J. clin. Path. (1966), **19**, 29

Folic acid deficiency in leukaemia and lymphomas

D. P. ROSE

From the University Department of Chemical Pathology, Royal Infirmary, Sheffield

SYNOPSIS Serum folic acid levels and the urinary excretion of Figlu have been studied in patients with leukaemia or lymphomas. The results indicate that folic acid deficiency is a common complication of these diseases. Bone marrow examinations of those with evidence of such a deficiency frequently show megaloblastic erythropoiesis.

The Figlu test appears to be a useful screening test for folic acid deficiency in patients with leukaemia or lymphomas.

Evidence that folic acid deficiency may develop in patients suffering from leukaemia or lymphomas has been obtained from studies of the excretion of folic acid in urine (Swendseid, Swanson, Meyers, and Bethell, 1952; Girdwood, 1953), of the rate of clearance of injected folic acid from the plasma (Hogan, Maniatis, and Moloney, 1964), and from serum folic acid assays (Hoogstraten, Baker, and Reizen-stein, 1961; Rao, Lagerlöf, Einhorn, and Reizen-stein, 1963; Kershaw and Girdwood, 1964).

The number of patients studied by previous authors has been too small to indicate the frequency with which folic acid deficiency is a complicating factor in the various forms of leukaemia and lymphomas. In the present study groups of patients with acute leukaemia, chronic lymphocytic leukaemia, lymphosarcoma, reticulum cell sarcoma, and Hodgkin's disease have been investigated for evidence of folic acid deficiency. In addition to serum folic acid estimations and bone marrow examinations, the excretions of formiminoglutamic acid (Figlu) in urine have been estimated. Increased excretions of Figlu have been reported in malignant diseases (Dymock, 1964), but the significance of these observations remains to be established.

MATERIAL AND METHODS

The composition of the patients studied is given in Tables I (acute leukaemia) and II (chronic lymphocytic leukaemia and lymphomas). The cases of acute leukaemia were investigated before starting treatment.

The haematological estimations were performed by standard methods, the fasting serum folic acid assays by the *Lactobacillus casei* method (normal range 5 to 22 mμg. per ml.), and the serum vitamin B_{12} assays by the *Euglena gracilis* technique (normal range 140 to 850 $\mu\mu$g. per ml.).

Received for publication 27 May 1965.

TABLE I

PATIENTS WITH ACUTE LEUKAEMIA

Case No.	*Sex*	*Age*	*Diagnosis*
1	M	15	Myeloblastic leukaemia
2	M	53	Myeloblastic leukaemia
3	F	64	Myeloblastic lauekemia
4	F	19	Myeloblastic leukaemia
5	F	76	Myeloblastic leukaemia
6	F	41	Myeloblastic leukaemia
7	M	52	Acute stem cell leukaemia
8	F	78	Subacute paramyeloblastic leukaemia
9	F	32	Acute monomyelocytic leukaemia

Formiminoglutamic acid (Figlu) excretion in urine was estimated by the method of Kohn, Mollin, and Rosenbach (1961). A five-hour collection of urine was made commencing three hours after giving a 15 g. oral dose of L-histidine, and the presence of Figlu was detected by cellulose acetate electrophoresis. The method is only semiquantitative and so the results have been grouped as follows:—Less than 2 mg. of Figlu excreted per hour (0, normal), 2 to 5 mg./hour ($\pm$), 5 to 10 mg./hour (+), 10 to 20 mg./hour (++), 20 to 30 mg./hour (+++), and more than 30 mg./hour (++++).

RESULTS

ACUTE LEUKAEMIA (NINE CASES) The results are given in Table III.

Six patients had reduced serum folic acid levels and four of these also had abnormal Figlu excretions. Two patients with normal serum folic acid results had raised outputs of Figlu. Megaloblasts or transitional megaloblasts were noted in the bone marrow smears of four cases, and the erythroblasts of another showed an abnormal degree of opening of the chromatin network which may have represented the earliest stage of megaloblastic change. Abnormal erythropoiesis was accompanied by a low serum folic acid level in all but one instance, and this

TABLE II

PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA OR LYMPHOMAS

Case No.	Sex	Age	Previous Therapy
Chronic lymphocytic leukaemia			
10	M	62	None
11	M	53	Proteolysed liver
12	M	79	None
13	M	56	Proteolysed liver, DXR[1] to spleen
14	F	49	Prednisolone. Splenectomy
15	M	56	Proteolysed liver
16	M	67	Prednisolone
17	F	68	None
18	M	49	Chlorambucil, DXR to spleen
19	F	69	Proteolysed liver
20	M	63	Chlorambucil
21	F	49	Prednisolone
Lymphosarcoma or reticulum cell sarcoma			
22	F	64	None
23	M	57	Nitrogen mustard
24	M	49	Abdominal DXR
25	F	39	Prednisolone
26	F	75	Abdominal DXR
27	F	61	Mediastinal and abdominal DXR
28	M	51[2]	None
29	M	48[3]	Abdominal DXR, nitrogen mustard
30	M	49[3]	DXR to superficial lymph glands
Hodgkin's disease			
31	M	68	None
32	M	48	Abdominal DXR
33	F	72	Nitrogen mustard. Prednisolone
34	F	47	Nitrogen mustard
35	M	28	Chlorambucil. Nitrogen mustard
36	M	33	Chlorambucil. Cyclophosphamide
37	M	46	None
38	M	48	Nitrogen mustard. DXR
39	M	54	DXR to cervical nodes
40	M	43	Abdominal DXR
41	F	31	DXR to superficial nodes and abdomen
42	M	39	None

[1]DXR = deep *x*-ray therapy.
[2]leukosarcoma.
[3]reticulum cell sarcoma.

patient (case 4) excreted Figlu in increased quantity.

CHRONIC LYMPHOCYTIC LEUKAEMIA (12 CASES) The results are shown in Table IV.

Four patients had reduced serum folic acid levels and three of these tested also excreted an abnormal amount of Figlu in the urine. Two other patients also had increased outputs of Figlu. Bone marrow examinations were carried out, at the time of the other investigations, in three patients. Erythropoiesis was normoblastic, although in one case (case 20) the serum folic acid level was reduced and the Figlu excretion was grossly abnormal.

LYMPHOSARCOMA AND RETICULUM CELL SARCOMA (TABLE IV, NINE CASES) Six patients had low serum folic acid levels. Figlu excretions were estimated in five of these and all gave abnormal results. Three patients with megaloblastic or transitional megaloblastic change in the bone marrow had evidence of folic acid deficiency.

HODGKIN'S DISEASE (TABLE IV, 12 CASES) Seven patients had both reduced serum folic acid levels and raised Figlu excretions. Two others had abnormal Figlu tests. The bone marrows of nine patients were examined and three showed transitional megaloblastic erythropoiesis. These changes were accompanied by low serum folic acid levels and increased Figlu excretions.

RESPONSE TO FOLIC ACID THERAPY The response to folic acid was studied in three patients with lymphomas.

Case 29 (reticulum cell sarcoma) showed a sudden fall in the haemoglobin level which was accompanied by the appearance of macrocytes in the peripheral blood and megaloblasts in the bone marrow. Intestinal malabsorption was indicated by a faecal fat excretion of 8·1 g. per day (average result for a four-day period; normal less than 6 g. daily) and impaired xylose absorption (1·3 g. xylose excreted in five hours following a 25 g. oral dose of xylose; lower limit of the normal range 4·0 g.). The serum folic acid level was low (1·5 mμg. per ml.) and the Figlu test was abnormal (+). Treatment with folic acid, given orally in a dose of 15 mg. a day, produced a rapid response with a maximum reticulocyte count of 14% followed by a rise in the haemoglobin level to 15·7 g./100 ml. The Figlu excretion was normal 12 days after starting folic acid therapy.

TABLE III

RESULTS IN ACUTE LEUKAEMIA

Case No.	Haemoglobin (*g. per 100 ml.*)	Total W.B.C. (*per c.mm.*)	Red Cell Precursors in Bone Marrow	Serum Folic Acid (*mμg. per ml.*)	Figlu Excretion
1	6·5	30,000	Transitional megaloblasts	1·4	+
2	6·9	73,000	Normoblasts	3·1	+
3	9·4	2,000	Megaloblasts	4·0	++
4	6·4	35,000	Megaloblasts	6·6	++
5	5·9	7,000	Normoblasts	9·6	0
6	6·9	28,000	Normoblasts	9·8	+
7	8·3	1,800	Normoblasts	2·0	0
8	8·5	77,000	Transitional megaloblasts	2·8	+++
9	7·0	12,000	Normoblasts with open chromatin network	3·7	0

0 = < 2 mg./hour. ± = 2-5 mg./hour. + = 5-10 mg./hour. ++ = 10-20 mg./hour. +++ = 20-30 mg./hour. ++++ = 30 mg./hour

TABLE IV

RESULTS IN CHRONIC LYMPHOCYTIC LEUKAEMIA AND LYMPHOMAS

Case No.	*Haemoglobin (g. per 100 ml.)*	*Total W.B.C.s (per c.mm.)*	*Red Cell Precursors in Bone Marrow*	*Serum*		*Figlu Excretion*
				Vitamin B_{12} ($\mu\mu$g./ml.)	*Folic Acid (mμg./ml.)*	
Chronic lymphocytic leukaemia						
10	13·0	33,000	—	—	9·6	0
11	10·6	410,000	—	424	10·5	0
12	11·1	70,000	Normoblasts	598	7·6	+
13	12·1	42,000	—	270	6·1[1]	0
14	10·1	36,000	—	—	2·0	++++
15	13·2	51,000	Normoblasts	226	13·0[1]	+
16	9·7	49,000	—	352	6·6	0
17	8·3	210,000	—	488	4·6	−
18	9·0	126,000	—	276	3·8	++
19	7·8	700,000	—	920	9·8[1]	−
20	7·7	40,000	Normoblasts	172	3·8	++++
21	6·8	65,000	—	272	5·9	0
Lymphosarcoma or reticulum cell sarcoma						
22	13·9	10,000	Normoblasts	434	7·0	−
23	12·2	2,500	Transitional megaloblasts	200	3·8	+++
24	10·0	18,000	Some megaloblasts	436	3·5	±
25	11·1	57,000	—	262	3·7	−
26	12·2	7,000	Normoblasts	284	2·1	+
27	13·2	5,000	Normoblasts	320	8·8	0
28	12·3	37,000	Normoblasts	362	9·2	0
29	7·6	5,000	Transitional megaloblasts	266	1·5	+
30	13·2	9,000	—	420	1·9	++
Hodgkin's disease						
31	9·7	6,000	Normoblasts	124	2·8	++
32	10·6	5,000	—	554	6·9	0
33	7·8	6,000	Aplastic	190	1·5	+
34	11·5	8,000	—	134	6·8	0
35	9·7	20,000	—	326	2·1	++
36	7·6	1,700	—	286	4·6	++++
37	12·2	15,000	Normoblasts	394	7·1	++++
38	8·8	4,000	Transitional megaloblasts	530	2·0	+++
39	13·8	9,000	Normoblasts	636	5·4	+
40	10·9	8,000	Transitional megaloblasts	378	1·2	+++
41	9·2	6,000	Micronormoblasts	340	5·0	0
42	8·6	7,000	Transitional megaloblasts	454	1·6	++

[1]Patients receiving proteolysed liver extract.

Case 40 (Hodgkin's disease) also presented with a rapidly developing megaloblastic anaemia. The results of the serum folic acid assay and the Figlu test were consistent with folic acid deficiency and he was treated with folic acid, 15 mg. daily. Within one month the haemoglobin level had risen from 6·0 to 10·2 g. per 100 ml. and three months later it was 15·2 g./ml. The Figlu excretion test became normal and the macrocytes disappeared from the peripheral blood.

Case 38 (Hodgkin's disease) had suffered from a microcytic anaemia, requiring blood transfusions, for several months. At the time of investigation, however, he had developed a severe glossitis and macrocytes were present in the peripheral blood. A bone marrow examination showed megaloblastic erythropoiesis and there was evidence of folic acid deficiency. The glossitis improved rapidly with folic acid therapy. There was, however, no improvement in the haemoglobin level, the haematological features being again those of a microcytic, normochromic anaemia.

DISCUSSION

The cause of folic acid deficiency in leukaemia and lymphomas has not been established with certainty. Leukaemia cells have a higher folic acid content than normal leucocytes (Swendseid, Bethell, and Bird, 1951) suggesting that one factor may be an abnormal demand for folic acid derivatives. Impaired intestinal absorption may also play a part in chronic lymphocytic leukaemia and lymphomas (Pitney, Joske, and Mackinnon, 1960, and case 29 in the present series).

Previous workers have reported low serum folic acid levels in leukaemias and lymphomas. Rao *et al.* (1963) estimated the fasting serum folic acid activity by the *Lactobacillus casei* method in nine cases of leukaemia or myelomatosis, considered as a single

group, and found the mean of the results (2·4 mμg. per ml.) to be significantly lower than the mean result for 16 control subjects (5·0 mμg. per ml.). Kershaw and Girdwood (1964) obtained low serum folic acid results in four of 12 patients with various forms of leukaemia, although the Figlu excretions were normal. These authors also investigated six patients with lymphomas. Three had low serum folic acid levels and two of these excreted Figlu in increased amounts.

In the present study four patients with acute leukaemia and three with chronic lymphocytic leukaemia had low serum folic acid levels and abnormal Figlu outputs. Two other cases of acute leukaemia had low serum folic acid levels but normal Figlu tests. Twenty patients suffering from a form of lymphoma were also studied and in 13 the serum folic acid level was reduced. All 12 of the 13 who were tested also excreted increased amounts of Figlu in the urine.

The frequency with which megaloblastic or transitional megaloblastic erythropoiesis was observed is of some interest. Reisner (1958) has described what he regarded as true megaloblasts in acute leukaemia. Dacie and White (1949), however, used the term 'megaloblastiform' to describe erythroblasts with an open chromatin network and advanced haemoglobinization which they observed in this condition. Their view was that these cells were not true megaloblasts, but that they arose from the 'leukaemic stimulus' which produced a disordered erythropoiesis as well as the malignant proliferation of leucocytes. Chanarin, Bennett, and Berry (1962) studied six untreated patients with acute myeloblastic leukaemia, three of whom showed megaloblastic change in the bone marrow. Five, including the three with megaloblastic erythropoiesis, had normal excretions of Figlu and urocanic acid in the urine. The other patient excreted large amounts of urocanic acid, but only a trace of Figlu. Villamil and McCracken (1963) found abnormal Figlu excretions in two of five patients with acute myeloblastic leukaemia and megaloblasts in the bone marrow. In the present series four patients with acute leukaemia and six with lymphomas showed megaloblastic or transitional megaloblastic change. All but one of those with acute leukaemia and all of those with lymphomas had low serum folic acid levels, suggesting that a deficiency of this vitamin is the usual cause of megaloblastic erythropoiesis in these diseases.

The clinical significance of folic acid deficiency in leukaemia and lymphomas remains to be elucidated. The administration of folic acid to patients with acute leukaemia may accelerate the progress of the disease (Farber, Diamond, Mercer, Sylvester, and Wolff, 1948). The responses of the three patients with lymphomas (cases 29, 38, and 40 in the present series) suggest that folic acid therapy may have a place in the management of these diseases when there is evidence of folate deficiency.

The results of the Figlu tests agreed well, on the whole, with the serum folic acid levels. There were, however, six patients with increased Figlu excretions but normal serum folic acid results. These may have resulted from malignant infiltration of the liver with impairment of the enzymes concerned in histidine metabolism. One of the six patients (case 37) had abnormal liver function tests.

I am grateful to Dr. E. K. Blackburn for his encouragement, the results of the haematological investigations and for access to his patients. I also wish to thank Dr. A. Jordan, in whose department this work was carried out, for his help and advice.

REFERENCES

Chanarin, I., Bennett, M. C., and Berry, V. (1962). *J. clin. Path.*, **15**, 269.
Dacie, J. V., and White, J. C., (1949). *Ibid.*, **2**, 1.
Dymock, I. W. (1964). *Lancet*, **2**, 114.
Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F. Jr., and Wolff, J. A. (1948). *New Engl. J. Med.*, **238**, 787.
Girdwood, R. H. (1953). *Brit. med. J.*, **2**, 741.
Hogan, J. A., Maniatis, A., and Moloney, W. C. (1964). *Blood*, **24**, 187.
Hoogstraten, B., Baker, H., and Reizenstein, P. (1961). *Ibid.*, **18**, 787.
Kershaw, P. W., and Girdwood, R. H. (1964). *Scot. med. J.*, **9**, 201.
Kohn, J., Mollin, D. L., and Rosenbach, L. M. (1961). *J. clin. Path.*, **14**, 345.
Pitney, W. R., Joske, R. A., and Mackinnon, N. L. (1960). *Ibid.*, 13, 440.
Rao, P. B. R., Lagerlöf, B., Einhorn, J., and Reizenstein, P. (1963). *Lancet*, **1**, 1192.
Reisner, E. H. Jr. (1958). *Blood*, **13**, 313.
Swendseid, M. E., Bethell, F. H., and Bird, O. D. (1951). *Cancer Res.*, **11**, 864.
——, Swanson, A. L., Meyers, M. C., and Bethell, F. H. (1952). *Blood*, **7**, 307.
Villamil, A., and McCracken, B. H. (1963). *Brit. med. J.*, **1**, 717.