Table 3. Effect of B1 and procarbazine on the leukopoiesis in mice

Substances mg/kg		Control	%	Day 3	%	5	%	Weeks 1	%	2	%	3	4	%	6	%
B1	Leukocytes	8900	100	5955	100	5545	100	7215	100	5715	100	6500			10330	
1×150	Lymphocytes	7085	79.6	4465	75.0	3620	65.3	4850	67.2	4380	76.6					
	Neutrophils	1750	19.7	1400	23.5	1845	33.3	2380	33.0	1325	23.2					
Procarbazine	Leukocytes	8900	100	3255	100	2835	100	3000	100	2400	100	1845			5965	
	Lymphocytes	7085	79.6	2590	80.3	2220	78.0	2315	77.1	1630	67.8					
1×165	Neutrophils	1750	19.7	620	19.2	600	21.3	665	22.9	760	32.0					
B1	Leukocytes	8900	100							9310	100		8300	100	6380	100
14×65	Lymphocytes	7085	79.6							5690	61.1		6690	80.6	4940	74.4
	Neutrophils	1750	19.7							3575	38.4		1585	19.1	1360	21.3
Procarbazine	Leukocytes	8900	100							3630	100		3080	100	2150	100
	Lymphocytes	7085	79.6							2350	64.8		2030	65.9	1150	53.6
14×50	Neutrophils	1750	19.7							1260	34.7		1020	33.1	975	45.3

causes a significant, long lasting shift from prophase to metaphase, an effect also found by Rutishauser and Bollag[9] in the case of 1-methyl-2-benzyl hydrazine. A similar but shorter shift is found for A1 after 8 h, whereas the distribution pattern of B1 does not differ from control. Thus, the prolongation of the interphase for B1 is not due to a direct influence on mitosis.

Leukopoiesis. We investigated the leukopoiesis after treatment with a single high equimolar dose of B1 and procarbazine and after 2 weeks of daily application of 1/10 DL_{50} (*mole B1/mole procarbazine* ≈ 1.5). Both drugs cause a depression of leukocytes, which is more intensive and lasts longer with procarbazine. The number of leukocytes reaches its minimum after 3 weeks of procarbazine treatment and is still below control values after 6 weeks, whereas B1 causes its maximal depression already after 5 days, but in this case the leukocytes recover at least after 6 weeks. Furthermore, the depression under B1 mainly concerns the differentiation of lymphocytes and barely affects the granuopoiesis. Compared with these effects, procarbazine causes a severe granulopenia. The lower toxicity of B1 becomes more distinct when the analysis is done after the 2 weeks' treatment. After application we could not determine any change of the leukocyte numbers. Under these conditions, procarbazine causes a long-lasting reduction of the myeloic and even a greater decrease of the lymphatic cells. The results are compatible with those obtained by Bollag in rats[10].

9 A. Rutishauser and W. Bollag, Experientia *19*, 131 (1963).
10 W. Bollag, Acta Genet. Med., Roma *XVII*, 158 (1968).

Folic acid and the inhibition of brain L-glutamic decarboxylase

G. Tunnicliff and T. T. Ngo[1]

Laboratory of Neurochemistry, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal (Quebec H2W 1R7, Canada), 11 June 1976

Summary. Folic acid competitively inhibited brain L-glutamic decarboxylase ($K_i = 1.62 \times 10^{-3}$ *M*). This inhibition could possibly be associated with epilepsy.

Introduction. There is a possible link between epilepsy and folic acid metabolism. The administration of folate to rats induces convulsions[2,3]. Moreover, it has been observed that the folic acid content is increased in experimental epileptic cobalt foci[4].

In epileptic patients undergoing anticonvulsant drug therapy a reduction in serum folate levels has been measured[5]. Indeed, the development of megaloblastic anaemia has been noted in such patients[6]. Folate administration, intended to counteract this deficiency, has been reported to result in an increased frequency of seizures[7,8].

An explanation of the biochemical basis for the convulsant action of folic acid has not yet been forthcoming. However, Roberts[9] has demonstrated that glutamate uptake by nervous tissue is competitively inhibited by folate. This is of interest since glutamate has been proposed as an excitatory transmitter in the brain[10]. Several drugs that can induce convulsions have been shown to inhibit brain L-glutamate decarboxylase (GAD)[11–14].

1 This work was supported by the United Parkinson Foundation.
2 O. R. Hommes and E. A. M. T. Obbens, J. Neurol. Sci. *16*, 271 (1972).
3 E. A. M. T. Obbens and O. R. Hommes, J. Neurol. Sci. *20*, 223 (1973).
4 A. Mayersdorf, R. R. Streiff, B. J. Wilder and R. H. Hammer, Neurology, Minneap. *21*, 418 (1971).
5 E. H. Reynolds, Psychiat. Neurol. Neurochir. *74*, 167 (1971).
6 E. H. Reynolds, in *Antiepileptic Drugs* (Eds. D. M. Woodbury, J. K. Penney and R. P. Schmidt; Raven Press, N. Y. 1972), p. 247.
7 E. H. Reynolds, Lancet I, 1086 (1967).
8 E. H. Reynolds, Lancet I, 1376 (1973).
9 P. J. Roberts, Nature *250*, 429 (1974).
10 J. L. Johnson, Brain Res. *37*, 1 (1972).
11 E. W. Maynert and H. K. Kaji, J. Pharmac. exp. Ther. *140*, 133 (1962).
12 R. W. Horton and B. S. Meldrum, Br. J. Pharmac. *49*, 52 (1973).
13 S. Simler, L. Ciesielski, M. Maitre, H. Randrianarisoa and P. Mandel, Biochem. Pharmac. *22*, 1701 (1973).
14 J. D. Wood, Progr. Neurobiol. *5*, 77 (1975).

We thought it would be of interest to investigate the action of folic acid on the activity of GAD in view of its convulsive action and its structural resemblance to glutamate, the GAD substrate.

In a typical experiment, whole brain from 3 male Charles River rats (200 g) was pooled and homogenised in ice-cold 0.32 *M* sucrose. GAD was partially purified by ammonium sulphate fractionation according to the procedure of WU et al.[15]. The enzyme was assayed[16] over a glutamate concentration range of 0.5 m*M* to 10 m*M* either in the absence or in the presence of 0.2 m*M* or 0.4 m*M* folic acid.

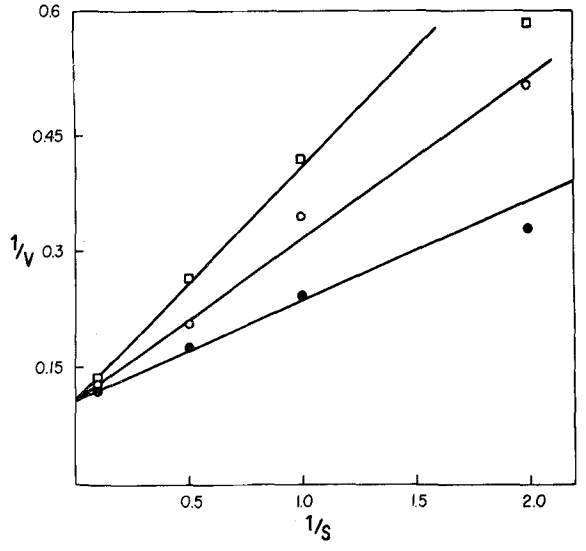

Fig. 1. Double reciprocal plot of the effects of substrate concentration on enzyme activity in the presence or absence of folate ●—● absence of folic acid, ○—○ presence of 0.2 m*M* folic acid, ■—■ presence of 0.4 m*M* folic acid. The enzyme was assayed at 37 °C for 30 min. The concentration range of glutamate was 0.5 m*M* to 10 m*M*. Each points represents the mean of 3 determinations. V: μ mol/h/mg protein; S: m*M*.

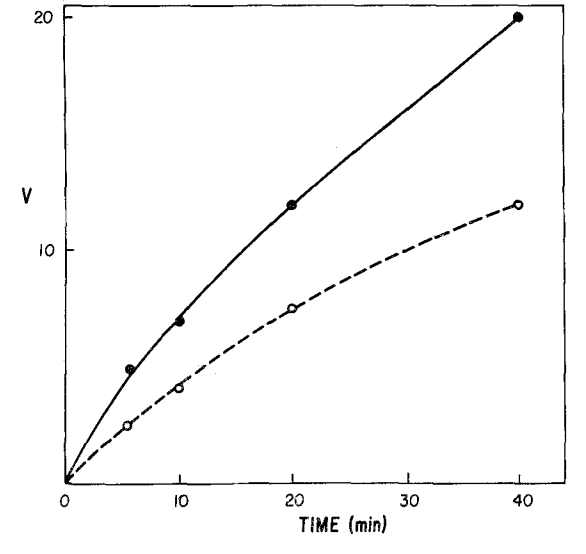

Fig. 2. Measurement of GAD activity against time in the absence (●—●) or presence (○—○) of 0.5 m*M* folic acid. Glutamate concentration was 0.5 m*M* and the temperature was 37 °C. Each point represents the mean of 3 determinations. V: μ mol/h/mg protein.

Results and discussion. The results were plotted by the method of LINEWEAVER and BURK[17] as shown in Figure 1. A classical competitive inhibition was observed and the K_i was calculated as 1.62×10^{-3} *M*. As we were employing a radioactive assay method that measured the formation of carbon dioxide from the decarboxylation of [1-^{14}C]-glutamate, we thought it possible that the competitive inhibition of GAD was merely an artifact and that peptidases might be present in our enzyme preparation which would liberate glutamate from the folic acid. In this case the apparent inhibition would simply be a result of a reduction in the specific radio-activity of our substrate. Two further experiments were carried out to test this theory.

GAD was measured at varying times over a period of 40 min in the absence or presence of 0.5 m*M* folic acid. The results are shown in Figure 2. It can be clearly seen that the degree of inhibition of the enzyme is almost the same throughout the incubation period. If glutamate were being hydrolysed from the folate it would be expected that there would be a steady increase in the rate of apparent inhibition over the 40 min period. A second experiment was carried out in which the formation of γ-aminobutyric acid (GABA) rather than carbon dioxide was measured. GAD was incubated for 30 min in the presence of 0.5 m*M* glutamate either with or without 0.5 m*M* folate. The reaction was stopped by applying the reaction mixture to a Bio-Rad-AG1-X4 anion exchange resin column and collecting the eulate. Folic acid remained on the column and was thus removed from the GABA fraction. The GABA formed during the incubation was determined by the method of JAKOBY[18]. The presence of folic acid reduced GABA production by 44%. The results of the latter two experiments indicate that the inhibition of GAD shown in Figure 1 is a genuine competitive inhibition by folate and not the result of folate hydrolysis which would provide additional unlabelled glutamate during the incubation period.

In light of the evidence linking folic acid with epilepsy[2-8] the present data are of interest. These provide evidence that folate has the ability to affect GAD activity. WOOD and PEESKER[19,20] have already demonstrated that a good correlation exists between the convulsant action of certain drugs and their ability to inhibit GAD. Consequently the seizures induced by folic acid may also be related to the inhibitory action of this compound on this enzyme. Both GAD[21] and folic acid[22] are associated mainly with nerve endings; it is thus conceivable that under certain circumstances GAD activity can be controlled by folic acid and that convulsions can occur when local folate leves become too high.

[15] J. Y. WU, T. MATSUDA and E. ROBERTS, J. Biol. Chem. *248*, 3029 (1973).
[16] E. ROBERTS and D. G. SIMONSEN, Biochem. Pharmac. *12*, 113 (1963).
[17] H. LINEWEAVER and D. BURK, J. Am. Chem. Soc. *56*, 658 (1934).
[18] W. B. JAKOBY, Meth. Enzym. *5*, 765 (1962).
[19] J. D. WOOD and S. J. PEESKER, J. Neurochem. *23*, 703 (1974).
[20] J. D. WOOD and S. J. PEESKER, J. Neurochem. *25*, 277 (1975).
[21] F. FONNUM, Biochem. J. *106*, 401 (1968).
[22] W. F. BRIDGES and L. D. MCLAIN, Adv. Biochem. Psychopharmac. *4*, 81 (1971).