form of a small tasteless tablet and because of the rapid clinical and haematological improvement which results even in cases refractory to parenteral liver therapy. Since the duration of treatment with folic acid is limited to a few weeks, the danger of producing subacute combined degeneration of the cord is greatly reduced as compared with Addisonian pernicious anaemia. Further time must elapse, however, before it can be stated confidently that neurological features will not occur in patients with pernicious anaemia of pregnancy receiving folic acid.

As many cases of pernicious anaemia of pregnancy are suffering from a dual deficiency of iron and the factor required for maintenance of normoblastic blood formation it is desirable that both deficiencies be corrected simultaneously. While our patients were receiving folic acid iron was deliberately withheld in order to simplify the interpretation of the therapeutic response.

(Anahaemin is a refined liver extract, and " plexan " and " campolon " are crude extracts.)

Summary

Three cases of pernicious anaemia of pregnancy and one case of Addisonian pernicious anaemia complicated by pregnancy responded excellently to treatment with folic acid. Three of the cases had failed to respond to the parenteral injection of liver extracts prior to the administration of folic acid.

We wish to thank Prof. Kellar, Dr. Fahmy, and others of our colleagues in the Simpson Memorial Maternity Pavilion of the Royal Infirmary, Edinburgh, for permission to investigate and treat the above cases.

REFERENCES

Callender, S. T. E. (1944). *Quart. J. Med.*, **13**, 75.
Davidson, L. S. P. (1948). *Blood*, **3**, 107.
—— Davis, L. J. (1947). *Advances in Internal Medicine*, **2**, 481. New York.
—— —— and Innes, J. (1942). *British Medical Journal*, **2**, 31.
Fullerton, H. W. (1943). Ibid., **1**, 158.
Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D. (1945). *J. Lab. clin. Med.*, **30**, 1056.
Spies, T. D. (1946). *J. Amer. med. Ass.*, **130**, 474.
Stevenson, E. M. K. (1938). *Trans. Edinb. Obstet. Soc.*, **58**, 81.

FOLIC ACID*

BY

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Idiopathic Steatorrhoea and Coeliac Disease

A number of reports have now appeared on the excellent effect of " folic acid " in the treatment of tropical sprue (Darby and Jones, 1945; Darby *et al.*, 1946a, 1946b; Spies, 1946a, 1946b; Spies *et al.*, 1946; Manson-Bahr and Clarke, 1946; Morrison and Johnston, 1947); while Davidson and Girdwood (1947) and Weir and Comfort (1947) have described their results using it on patients with non-tropical sprue, particularly those with normoblastic marrows.

*The conclusion of the lectures (abridged) given to the Section of Experimental Medicine of the Royal Society of Medicine on May 13, 1947, and to the Medical Society of London on Feb. 9, 1948.

The following four cases illustrate my results when pteroylglutamic acid is used on patients with idiopathic steatorrhoea (non-tropical sprue) and coeliac disease (Table V).

Idiopathic Steatorrhoea

The diagnostic criteria for patients with idiopathic steatorrhoea (non-tropical sprue) were: (1) a macrocytic anaemia with a megaloblastic or normoblastic marrow; (2) frequent loose, bulky, offensive stools, often yellowish in colour; (3) high total fat content of stools with or without a prior fat test meal; (4) achlorhydria gastrica or normal acidity; (5) soreness or ulceration of the mouth or tongue; (6) severe loss of weight; and (7) a flat glucose-tolerance curve.

Case I.S.—A housewife aged 53 had an eight-months history of tiredness, lack of energy, palpitation, intermittent soreness of the tongue, increasing pallor, and frequent loose, bulky, offensive, yellowish stools up to eight in 24 hours. She had lost at least 3 st. (19 kg.) in weight. The bone marrow was very cellular and active, with a mixed megaloblastic (5%)–normoblastic (27%) hyperplasia. A blood count showed: R.B.C., 2,610,000; Hb, 72%; C.I., 1.33; W.B.C., 6,600 (polymorphs, 73%, lymphocytes 22%, large monocytes 3%, eosinophils 2%); marked anisocytosis and poikilocytosis; reticulocytes, 3.2%. Stools: total fat, 55% (split fat 83%, unsplit 17%). She was given 20 mg. of pteroylglutamic acid orally per day. There was slight reticulocytosis (4.2% on the 8th day) and the haematological response was slow but ultimately (after 64 days) reached R.B.C. 4,140,000, Hb 82%, C.I. 1, W.B.C. 7,100, with a normal differential white cell count. The stools rapidly improved, became formed, and ceased to be frequent, bulky, or offensive; total fat, 28% (split 84%, unsplit 16%). Clinically the patient had improved very much indeed; she was free from symptoms and was gaining weight steadily when discharged from hospital.

Case B.C.—A housewife aged 35 had had tetany intermittently since the age of 24; she had bilateral cataracts and frequent bulky offensive stools in addition to the usual general symptoms. Examination revealed achylia gastrica, a flat glucose-tolerance curve, a negative Wassermann reaction, and normal C.S.F.; serum calcium varied between 6.4 and 9.8 mg. per 100 ml., blood urea 22 mg. per 100 ml., urea clearance 85%, serum phosphorus 3.6%, plasma alkaline phosphatase 4–14 units; radiologically there was some generalized osteoporosis of the long bones; weight, 4 st. 12 lb. (30.84 kg.); blood pressure, 100/70. A blood count on June 27, 1946, showed: R.B.C., 3,210,000; Hb, 74%; C.I., 1.16; W.B.C., 3,600 (polymorphs 59%, lymphocytes 36%, large monocytes 4%, eosinophils 1%); anisocytosis and poikilocytosis marked; platelets 97,000. The marrow was very active and cellular and showed a mixed megaloblastic (4.7%)–normoblastic (9.7%) reaction. Stools: total fat, 41% (split fat 82%, unsplit fat 18%). Bacteriological examination of faeces did not disclose any abnormality.

The patient had 15 mg. of pteroylglutamic acid intramuscularly daily for 10 days without any clinical or haematological response, and was then given 1 oz. (28 g.) orally of proteolysed liver extract daily without improvement, the blood count on Aug. 9 being rather worse: R.B.C., 2,820,000; Hb, 62%; C.I., 1.11; W.B.C., 7,200. At this stage the sternal marrow showed a normoblastic (49.25%) hyperplasia. Continued treatment with liver extract parenterally, supplementary synthetic vitamins of the B complex, hog's stomach, etc.,

TABLE V.—*Idiopathic Steatorrhoea*

Case	Sex	Age	Marrow	Gastric Acidity	R.B.C. × 10^6	Hb %	W.B.C.	Folic Acid
I.S.	F	53	Mbl 5% Nbl 27%	Achylia	2·61 4·14 (69)	72 82 (69)	6,600 7,100	20 mg. orally
B.C.	F	35	Mbl 4·7% Nbl 9·7% Mbl 0 Nbl 49·25%	Achylia	3·21 3·11 (14) 3·49 4·31 (32) 2·80 (187) 3·08 (201)	74 72 (14) 70 84 (32) 74 (187) 64 (201)	3,600 5,200 (14) 9,700 7,400 (32) 9,000 (187) 12,800 (201)	15 mg. [10] i.m. 50 mg. [2] i.v. 50 mg. [25] i.v. 40 mg. [187] orally
H.C.	F	27	Mbl 10·4% Nbl 10·4%	Normal	1·97 5·35 (107)	50 108 (107)	3,200 5,700	20 mg. [107] orally
J.M.	F	3	Mbl 2·25% Nbl 2·5%	Normal	3·12 3·81 (129)	63 44 (129)	4,500 4,300 (129)	5 mg. [129] orally

Reticulocytes: Case I.S. 4·3% (8); Case H.C. 25·4% (7). Nbl = Normoblasts. Mbl = Megaloblasts.)Day after beginning treatment. [ ] No. of days on treatment

duced a slightly better response, with a rise on Sept. 13 to R.B.C. 3,490,000 and Hb 70%. She was again given 50 mg. of pteroylglutamic acid intravenously on two successive days, and although there was no reticulocyte response the count rose to R.B.C. 4,310,000 and Hb 84% on Oct. 11. A further 50 mg. was given intravenously, and she was transferred to the convalescent home distinctly better with almost normally formed stools. After return home the pteroylglutamic acid was discontinued and she gradually relapsed; the bowels again became loose and the stools fluid, offensive, and yellow; she lost weight (4 st. 9 lb.—29.48 kg.); serum calcium was 8.5 mg. per 100 ml.; and on April 23, 1947, the blood count was: R.B.C., 2,800,000; Hb, 74%; C.I., 1.3; W.B.C., 9,000. The sternal marrow was still normoblastic.

Pteroylglutamic acid was recommenced at 40 mg. orally per day, but this apparently made the stools worse again and they remained very troublesome. The blood count rose very slowly, and on Sept. 10 it was R.B.C., 4,180,000; Hb, 80%; W.B.C., 11,900. She was put on to proteolysed liver, "sprulac," calcium gluconate, and calciferol, the blood count reaching: R.B.C. 4,400,000, Hb 84%, W.B.C. 11,200 on Oct. 8.

Case H.C.—A housewife, then aged 20, was first seen in 1940 with a refractory macrocytic anaemia for which various treatments and frequent blood transfusions at intervals of two to three weeks were tried with moderate to poor results. In 1945 she attended my clinic complaining of some intermittent diarrhoea which cleared up with treatment, but in January, 1946, it had become very troublesome and less responsive to treatment. In April, 1946, for the first time the stools were bulky, greasy, and soft, contained total fat 74% (split fat 75%, unsplit fat 25%), while the occult blood reaction was negative. There was a normal gastric acidity. *X*-ray examination showed general osteoporosis of skull, pelvis, and long bones, and bilateral fractures of the ischio-pubic junctions. She was treated with high supplementary vitamins, calcium, oral liver extract, etc., and some clinical improvement was obtained, but the blood count reached R.B.C. 3,840,000, Hb 92%, W.B.C. 5,000 on only one occasion; *x*-ray examination of the skeleton revealed marked improvement in the osteoporosis, which had nearly gone. Thereafter she relapsed again and on Nov. 15, 1946, had a count of R.B.C. 1,970,000, Hb 50%, C.I. 1.25, W.B.C. 3,200.

She was given pteroylglutamic acid (20 mg. orally per day), with a very good haematological response—reticulocytes 25.4% on the 7th day—while the count rose steadily on 10 mg. daily to R.B.C. 5,350,000, Hb 108%, C.I. 0.99 on March 12, 1947; radiologically the skeleton did not show any osteoporosis. She became pregnant but aborted in October, 1947 (R.B.C. 4,810,000, Hb 98%). She remained quite well until Jan. 19, 1948, when the blood had relapsed to R.B.C. 2,940,000, Hb 70%, W.B.C. 3,400, and she complained of a recurrence of the old symptoms; the bone marrow was of an active mixed megaloblastic-normoblastic type.

Coeliac Disease

Case J.M.—A girl aged 3 (under care of Dr. M. Egan) was diagnosed as having coeliac disease, with enlarged abdomen, general wasting (weight 1 st. 7 lb.—9.53 kg.), frequent bulky foul stools—total fat 45.6% (split fat 88%, unsplit fat 12%). The spleen, liver, and glands were not enlarged; the nervous system and gastric acidity were normal. The glucose-tolerance curve was: 100, 110, 110, 100—no glycosuria. The sternal marrow contained 2.25% megaloblasts and 2.5% normoblasts. A blood count showed: R.B.C., 3,120,000; Hb, 63% (8.7 g.); C.I., 1.10; W.B.C., 4,500. She was given 5 mg. of pteroylglutamic acid daily for 19 weeks, which was supplemented with ferric ammonium citrate or ferrous sulphate and vitamin C (50 mg. daily) without any haematological improvement; at the end of that time the blood count was: R.B.C., 3,810,000; Hb, 44%; C.I., 0.5; W.B.C., 4,300. Nevertheless, although there was some slight improvement in the stools, which before discharge from hospital had become more formed, she had recurrences of the frequent bulky offensive stools.

The response in this case was disappointing and contrasts with the two cases described by Dalton, Thomson, and Wilson (1946)—both of which had megaloblastic marrows and responded well—and the case of Brody and Gore (1946). On the other hand, Davidson and Girdwood's cases had normoblastic marrows and did not do well, while six of Tegelaers and Weyers's (1947) children improved clinically without haematological improvement.

Of my three patients with steatorrhoea, Cases I.S. and H.C. did very well, showing steady improvement after folic acid, with relief of symptoms and the intestinal steatorrhoea. Case H.C. was particularly good, and early osteoporosis in the skeleton also cleared up, but the patient relapsed later. Case B.C. was less satisfactory, but did ultimately show marked improvement, although relapsing later when the pteroylglutamic acid was discontinued and failing to give much response to a second course.

The cases of non-tropical sprue of Darby *et al.* (1946a, 1946b) also showed good responses, but in the seven cases of Weir and Comfort (1947), and nine out of 10 cases of steatorrhoea and sprue described by Davidson and Girdwood (1947), the haematological responses were unsatisfactory: macrocytosis persisted, yet there was clinical improvement, and the diarrhoea was better although fat absorption was still abnormal. The excellent claims for folic acid in the treatment of sprue by Spies and collaborators and other American authors would appear to require further explanation, and it is probable that their cases were suffering from severe nutritional deficiencies as well—a complication not often seen in this country.

Chronic Ulcerative Colitis

Following the variable results in coeliac disease, the good results claimed by Carruthers (1946) in the treatment of six patients with severe recurrent diarrhoea with folic acid lead naturally to a consideration of other intestinal disturbances. A patient with severe chronic ulcerative colitis of long standing failed to show the slightest evidence of relief when given 20–50 mg. of folic acid daily for three weeks, the blood count falling further and the colitis continuing without remission. Similar results have been obtained by others (Davidson and Girdwood, 1947).

Aplastic Anaemia, Agranulocytosis, Neutropenia, and Thrombocytopenia

One of the earliest observations (Endicott, Daft, and Ott, 1945) on folic acid was its importance in preventing or rapidly curing the marrow hypoplasia or aplasia produced in rats fed on folic-acid-free diets or given diets containing 1% sulphasuxidine, which inhibits the synthesis of folic acid by intestinal bacteria. This raised hopes that folic acid might also be of value in relieving the aplastic or hypoplastic conditions of the human marrow as seen in aplastic anaemia, agranulocytosis, chronic neutropenia, and thrombocytopenia.

In my experience and that of others (Doan, 1946, 6 hypoplastic patients; Goldsmith, 1946, 2 aplastic cases; Kaufmann and Schwager, 1946, 1 aplastic case; Peat and Branch, 1946, 1 aplastic case; Spies, 1946a, 1946b, 3 aplastic patients; Watson *et al.*, 1945, 6 refractory aplastic anaemias, 1 neutropenia; Zuelzer, 1946, 3 aplastic or hypoplastic children) the treatment of aplastic or hypoplastic anaemias with natural or synthetic folic acid has been most disappointing. Gendel (1947), however, has recently claimed to have had some slight responses in three cases of "aplastic" anaemia treated with enormous doses (400–500 mg. daily) of synthetic pteroylglutamic acid for long periods of time, with a reduction in the need for blood transfusions. His results were not very convincing, and one of his patients had a normoblastic marrow.

In my experience similar large doses have not produced any obvious effects. Pteroylglutamic acid in lesser doses

TABLE VI.—*Aplastic Anaemia*

Case	Sex	Age	Marrow	R.B.C. × 10^6	Hb %	W.B.C.	Platelets	Folic Acid Dosage
J.S.	M	44	Aplastic	1·52 1·60 (7)	30 30 (7)	4,200 2,700 (7)	40,000 25,000 (9)	150 mg. i.v.
P.S.	M	6	,,	1·37 0·85 (56) 1·08 (308)	18 20 (56) 24 (308)	1,800 1,400 (56) 1,800 (308)	35,000 246,000 (56)	5–100 mg. orally daily
M.L.	F	53		1·38	26	3,800	30,000	100 mg. i.v. 20 mg. orally daily

was given to three patients with typical aplastic anaemia (see Table VI): all had aplastic marrows and all failed to show the slightest clinical or haematological responses after doses varying up to 150 mg. a day intravenously and orally. Similar negative results were noted in patients with agranulocytosis and the cases of chronic neutropenia given up to 150 mg. daily, intravenously or orally, in contradistinction to the changes noted in rats (see above), while thrombocytopenia purpura (two cases secondary to gold therapy) also resisted this treatment.

Of course, as shown above, where there is haematological response to treatment, as in pernicious anaemia and similar megalocytic anaemias, all the cellular elements of the blood benefit, so that with the red cells there is concomitant improvement in the numbers of white cells and platelets, as occurs after liver and stomach therapy.

Leukaemias

A large number of cases of acute and chronic leukaemia were given synthetic pteroylglutamic acid without benefit. Thus patients with acute myeloblastic, lymphoblastic, and monocytic leukaemias who received up to 150 mg. intravenously and 20–80 mg. orally daily did not show improvement in their haematological or in their general clinical conditions. In chronic myeloid and lymphatic leukaemias no better results were seen, but two of my old patients with pernicious anaemia who developed chronic myeloid leukaemia showed marked general improvement in the pernicious anaemia but subsequently failed to maintain it.

Patient E.O., a woman aged 40, was first seen by me in 1932, when pernicious anaemia was diagnosed: her case has been reported by my chief assistant, Dr. Woolley (1944). She remained well until 1942 when, after five years' absence, she was found to have a large spleen—6 in. (15 cm.) below the costal margin—due to a chronic myeloid leukaemia, with R.B.C., 2,680,000; Hb., 48%; C.I., 0.92; W.B.C., 85,600 (polymorphs 59.8%, lymphocytes 7.2%, large monocytes 0.4%, eosinophils 2.4%, basophils 6.6%, metamyelocytes 14%, myelocytes 9%, myeloblasts 0.6%); normoblasts 6 in 500, megaloblasts 8 in 500 white cells. The sternal marrow puncture showed a chronic myeloid leukaemia picture with also normoblasts 6.6% and megaloblasts 6.6%. She did quite well on anti-pernicious-anaemia treatment with liver extract and additional *x*-ray therapy, and later urethane (1–3 g. daily), but on Feb. 13, 1947, the blood count had become worse, with R.B.C., 3,720,000; Hb, 74%; C.I., 1; W.B.C., 120,500. No improvement was shown haematologically on 20–40 mg. of pteroylglutamic acid daily.

Patient G.G., a woman aged 52, was originally seen in 1940 with pernicious anaemia which responded normally to routine treatment for four years, but on Oct. 16, 1945, she returned to my clinic with a spleen 2 in. (5 cm.) below the costal margin and a blood count of R.B.C., 3,610,000; Hb, 82%; C.I., 1.12; W.B.C., 82,600 (polymorphs 54.5%, lymphocytes 9.5%, large monocytes 4.5%, eosinophils 1.5%, basophils 4.5%, myelocytes 15.5%, metamyelocytes 3.5%, myeloblasts 6.5%); nucleated red cells, 4 in 200 white cells. Marrow biopsy showed a typical chronic myeloid leukaemia with no megaloblasts to be seen. The patient responded very well to liver extract and urethane, but after severe haematuria she had (Feb. 15, 1947) R.B.C., 1,710,000; Hb, 34%; C.I., 1; W.B.C., 219,500; and a typical myeloid leukaemia marrow with very few megaloblasts and normoblasts. She was given pteroylglutamic acid, 30 mg. daily orally, with improvement to April 9 (R.B.C., 3,260,000; Hb, 64%; C.I., 0.98), without any further increase subsequently. The white cell count fell to 7,900 (polymorphs 53%, lymphocytes 5.5%, monocytes 2%, eosinophils 1.5%, basophils 3%, myelocytes 24%, metamyelocytes 10%, myeloblasts 1%) following a course of tri-β-chlorethylamine hydrochloride.

Two patients with leuco-erythroblastic anaemia did not show any clinical or haematological response to synthetic pteroylglutamic acid in daily doses of 20–30 mg. orally for periods up to 38 days.

Miscellaneous Blood Diseases

Synthetic pteroylglutamic acid has also failed to initiate any clinical or haematological improvement in many other blood conditions not having megaloblastic marrow hyperplasia, such as acute and chronic haemolytic anaemias, microcytic hypochromic anaemias, anaemias secondary to hyperthyroidism and myxoedema, toxic anaemias, etc.

Recently Farber *et al.* (1947) reported their preliminary and somewhat favourable results in the treatment of 100 cases of malignant disease with two synthetic polyglutamates—pteroyldiglutamic acid ("diopterin") and pteroyltriglutamic acid ("teropterin"). Apparently pteroylglutamic acid was without effect, and these observers say that there is no evidence to indicate that those substances should be used in routine therapy.

Discussion

Evidence has been accumulating as to the value of this new synthetic anti-macrocytic-anaemia constituent of the vitamin B complex. It is quite clear that in general only those anaemias with megaloblastic marrows respond to pteroylglutamic acid, however administered, while those with normoblastic, aplastic, or hypoplastic marrows do not.

Thus of the megaloblastic anaemias that respond we have pernicious anaemia, possibly achrestic anaemia, and macrocytic anaemias of pellagra, sprue, idiopathic steatorrhoea, pregnancy, infancy, and nutritional deficiencies. In the sprue syndrome, coeliac disease and steatorrhoea may vary in response according to the type of marrow activity: with a megaloblastic marrow the response may be good for a time, but these patients tend to relapse with recurrence of the loose, bulky, fatty stool. Less satisfactory results are obtained when the marrow is normoblastic, and although these patients do not respond haematologically they may show some improvement in their intestinal functions. This has led Davidson and Girdwood to suggest that pteroylglutamic acid has two functions: to maintain normoblastic marrow action, and to control the behaviour of the alimentary tract.

The great difference between the sprue cases of American authors and those described in this country does suggest that the American patients may have had more gross nutritional deficiencies with superimposed gastro-intestinal disturbances, while the British ones are primarily due to a failure of absorption from the intestinal tract or else failure to utilize essential nutritional factors—in particular, members of the vitamin B group.

Among the various haematological conditions not responding to pteroylglutamic acid I have noted hypochromic microcytic and secondary anaemias, aplastic and hypoplastic types of anaemia, many "liver-refractory" anaemias, haemolytic anaemias, acute and chronic leukaemias, idiopathic and toxic thrombocytopenic purpura, agranulocytosis, chronic and toxic leucopenias.

However administered, the immediate effects of pteroylglutamic acid on patients with pernicious anaemia are good, although not quite so striking as may follow highly potent liver extracts or hog's stomach preparations. A good reticulocyte crisis is obtained in most but not all cases at about the seventh to ninth day, with conversion of the megaloblastic marrow reaction to a normoblastic one and a steady rise in the haemoglobin percentage and the red cell, white cell, and platelet values. Clinically, the patient begins to feel very much better; appetite and colour return, weight increases, gastro-intestinal symptoms improve, but there is a tendency for macrocytosis to remain, and the symptoms of peripheral polyneuritis or postero-lateral sclerosis either persist unchanged or become worse.

After only a few months eight of 20 patients with pernicious anaemia have neurological symptoms and signs—four coming on acutely in the course of one to three months after discharge from hospital. Similar disappointing results are now being obtained by others, and it is therefore very important that all patients with pernicious anaemia on pteroylglutamic acid should be kept under very frequent and constant haematological and clinical observation. Maintenance doses must not be reduced too much (5–15 mg. daily are commonly required), otherwise the haematological condition deteriorates and macrocytosis becomes more pronounced, with an even greater tendency to produce neurological changes.

Thus, while we recognize that pteroylglutamic acid has a very great and prompt haematological influence, in particular with the macrocytic megaloblastic anaemias, nevertheless we are compelled to take note of the very disquieting and disappointing fact that it has no influence at all in curing, relieving, or preventing the onset or progression of neurological symptoms and signs. There are now records of neurological changes developing or getting worse in 59 out of 184 patients treated. In fact, I have been particularly struck by the acuteness of the onset of signs and symptoms of subacute combined degeneration of the cord with changes in the reflex reactions that may come on within one to four months or so of the institution of pteroylglutamic acid therapy.

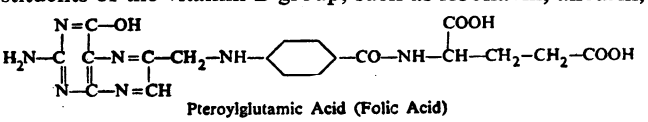
It is quite clear that pteroylglutamic acid has definite limitations and alone is not a safe treatment for pernicious anaemia owing to the very real risk (at least 30% of our cases) of neurological changes persisting or appearing for the first time—sometimes very acutely in the course of a few months. Therefore liver extracts or hog's stomach preparations must still always be employed in adequate dosage in treating pernicious anaemia with or without neurological changes, so that there is no indication for or advantage in using pteroylglutamic acid for this purpose. I deprecate very much the recurring efforts to offer "blunderbuss" mixtures of folic acid with liver, stomach, and iron—since they never contain any of the individual constituents in adequate therapeutic doses, the individual doses cannot be varied as required, and they are of no value in most blood diseases. On the other hand, in the other macrocytic and achrestic anaemias pteroylglutamic acid might sometimes be used with advantage, since neurological symptoms do not occur, although the cost is still an important feature.

The value of pteroylglutamic acid in treatment is also limited in the sprue syndrome, for patients with steatorrhoea and coeliac disease have given irregular response (from good to not at all) and have often required liver preparations to complete the clinical and haematological remission. The presence of a normoblastic marrow indicates that pteroylglutamic acid will be ineffective, since it is apparently effective only in the treatment of conditions with megaloblastic erythropoiesis. From the evidence it is quite clear that, whatever the part played by pteroylglutamic acid in erythropoiesis, there must be at least another factor concerned with the prevention and cure of the neurological features in pernicious anaemia, as I have suggested from my experience with hog's stomach preparations in the treatment of subacute combined degeneration of the cord and pernicious anaemia (Wilkinson, 1933). The role of pteroylglutamic acid in haemopoiesis is of course not at all clear, and we are still very short of clear-cut unequivocal facts.

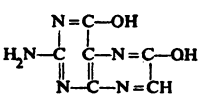
It was observed by Stokes (1944a, 1944b) from substitution experiments on the growth of *L. casei* and *Str. faecalis*, that folic acid could be replaced by certain pyrimidines and purines; of these, thymine (5-methyluracil) in a 5,000-fold concentration was effective. Taking up this observation, Spies *et al.* (1946, 1947) found that 7–15 g. of thymine daily was required to produce reasonably good haematological and clinical improvement, which was certainly not so good as with pteroylglutamic acid; it failed to prevent or relieve any neurological changes, glossitis, and stomatitis.

No haematological responses followed administration of many purines, pyrimidines, and members of the vitamin B complex, and Spies thinks the 5-methyl group in thymine has specificity of some kind in haemopoiesis (Spies *et al.*, 1945).

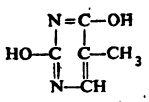
In past years I have also tried, with and without incubation with gastric juice or haemopoietin, many of the constituents of the vitamin B group, such as riboflavin, aneurin,



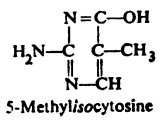
Pteroylglutamic Acid (Folic Acid)



Xanthopterin



5-Methyluracil (Thymine)



5-Methyl*iso*cytosine

pyridoxin, pantothenic acid, and xanthopterin, with negative results; the similarity between pteroylglutamic acid and 5-methyl*iso*cytosine led to a trial of this substance in doses of 100–200 mg. intravenously, and 5 g. orally, per day. With these doses slight reticulocytosis and a slow rise in the number of red cells and in haemoglobin percentage were obtained—results similar to those with thymine in similar dosage—and maybe larger doses are required to give full remissions.

It is perfectly obvious that pteroylglutamic acid is not: (*a*) the extrinsic factor of Castle (it can be given parenterally without the need for digestion with gastric juice); (*b*) haemopoietin, the anti-pernicious-anaemia stomach enzyme (Wilkinson and Klein, 1932); (*c*) the liver anti-pernicious-anaemia principle, which is highly potent in very small doses free from pteroylglutamic acid—thus, on the basis of dosage, 10–20 mg. of pteroylglutamic acid is required daily, while the maximal amount of it present in the corresponding dose of liver extracts is of the order of 0.02–3.7 μg. (i.e., a 5,000–20,000-fold difference) (Clark, 1945); pteroylglutamic acid will not mature marrow cells if incubated with them to form normal red cells from the precursors, although liver extracts will do so; or (*d*) the anti-subacute-combined-degeneration factor, whether it is or is not a different or separate factor from the anti-anaemia factor.

While free folic acid itself is almost universally active in relation to bacterial growth and the relief of chick, animal, and human anaemias and certain rat leucopenias, this is not so with the so-called vitamin B_c conjugates or pteroylglutamic acid conjugates. These conjugates consist essentially of pteroylglutamic acid combined with extra glutamic acid molecules to form polyglutamates, which are not universally active like the free folic or pteroylglutamic acid until the extra glutamic acid molecules have been removed by enzyme action or incubation with *Str. faecalis*. Nevertheless where they do act—as when given to rats, chicks, or monkeys suffering from *L. casei* factor, "vitamin B_c" deficiency, or "vitamin M" deficiency, respectively—their activities are proportional to their respective free folic acid contents.

The two crystalline polyglutamates so far examined are: (1) the vitamin B_c conjugate prepared from yeast (pteroylhexaglutamyl-glutamic or pteroylheptaglutamic acid), which has no effect on the growth of *L. casei* and *Str. faecalis*, and (2) the fermentation or *L. casei* factor (pteroylglutamic acid conjugate), obtained by the extraction of certain corynebacteria residues as pteroyl-diglutamyl-glutamic (or pteroyl-triglutamic acid), which promotes the growth of *L. casei*, but is only 1/50 as active as *Str. faecalis*.

It has been suggested that patients with pernicious anaemia are able to utilize only free pteroylglutamic acid and not the polyglutamates, probably owing to the gastric deficiency (Bethell *et al.*, 1947; Heinle *et al.*, 1947), while patients with other macrocytic anaemias having normal gastric secretions could use the polyglutamates. Spies (1946a, 1946b) claims that the doses the above authors employed were too small, and he has obtained moderate haematological responses in pernicious anaemia using the natural polyglutamates and also one synthetic diglutamate. Others (e.g., Sharp *et al.*, 1947) have also obtained responses with natural conjugates. This requires further confirmation using synthetic conjugates, as it is of the greatest importance.

Chicks, dogs, rats, and monkeys apparently utilize the polyglutamates because the pteroylglutamic acid is liberated by certain enzymes present in their livers or kidneys and probably by enzymes or bacteria in their intestinal tracts. It is difficult to believe that the human organs behave so differently from these. In fact, Denko *et al.* (1946) have shown that folic acid can be synthetized in quite large amounts in the human gut.

Summary

The relationship of "folic acid" (pteroylglutamic acid) and the "folic acid conjugates" (pteroyltriglutamic and pteroylheptaglutamic acids) are considered with their effects on the haematological changes induced in chicks, rats, and monkeys.

The uses of synthetic pteroylglutamic acid in the oral and parenteral treatment of certain diseases involving the haemopoietic system in man are described.

Pteroylglutamic acid produces good haematological and clinical effects only in macrocytic anaemias with some degree of megaloblastic change in the bone marrows; it has little or no effect on patients with normoblastic marrows. Consequently the following anaemias respond to pteroylglutamic acid: pernicious anaemia; macrocytic megaloblastic anaemias of pregnancy, infancy, pellagra, sprue, steatorrhoea, and coeliac disease; nutritional macrocytic anaemias (with or without nutritional leucopenia), and possibly achrestic anaemia.

Pteroylglutamic acid acts equally well when given orally or parenterally and has no ill effects on the patient. The initial dose varies with the individual patient, but is usually 15–20 mg. a day orally, or 50–150 mg. intravenously.

In pernicious anaemia a prompt reticulocyte response is produced after the administration of pteroylglutamic acid, followed by general haematological and clinical improvement, but not quite so well or so rapidly as with a highly potent liver extract. A maintenance treatment of 5–20 mg. daily is necessary, but after months of treatment a lower dose often shows a fall in the blood count and haemoglobin that will often respond to increased doses. Macrocytosis still tends to persist even after one to two years' treatment.

Pteroylglutamic acid is of no value in preventing the onset and progression or the development (which may be acute) of subacute combined degeneration of the cord in pernicious anaemia. For this reason it is not a satisfactory treatment for pernicious anaemia, and, since it has no advantages over present-day highly potent liver extracts or stomach preparations, it should not be given alone as routine to patients with pernicious anaemia.

Refractory macrocytic normoblastic anaemias do not respond to pteroylglutamic acid, but refractory megaloblastic anaemia may give responses.

It often relieves the symptoms of the sprue syndrome, leading to better-formed stools and some improvement in blood count, but only in the megaloblastic cases: the responses are irregular in steatorrhoea, and liver extracts may be required additionally to produce complete remissions. The response in coeliac disease may be very slow or poor; those with normoblastic marrows do not respond, since pteroylglutamic acid is effective only where megaloblastic erythropoiesis is present.

Pteroylglutamic acid has no beneficial clinical or haematological effects on hypochromic microcytic anaemias, haemolytic anaemias, refractory megalocytic anaemias with normoblastic marrows, acute and chronic leukaemias of all types, leucoerythroblastic anaemia, chronic ulcerative colitis, anaemias secondary to myxoedema or hyperthyroidism, toxic or idiopathic aplastic and hypoplastic anaemias, agranulocytosis, neutropenias, and thrombocytopenic purpura, but it relieves leucopenia and thrombocytopenia when these are features of a nutritional deficiency or a megaloblastic anaemia.

There is no place in therapeutics for the "blunderbuss" preparations of pteroylglutamic acid plus iron, liver extract, etc.

Pteroylglutamic acid is not the extrinsic factor, haemopoietin (the stomach enzyme or intrinsic factor), or the liver anti-pernicious-anaemia principle, nor is it the possible "neuropoietic" principle (anti-subacute combined degeneration of the cord). The effects of pteroylglutamic acid on haemopoiesis are discussed.

There may possibly be a separate factor in liver and hog's stomach preparations with neuropoietic properties for preventing or curing the neurological symptoms in pernicious anaemia.

The anti-pernicious-anaemia potencies of substances related to pteroylglutamic acid are considered.

References

Amill, L. A., and Wright, M. (1946). *J. Amer. med. Ass.*, **131**, 1201.
Angier, R. B., and 15 others (1945). *Science*, **102**, 227.
—— (1946). Ibid., **103**, 667.
Axelrod, A. E., Gross, P., Bosse, M. D., and Swingle, K. F. (1943). *J. biol. Chem.*, **148**, 721.
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.
—— Swendseid, M. E., Bird, O. D., Meyers, M. C., Andrews, G. A., and Brown, R. A. (1946). *Univ. Hosp. Bull., Ann Arbor*, **12**, 42.
Binkley, S. B., Bird, O. D., Bloom, E. S., Brown, R. A., Calkins, D. G., Campbell, C. J., Emmett, A. D., and Pfiffner, J. J. (1944). *Science*, **100**, 36.
Briggs, G. M., Luckey, T. D., Elvehjem, C. A., and Hart, E. B. (1943). *J. biol. Chem.*, **148**, 163.
—— —— —— —— (1944). Ibid., **153**, 423.
Brody, H. P., and Gore, L. (1946). *Lancet*, **2**, 618.
Campbell, C. J., Brown, R. A., and Emmett, A. D. (1944). *J. biol. Chem.*, **152**, 483.
—— McCabe, M. M., Brown, R. A., and Emmett, A. D. (1945). *Amer. J. Physiol.*, **144**, 348.
Carruthers, L. B. (1946). *Lancet*, **1**, 849.
Clark, G. W. (1945). *Amer. J. Med. Sci.*, **209**, 520.
Cooperman, J. M., McCall, K. B., and Elvehjem, C. A. (1945). *Science*, **102**, 645.
Daft, F. S., Kornberg, A. Ashburn, L. L., and Sebrell, W. H. (1945). *Publ. Hlth Rep., Wash.*, **60**, 1201.
—— —— —— —— (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 154.
—— and Sebrell, W. H. (1943). *Publ. Hlth Rep., Wash.*, **58**, 1542.
Dalton, H. W., Thomson, M. L., and Wilson, V. K. (1946). *Lancet*, **2**, 652.
Darby, W. J., and Jones, E. (1945). *Proc. Soc. exp. Biol., N.Y.*, **60**, 259.

Darby, W. J., Jones, E., and Johnson, H. C. (1946a). *Science*, **103**, 108.
—— —— —— (1946b). *J. Amer. med. Ass.*, **130**, 780.
Davidson, L. S. P., and Girdwood, R. H. (1946). *Lancet*, **2**, 373.
—— —— and Innes, E. M. (1947). Ibid., **1**, 511.
Day, P. L., Langston, W. C., and Shukers, C. F. (1936). *J. biol. Chem.*, **114**, xxv.
—— Mims, V., Totter, J. R., Stokstad, E. L. R., Hutchings, B. L., and Sloane, N. H. (1945). *J. biol. Chem.*, **157**, 423.
Denko, C. W., *et al.* (1946). *Arch. Biochem.*, **10**, 33.
Doan, C. A. (1946). *Amer. J. med. Sci.*, **212**, 257.
Endicott, K. M., Daft, F. S., and Ott, M. (1945). *Arch. Path.*, **40**, 364.
Farber, S., Cutler, E. C., Hawkins, J. W., Harrison, J. H., Pearce, E. C., and Lenz, G. G. (1947). *Science*, **106**, 619.
Frommeyer, W. B., jun., and Spies, T. D., Vilter, C. F., and English, A. (1946). *J. Lab. clin. Med.*, **31**, 643.
Gendel, B. R. (1947). Ibid., **32**, 139.
Goldsmith, G. A. (1946). Ibid., **31**, 1186.
Hall, B. E., and Watkins, C. H. (1947). Ibid., **32**, 622.
—— —— and Hargreaves, M. M. (1946). *Proc. Cent. Soc. clin. Res.*, **19**, 55.
Harrison, R. J., and White J. C. (1946). *Lancet*, **2**, 787.
Heinle, R. W., Dingle, J. T., and Weisberger, A. S. (1947). *J. Lab. clin. Med.*, **32**, 970.
—— and Welch, A. D. (1947). *J. Amer. med. Ass.*, **133**, 739.
Hogan, A. G., and Parrott, E. M. (1940). *J. biol. Chem.*, **132**, 507.
Hutchings, B. L., Bohonos, N., and Peterson, W. H. (1941). Ibid., **141**, 521.
—— Stokstad, E. L. R., Bohonos, N., and Slobodkin, N. H. (1944). *Science*, **99**, 371.
Kaufmann, J., and Schwager, P. G. (1946). *Canad. med. Ass. J.*, **54**, 539.
Langston, W. C., Darby, W. J., Shukers, C. F., and Day, P. L. (1938). *J. exp. Med.*, **68**, 923.
Levy, H. (1947). *British Medical Journal*, **1**, 412.
Manson-Bahr, P., and Clarke, O. (1946). *Lancet*, **2**, 903.
Martin, G. J. (1942). *Proc. Soc. exp. Biol., N.Y.*, **51**, 353.
Meyer, L. M. (1947). *Blood*, **2**, 50.
Mitchell, H. K., Snell, E. E., and Williams, R. J. (1941). *J. Amer. chem. Soc.*, **63**, 2284.
—— —— —— (1944). Ibid., **66**, 267.
Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D. (1945). *J. Lab. clin. Med.*, **30**, 1056.
Morrison, R. J. G., and Johnston, C. R. St. (1947). *Lancet*, **1**, 636.
O'Dell, B. L., and Hogan, A. G. (1943). *J. biol. Chem.*, **149**, 323.
Peat, G. B., and Branch, A. (1946). *Canad. med. Ass. J.*, **55**, 287.
Petering, H. G., Delor, R. A., and Murray, H. C. (1947). *Blood*, **2**, 440.
Pfiffner, J. J., Binkley, S. B., Bloom, E. S., Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. C. (1943). *Science*, **97**, 404.
Ransone, B., and Elvehjem, C. A. (1943). *J. biol. Chem.*, **151**, 109.
Richardson, L. R., Hogan, A. G., and Karrasch, R. J. (1942). *J. Nutrit.*, **24**, 65.
Ross, J. F., Belding, H., and Paegel, B. L. (1948). *Blood*, **3**, 68.
Scott, M. L., Norris, L. C., and Heuser, G. F. (1946). *Science*, **103**, 303.
—— —— —— and Bruce, W. F. (1945). *J. biol. Chem.*, **158**, 291.
Sharp, E. A., and Vonder Heide, E. C. (1947). *Amer. J. clin. Path.*, **17**, 761.
Snell, E. E., and Peterson, W. H. (1940). *J. Bact.*, **39**, 273.
Spies, T. D. (1946a). *Sth. med. J., Birmingham, Ala.*, **39**, 634.
—— (1946b). *Lancet*, **1**, 225.
—— Frommeyer, W. B., jun., Lopez, G. G., Toca, R. L., and Gwinner, G. (1946). Ibid., **1**, 883.
—— —— Vilter, C. F., and English, A. (1946). *Blood*, **1**, 185.
—— Lopez, G. G., Menendez, J. A., Minnich, V., and Koch, M. B. (1946). *Sth. med. J., Birmingham, Ala.*, **39**, 30.
—— Milanes, F., Menendez, J. A., Koch, M. B., and Minnich, V. (1946). *J. Lab. clin. Med.*, **31**, 227.
—— and Stone, R. E. (1947). *Lancet*, **1**, 174.
—— Suarez, R. M., Suarez, R. M., jun., and Hernandez-Morales, F. (1946). *Science*, **104**, 75.
—— Vilter, C. F., Cline, J. K., and Frommeyer, W. B., jun. (1946). *Sth. med. J., Birmingham, Ala.*, **39**, 269.
—— —— Koch, M. B., and Caldwell, M. H. (1945). Ibid., **38**, 707.
Stokes, J. L. (1944a). *J. Bact.*, **47**, 433.
—— (1944b). Ibid., **48**, 201.
Stokstad, E. L. R. (1941). *J. biol. Chem.*, **139**, 475.
—— (1943). Ibid., **149**, 573.
Tegelaers, W. H. H., and Weyers, H. A. (1947). *Maandschr. Kindergeneesk.*, **15**, 107.
Totter, J. R., and Day, P. L. (1943). *J. biol. Chem.*, **147**, 257.
—— Shukers, C. F., Kolson, J., Mims, V., and Day, P. L. (1944). Ibid., **152**, 147.
Vilter, C. F., Spies, T. D., and Koch, M. B. (1945). *Sth. med. J., Ala.*, **38**, 781.
—— Vilter, R. W. and Spies, T. D. (1947). *J. Lab. clin. Med.*, **32**, 262.
Waisman, H. A., and Elvehjem, C. A. (1943). *J. Nutrit.*, **26**, 361.
Watson, C. J., Sebrell, W. H., McKelvey, J. L., and Daft, F. S. (1945). *Amer. J. Med. Sci.*, **210**, 463.
Weir, J. F., and Comfort, M. W. (1947). *J. Lab. clin. Med.*, **32**, 1231.
Wilkinson, J. F. (1931a). *British Medical Journal*, **1**, 85.
—— (1931b) *Lancet*, **2**, 791.
—— (1932). *British Medical Journal*, **1**, 325.
—— (1933). *Proc. R. Soc. Med.*, **26**, 42, 1341.
Wilkinson, J. F. (1940). *British Medical Journal*, **1**, 414.
—— and Israëls, M. C. G. (1935). *British Medical Journal*, **1**, 139, 194.
—— —— (1936). *Quart. J. Med.*, **5**, 69.
—— —— and Fletcher, F. (1946). *Lancet*, **2**, 156.
—— and Klein, L. (1932). Ibid., **1**, 719.
Wills, L., and Bilimoria, H. S. (1932). *Indian J. med. Res.*, **20**, 391.
—— Clutterbank, P. W., and Evans, B. D. F. (1937). *Biochem. J.*, **31**, 2136.
—— and Evans, B. D. F. (1938). *Lancet*, **2**, 416.
—— and Stewart, A. (1935). *Brit. J. exp. Path.*, **16**, 444.
Wilson, H. E., Doan, C. A., Saslaw, S., and Schwab, J. L. (1942). *Proc. Soc. exp. Biol., N.Y.*, **50**, 341.
—— Saslaw, S., and Doan, C. A. (1946). *J. Lab. clin. Med.*, **31**, 631.
Woolley, P. B. (1944). *Lancet*, **1**, 85.
Wright, L. D., and Welch, A. D. (1943). *Science*, **98**, 179.
Zuelzer, W. W. (1946). *J. Amer. med. Ass.*, **131**, 7

CONGENITAL MEGACOLON

RESULTS OF TREATMENT BY SPINAL ANAESTHESIA

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In the milder cases of megacolon in childhood it may be that diet and medical measures will secure a sufficient action of the bowel, but where diet and drugs alike fail and frequent recourse has to be had to enemata and mechanical evacuation the need for surgical treatment is clear. Without such treatment the trouble tends to worsen, and in the more severe forms death is likely before the age of 20.

The fashion of surgical intervention has changed from time to time. In earlier days short-circuiting operations were tried and found wanting, while heroic resections of the dilated colon were attended by a high mortality even in experienced hands: 25% by Adson (1937). The mortality should certainly be lower to-day as a result of the better pre-operative preparation of the patient and his colon. There is undoubtedly a place for resection in the most severe varieties of the trouble which show no response to other forms of treatment.

The conception that megacolon is due to an achalasia—i.e., an imbalance between the sympathetic (filling) and the parasympathetic (emptying) mechanism which results in a relative inadequacy of the latter action—at once altered the outlook by suggesting an attack on the nerve supply rather than on the bowel itself. The sympathetic supply was interrupted in various places by bilateral lumbar, unilateral lumbar, presacral, and inferior mesenteric sympathectomies. On the whole, good results were obtained at small risk. But in 1935 Stabins, Morton, and Scott showed that success followed the use of a spinal anaesthetic alone. Since that date a number of successful results have been reported by various workers. These reports are open to criticism in that the follow-up period is too short. We have therefore re-examined those patients who were treated by spinal anaesthesia in the neurovascular clinic of the Manchester Royal Infirmary between the years 1938 and 1945. Cases treated within the last two years have been rejected as being too recent to be included.

The number of patients treated during the period is 17. We saw 11 of these patients during December, 1947, and obtained a detailed account of another: five were not traced. These 12 children comprised seven boys and five girls. The youngest was aged 6 months and the oldest 13 years when treated. The average age of the group was 7 years.