myosine ATPase, might possibly attach itself to another site of lower affinity, inhibiting the ATPase.[12,13] At low concentration of ATP, contraction may proceed without release of Ca^{2+}. The function and turnover of the actin bound ADP is not yet clear, but it is well known that ADP is a competitive inhibitor of pure myosin ATPase.[14]

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Folic acid and convulsions in the rat

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MEGALOBLASTIC anaemia may occur in patients undergoing drug treatment for major epilepsy.[1] This anaemia responds to folic acid, but not to Vitamin B_{12}.[2] In fact the majority of drugs which are effective in preventing convulsions in man have been reported to produce a folic acid deficiency anaemia. It is therefore possible that folic acid plays a role in the function of the nervous system and affects its susceptibility to epilepsy. In support of this is the observation that children treated with anti-epileptic drugs may suffer progressive mental deterioration, which is accompanied by low serum folate levels. Mental function may improve in these patients when folic acid is administered[3] but there is evidence that this may aggravate the epilepsy.[4] However, this deleterious effect of folic acid in epilepsy has recently been disputed.[5] The following is an experiment in which an antimetabolite, methotrexate, is used to decrease utilisation of folic acid in the tissues. The effect of the drug is to inhibit folate reduction,[6] so that dihydrofolate and tetrahydrofolate production is inhibited, but folate absorption from the intestine is primarily unimpaired. The susceptibility to chemically induced seizures was estimated by measuring the time of onset and severity of fits following administration of 50 mg/kg of leptazol. Adult white Wistar rats of either sex, weighing 105–155 g were used. Methotrexate 4 mg/kg was injected intraperitoneally into test animals and isotonic saline was injected into controls. Two further groups of controls were used: (i) 20 mg/kg folic acid was injected with 4 mg/kg

methotrexate (ii) 6 mg/kg formyl tetrahydrofolate was injected with 4 mg/kg methotrexate. These amounts were administered twice daily for 2–4 days before the single dose of leptazol was given.

In the first group of experiments (Table 1) only one observation was made—whether or not fits occurred following administration of leptazol. The methotrexate-treated animals had a significantly

TABLE 1. THE OCCURRENCE OF LEPTAZOL-INDUCED FITS IN RATS PRETREATED WITH SALINE (CONTROLS), METHOTREXATE (mtx), METHOTREXATE WITH FORMYL TETRAHYDROFOLIC ACID(mtx + L) AND METHOTREXATE WITH FOLIC ACID (mtx + F)

Response	Controls	mtx	mtx + L	mtx + F
Fits	10	3	10	8
No fits	3	10	3	5
Significance of difference from controls		P = 0·005–0·01	N.S.	N.S.

Significance of difference from controls estimated by calculation of χ^2. N.S. = no significant difference.

lower incidence of fits compared with controls (P = 0·005–0·01). Administration of formyltetrahydrofolate with the methotrexate resulted in exactly the same incidence of fits as in controls. A small decrease in the occurrence of fits compared with controls was observed in the animals treated with methotrexate with folate, but this was not significant (χ^2 test: P = 0·15–0·20). Similarly, comparing the incidence of fits in the methotrexate- and formyltetrahydrofolate-treated group with that of the methotrexate- and folate-treated group no significant difference occurred (χ^2 test: P = 0·15–0·20).

The influence of the same procedure on the time of onset of fits measured from the time of injection of the leptazol, and on the severity of the convulsions also showed significant protection by methotrexate pre-treatment: both a delay in the onset of fits and a decrease in their severity occurred (Table 2). Also, additional administration of formyltetrahydrofolate or folate abolished both of these protective effects (Table 2).

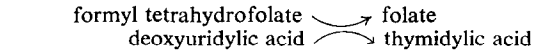
Administration of high doses of folic acid (60 mg/kg intraperitoneally) did not alter the time of onset or severity of leptazol-induced convulsions 2 hr afterwards (mean time of onset of fits: test = 3·78, control = 3·84, N.S.; severity: test = 1·0, control = 1·5, N.S.; n: test = 6, control = 2). Thus folic acid itself does not appear to provoke fits or make the rats more susceptible to the effects of

TABLE 2. THE EFFECT OF METHOTREXATE, METHOTREXATE PLUS FORMYL TETRAHYDROFOLIC ACID AND METHOTREXATE PLUS FOLIC ACID ON THE SEVERITY AND TIME OF ONSET OF LEPTAZOL-INDUCED FITS

	Control (n = 12)	mtx (n = 12)	mtx + L (n = 12)	mtx + F (n = 12)
Mean time of onset of fits (min)	3·84	8·11	4·08	5·72
Significance of difference from controls		P = 0·005–0·010	P > 0·3	P = 0·10–0·15
Mean degree of severity of fits	1·50	0·42	1·67	1·42
Significance of difference from controls		P = 0·005–0·010	P > 0·3	P > 0·3

Significance obtained from student's t-test. n = 12 in each treatment group. Scale of severity: 0 = no fits; 1 = convulsion lasts 15 sec or less; 2 = convulsion lasts 1 min or less; 3 = convulsion lasts over 1 min; 4 = prolonged convulsions lead to death.

leptazol. This suggests that the effects of methotrexate in this experiment are due to a deficiency of some of the metabolic products of folic acid and not to changes in folic acid itself. Folic acid is ultimately converted to formyl tetrahydrofolate which denotes a one carbon fragment (1C) to several metabolic steps—one of which is the methylation of deoxyuridylic acid to form thymidylic acid. If the latter step is inhibited, not only does a deficiency of thymidylic acid develop, but it is possible that more 1C fragments are available for other metabolic transformations for which formyl tetrahydrofolate is necessary. 5-fluoro uracil competitively blocks this reaction[6]



5-fluoro uracil (75 mg/kg) was administered intravenously to six rats. In each case there was a prolongation of the length of the leptazol convulsions (mean = 2·4 min; significance of difference from controls $P < 0{\cdot}001$) and even after the completion of the major seizure, the animal showed repeated jactitations for at least 1 hr with a generalised fit at 20–30 min. Administration of 150 mg/kg intravenously of 5-fluoro uracil produced convulsions in three out of eight animals 2–4 hr afterwards, without administration of leptazol. This indicates that a deficiency of thymidylic acid is not the means by which methotrexate exerts its protection action against leptazol fits, and suggests that 1C transfer may be the process involved in alterations of convulsion succeptibility produced by these antimetabolite drugs. The question remains whether therapeutically useful anti-convulsant drugs operate in this way. Suggestive evidence in support of this is that a wide range of different drugs which inhibit grand mal may produce a folic acid deficiency anaemia. Although the administration of folic acid may possibly aggravate the epilepsy, the addition of vitamin B_{12} to the drugs given enables the anaemia to be cured without deterioration of the epilepsy.[7,8] Vitamin B_{12} acts at several metabolic sites, of which two are also affected by folate: the biosynthesis of methionine from homocysteine and the conversion of cytidine diphosphate to deoxycytidine diphosphate. In the first of these reactions an increase in N5, N10 methylene tetrahydrofolate (M-THF) with or without additional vitamin B_{12} will result in the same action—an acceleration of the formation of methionine. In the second reaction, however, the relationship is more complex in that M-THF and vitamin B_{12} act at different points in the synthetic pathway of thymidylate (TMP):

$$\text{CMP} \xrightarrow{B_{12}} \text{CDP} \rightarrow \text{d CDP} \rightarrow \text{d CMP} \rightarrow \text{d UMP} \xrightarrow{\text{M-THF}} \text{TMP}.$$

An increase in M-THF will not necessarily result in a progressive increase in the formation of TMP because this reaction will be limited by the availability of d UMP. When the latter becomes rate limiting, more 1C fragments will be available for other reactions dependent on M-THF, such as the biosynthesis of methionine and choline and the glycine $\rightleftharpoons$ serine interconversion. If one of these substances is epileptogenic in raised concentrations, the convulsive threshold will be lowered. When vitamin B_{12} is administered in addition to the folate, increased amounts of d UMP will become available to accept some of the additional M-THF and thus result in a decreased diversion of 1C fragments into the other synthetic reactions.

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