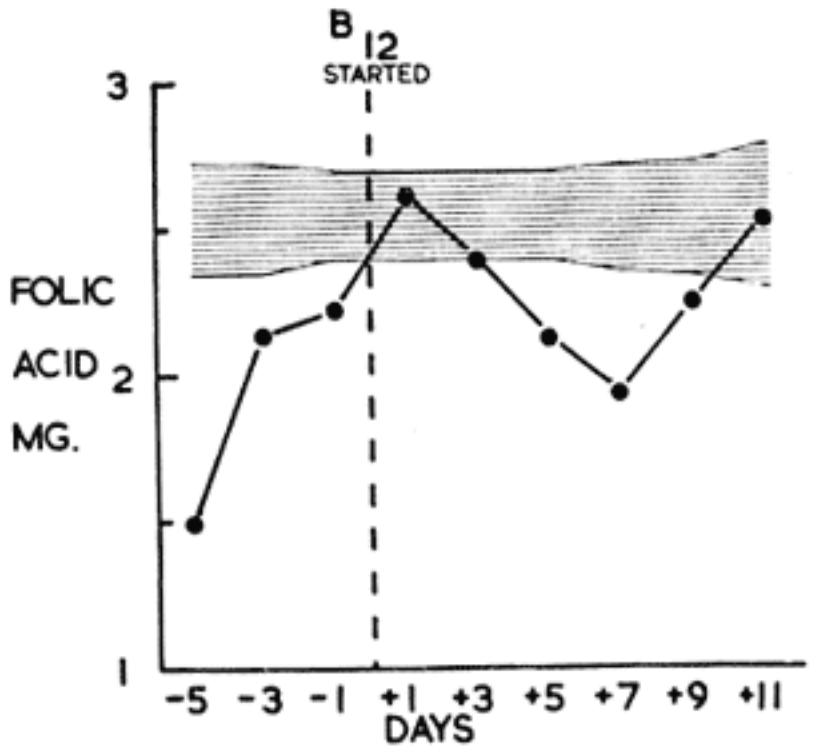

Fig. 1. Effect of B.12 therapy on the mean values of the urinary excretion of folic acid following 5 mg. intramuscularly on alternate days in 10 patients with pernicious anæmia are shown. The shaded area represents the mean value ± twice the corrected standard error of the urinary folic acid excretion by 5 control subjects (30 observations).

Discussion

As a group, patients with pernicious anæmia in relapse, excrete a smaller proportion of a test dose of folic acid than the controls. Girdwood (3, 4) had made similar observations and Neiweg, Faber, de Vries and Kroese (12) found reduced blood levels of folic acid in 9 out of 16 patients. These observations would suggest that some tissue unsaturation of folic acid occurs in pernicious anæmia in relapse. This can be of little importance in the production of the variable clinical picture of pernicious anæmia since there is no significant correlation between the degree of anæmia and the amount of folic acid excreted after the test dose. The patient with the highest excretion had a hæmoglobin of 49% (7·3 g.%) and a grossly megaloblastic erythropoiesis. Even more significant, the amount of folic acid required to restore this excretion to normal in the patients with initially reduced excretions was only 5 mg. in 4 patients and 15 mg. in the fifth. This ease in saturating these patients has been observed by Girdwood (3). In view of the trivial hæmatological response to the amount of folic acid required to restore the excretion to normal, and the rarity with which it is necessary to supplement B.12 therapy with folic acid in pernicious

TABLE IV

Effect of therapy with cyanocobalamin commencing at 0 day on the excretion of material with folic acid-like activity (P.G.A.) and citrovorum factor (C.F.) after 3 mg. test doses of leucovorin in 2 patients with pernicious anæmia

Case	Day of test	−3	−1	+1	+3	+5	+7	+9	+11	+13	+15
Case 11	C.F. mg.	0·09	0·27	0·28	0·25	0·31	0·23	0·18	0·45	—	—
Serum B.12	P.G.A. μg.	0·09	0·33	0·36	0·26	0·36	0·25	0	0	—	—
30 μμg./ml.	Hb. %	45	—	—	54	—	60	—	64	—	—
	Retics. %	1·0	1·4	7·2	9·4	28	18	—	—	—	—
Case 12	C.F. mg.	0·08	0·10	0·16	0·14	0·11	0·09	0·10	0·12	0·15	0·25
Serum B.12	P.G.A. μg.	0·09	0·08	0·08	0·04	0·02	0·03	0·04	0·01	0·01	0·009
20 μμg./ml.	Hb. %	27	—	27	—	44	—	50	—	—	58
	Retics. %	0·5	—	13·0	39·0	27·0	8·6	—	—	—	—

anæmia (18), it seems reasonable to conclude that although some tissue unsaturation can be detected, this is unlikely to play any significant role in the development of the megaloblastic anæmia. Anorexia, which is a variable feature of pernicious anæmia may account for this apparent deficiency of folic acid metabolism.

Vilter, Horrigan, Mueller, Jarrold, Vilter, Hawkins and Seamen (20) suggested that the demand for folic acid may be increased in B.12 deficiency. The reduction in the urinary excretion of folic acid after B.12 therapy which we observed and the lowering of the blood folic acid levels following B.12 injections (12) would suggest that this therapy increases the utilisation of folic acid. This could be the result of the increased or altered erythropoiesis, but this view, though attractive, is unlikely since 4 of our patients had a significant reticulocytosis before the commencement of B.12 therapy and 7 out of 9 patients showed the fall in excretion after the reticulocyte peak. Thus, the fall also occurs much later than the change from megaloblastic to the normoblastic form of erythropoiesis and it occurs at a time after B.12 therapy similar to that of the complete disappearance of the erythropoietic inhibitor from pernicious anæmia serum as demonstrated by marrow culture (2).

Generally speaking greater reduction in folic acid excretion following B.12 therapy occurred in the most anæmic patients but this cannot be attributed to a significantly greater increase in the hæmoglobin level. Possibly a disorder of folic acid metabolism occurring in B.12 deficiency is more marked in the more anæmic patients. Correction of the B.12 deficiency may correct this defect of folic acid metabolism and temporarily increases the demand for it. It is also possible that a disturbance of folic acid metabolism may arise in a B.12 deficient state to a variable degree thus accounting for some of the variations in the clinical pictures of pernicious anæmia. The smaller proportional reduction in the folic acid excretion in the less anæmic patients favours this.

Since there is a good deal of evidence to suggest that folic acid is converted into citrovorum factor, the reduction of the urinary excretion of C.F. after test doses of leucovorin following B.12 therapy to pernicious anæmia patients seems to favour the view that an increased demand for the whole pteroylglutamate complex is created temporarily. Again, the delay in the fall in the urinary excretion until after the reticulocyte peak would suggest that the increased demand is not just due to the change in the nature or rate of erythropoiesis. In the two patients given leucovorin, the virtual disappearance of P.G.A. (material with only *Str. fæcalis* activity) from the urine after the excretion of C.F. had returned to normal suggests that the P.G.A. was a more necessary fraction of the pteroylglutamate complex. This would confirm the view that citrovorum factor is not the only active principle of the pteroylglutamate complex.

Summary

1. Using a folic acid excretion test, evidence of a deficiency of folic acid was found in five out of ten patients with pernicious anæmia. The degree of unsaturation appeared to be slight, and probably does not contribute to the dyshæmopoiesis of pernicious anæmia.

2. Following treatment with cyanocobalamin, a reduced excretion of P.G.A. and C.F. occurred following either administration of 5. mg folic acid or 3 mg. leucovorin. The changes in the nature and rate of the hæmatopoiesis were not solely responsible for this fall since in all but two patients it occurred after the reticulocyte peak.

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