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Implications of Methionine and S-Adenosylmethionine for the Brain Function

By

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Abstract

Clinical trials during the last decade have established that S-adenosylmethionine (SAM) is an effective antidepressant with few side effects. SAM causes an increase in brain 5-hydroxytryptamine (5-HT), but few studies have explored the behavioral effects of SAM in experimental animals. Because tail flick latency in response to the thermal stimulus, an animal measure of pain sensitivity, is influenced by 5-HT, and antidepressant drugs often have an effect on pain, we have now studied the effect of SAM on tail flick latency in the rat. We also studied the effect of methionine the immediate precursor of SAM. Administration of methionine to the rat increases brain SAM, but little is known about its behavioral effects. Long-Evans rats were given SAM and methionine orally at different doses and tail-flick latency was measured at various times. Both methionine and SAM increased tail-flick latency, but methionine did so at a lower dose. A biochemical study showed that methionine was more effective than SAM in raising brain SAM probably because it is transported better into brain. The biochemical measurements were not consistent with the idea that the effects of SAM and methionine were mediated by an increase in brain 5-HT.

Folate deficiency can lower brain SAM levels and cause depression. Thus, methionine, which raises brain SAM, may overcome the effects of folate deficiency. Seven day food records were done by 26 psychiatric outpatients who were stable on lithium treatment. Eight patients had mean daily folate intakes below those recommended. Some of those with low folate intake had high methionine intake consistent with the idea that methionine could substitute metabolically for folate deficiency. Daily methionine intakes ranged from 13 to 304% of the recommended intake. As methionine had behavioral effects in the rat at doses much less than the daily dietary intake this raises the question of whether varying daily intakes of methionine in humans have behavioral implications.

Résumé

Les essais cliniques effectués ces dix dernières années ont permis d'établir que la S-adénosylméthionine (SAM) était un agent antidépresseur efficace avec peu d'effets secondaires. La SAM entraîne une augmentation des concentrations de 5-hydroxytryptamine (5-HT) dans le cerveau mais très peu de recherches ont étudié ses effets sur le comportement des animaux de laboratoire. Dans la mesure où le temps de latence de rétraction de la queue à une stimulation photothermique (mesure de la sensibilité de l'animal à la douleur) est déterminée par les concentrations de 5-HT et que les antidépresseurs ont souvent un effet sur la douleur, nous avons étudié l'effet de la SAM sur le temps de latence de rétraction de la queue chez le rat. Nous avons également étudié l'effet de la méthionine, précurseur immédiat de la SAM. L'administration de méthionine au rat augmente les concentrations de SAM dans le cerveau, mais l'on ne sait pas exactement quels sont ses effets sur le comportement. Des rats Long-Evans ont reçu par voie orale des doses différentes de SAM et de méthionine et nous avons mesuré le temps de latence de rétraction de leur queue à différents moments. La méthionine et la SAM ont augmenté le temps de latence de rétraction de la queue, la méthionine à des doses cependant inférieures. Une étude biochimique a permis de démontrer que la méthionine était plus efficace que la SAM au titre de l'augmentation des concentrations de SAM dans le cerveau probablement parce qu'elle se rend mieux au cerveau. Les mesures biochimiques ne confirment pas l'hypothèse voulant que les effets de la SAM et de la méthionine soient attribuables à une augmentation des concentrations de 5-HT dans le cerveau.

La carence en folate peut faire fléchir les concentrations de SAM dans le cerveau et causer des accès de dépression. La méthionine, qui augmente les concentrations de SAM dans le cerveau, doit par conséquent pouvoir contrer les effets de la carence en folate. Des relevés alimentaires sur sept jours ont été obtenus auprès de 26 patients de consultation externe en psychiatrie,

suivant un traitement stabilisateur au lithium. Huit patients avaient des apports quotidiens de folate inférieurs aux apports recommandés. Parmi les patients dont les apports en folate étaient insuffisants, quelques-uns avaient des apports en méthionine importants, confirmant l'hypothèse voulant que la méthionine se substitue à la carence en folate sur le plan métabolique. Les apports quotidiens de méthionine variaient entre 13 et 304 % des apports recommandés. La méthionine ayant des effets comportementaux chez le rat à des doses bien inférieures à l'apport alimentaire quotidien, on peut par conséquent se demander si des apports quotidiens variables de méthionine chez les êtres humains ont des répercussions sur leur comportement.

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LIST OF ABBREVIATIONS

ami.	amitriptlyline
ANOVA	analysis of variance
BBB	blood-brain barrier
chlorimi.	chlorimipramine
CNS	central nervous system
CSF	cerebrospinal fluid
EDTA	ethylenediaminetetraacetate
Fig.	figure
$HCLO_4$	perchloric acid
HDRS	Hamilton Depression Rating Scale
HPLC	high performance liquid chromatography
g	gram
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenouse
kg	kilogram
LNAA	large neutral amino acid
M	Molar
mM	millimolar
mmole	millimole
mg	milligram
ml	milliliter
mm	millimeter
ng	nanogram
nm	nanometer
nomif.	nomifensine

REM	rapid eye movement
RNI	recommended nutrient intake
SE	standard error
SEM	standard error of the mean
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SD	standard deviation
TCA	tricyclic antidepressant
THF	tetrahydrofolate
µg	microgram
UV	ultraviolet
µl	microliter
µmol	micromole

I. LITERATURE REVIEW

1. Introduction

Over years, people have believed that food can influence mental well-being and behavior. One of the first treatments for a major psychiatric disorder, pellagra, was nutritional (Sebrell, 1981), and interest in the mental effects of dietary deficiencies still exists today. Although pellagra is now limited mainly to undeveloped countries, other nutritional deficiencies can contribute to psychopathology in developed countries. One of the more common nutritional deficiencies in developed countries is folic acid, and folic acid deficiency seems to be associated with a wide array of mental symptoms (Young and Ghadirian, 1989). This may be due, in part, to the wide variety of metabolic processes that folate can influence. Folic acid is involved in one carbon metabolism and donates methyl groups to homocysteine to form methionine, the immediate precursor of S-adenosylmethionine (SAM). SAM is involved in many methylation reactions in the brain. The relationship of folate, methionine and SAM is of great interest. SAM has been shown to have antidepressant properties, and folic acid deficiency is associated with depression. Under some circumstances folic acid may be a useful adjunct in the treatment of depression. Administration of methionine to the rat can increase brain SAM levels (Rubin et al 1974). Thus, the antidepressant action of SAM raises the possibility of

psychopharmacological actions of methionine.

The present investigation is nutritional in nature but has relevance to an area of psychiatry, the affective disorders (mania and depression). Part of this research work is on rats, and is on the neurochemical and behavioral effects of SAM and methionine. Insights into the mechanism involved may provide strategies for influencing brain function for therapeutic purposes. In the other part of this study, the dietary intake of patients with bipolar affective disorders (popularly known as manic-depressives) with particular reference to the intakes of folic acid and methionine is investigated. Although, mental disorders are unlikely to be treated by dietary means alone, an understanding of how diet and dietary components alter brain metabolism and function may lead to non-dietary treatments. An example of this is the use of tryptophan, given in isolated form as a tablet, which can have some limited therapeutic effect in psychiatric disorders (Young, 1986).

2. Folate deficiency and depression

A. Effect of folate on depression

Folic acid deficiency is associated with a variety of neuropsychiatric disturbances, most commonly depression (Reynolds and Stramentinoli, 1983). Many studies have been done to explore the association between folic acid deficiency and depression. Surveys of psychiatric in-patient populations indicate

that between 10 and 30% may have low serum folate levels (Reynolds and Stramentinoli, 1983). Folate deficiency may be associated with various diagnostic categories but it is most commonly associated with depression. A direct comparison of the different studies to determine overall rates of folate deficiency in different diagnostic groups is not possible for several reasons (Young and Ghadirian, 1989). Since an arbitrary cutoff point for folate deficiency was set in most of the studies of folate deficiency the threshold level for folate deficiency varies from one study to another ranging from 2 to 6 ng/ml. Second, assay techniques vary and different assays tend to give different results. Several comparisons have been made between competitive binding methods and microbiological assays and between microbiological assays employing different modes of enzymatic deconjugation (Gregory 1992). Results of these studies indicate a substantial variabilities between these methods. In addition, studies vary both over time and geographic location, so diagnostic criteria would differ, and lastly incidence of folate deficiency may be different in different places.

B. Effect of depression on folate

As psychiatric disorders, including depression, may lead to loss of appetite it is reasonable to suppose that often folate deficiency is a secondary phenomenon. Low folate levels could be the result of inadequate dietary intake, diminished absorption from the gastrointestinal tract or increased folate

utilization. No attempts to determine dietary folate intake have been made in any of the studies done on patients with folate deficiency. Two studies involved assessment of the overall adequacy of the patients' diet through 24 hour recall which may not be a very reliable method for these patients. One of them (Reynolds et al 1970) found no difference in serum folate levels between patients whose diet was assessed as poor, moderate or good, while the other (Thornton and Thornton, 1978) found that inadequate diet occurred frequently among the patients, but at the same frequency among those with or without folate deficiency. Although results of these studies indicate that poor diet does not explain the increased likelihood of low serum folate levels it would be surprising, in view of the decreased food intake known to occur in various psychiatric disorders, specially depression, if diet did not play some role.

C. Effect of folate administration

The role of folate deficiency in depression has been tested by looking at the response of symptoms of depression to folate administration. Three double-blind placebo-controlled studies produced positive results but were not unequivocal. Botez et al (1982) studied patients who were referred because of neuropsychiatric illness and were found to have folate deficiency. They defined a syndrome associated with folate deficiency which consists of depression, easy fatiguability, anorexia, insomnia, irritability and neuropsychological changes. After 4 months of treatment with folate 15 mg/day, folate, but not

placebo, produced a significant improvement on neuropsychological testing. However, although the patients were depressed mood was not mentioned in this study. Coppen et al (1986) looked at affective disorder patients on long-term lithium therapy. After 1 year of treatment with 0.2 mg/day of folate or placebo patients with the highest plasma folate during the trial showed a significant reduction in their affective morbidity. In another study (Godfrey et al 1990) 41 (33%) of the 123 patients with depression and schizophrenia, all of who were treated with antidepressants or antipsychotic drugs in addition to methylfolate or placebo who had red-cell folate levels below 200 μg/l took part in a trial of methylfolate, 15 mg/day, for six months. They observed a significant improvement in clinical state and social function on folate. The dose of folic acid used in this study was considerably higher than the recommended daily allowance of 180-200μg in the U.S.A. Whether doses up to 75 times the recommended daily allowance are really needed is uncertain (Young and Ghadirian, 1989). Studies on lower doses are needed because of indications that folic acid at a dose of 15 mg/day can cause toxic effects in human after 1 month of administration (Hunter et al 1970). In this study subjects experienced irritability, overactivity, altered sleep patterns and gastrointestinal symptoms.

D. Conclusions concerning folic acid deficiency and mood

Further controlled studies of folate administration are needed in patients

with psychiatric symptoms that are thought to be associated with folate deficiency. An important problem that needs study is whether folate deficiency in psychiatric patients is the result of low intake, low absorption or high utilization. Finally, studies of the mechanism of action of folate and the control of the biochemical pathway in which folate is involved are necessary.

3. Antidepressant effect of SAM

Over twenty studies on the antidepressant effect of SAM have been performed in the last two decades. There are three main types of study. The least effective test is an open trial, but several such studies suggest that SAM can cause an improvement in depression. A somewhat more demanding test is a comparison with a standard antidepressant, and such tests have demonstrated that SAM is equal, or in some cases possibly even superior, in its antidepressant action to standard antidepressants. However, unless the sample size is very large, the conclusion that a drug is not significantly different from a standard antidepressant does not necessarily imply that the drug is better than placebo, because of the large placebo response in depressed patients. The most demanding comparison for any potential antidepressant drug is a comparison with placebo. SAM has been put to this test in several studies. All of the earlier studies were carried out in Europe, especially Italy, where the clinical use of SAM was pioneered. Only recently have North

American researchers shown any interest in this topic.

A. Open trials of SAM

In general, results of the open studies indicate an effective response of SAM to depression (Table 1a). In a study of 51 patients, Agnoli et al (1978) found SAM to be significantly effective after the first week of treatment, and the percentage of improvement increased by the end of the second week. Salvadorini et al (1980) found a significant improvement in core depression scores in patients treated with SAM. Of 39 patients, 86% showed improvement. The authors concluded that the patients who did not improve appreciably were those affected with the most severe illnesses and who had been hospitalized for several years. In a study of 9 patients, Lipinski et al (1984) observed varying degrees of improvement; three patients showed complete remission, three were much improved, and one was slightly improved. De Leo (1985) studied the effects of SAM in a large population of out-patients (n=75) with minor depression. There was a significant improvement on depression scale scores. SAM did not have any appreciable side effects during the one month trial.

While earlier open studies used i.v. or i.m. SAM, Rosenbaum et al (1990) looked at the antidepressant potential of oral SAM in 20 patients who were categorized according to their prior history of treatment resistance (treatment resistance subjects n=9, non-treatment resistance n=11). The

group as a whole improved significantly, with the patients who had responded previously to other antidepressants showing the best response.

When positive results are seen in open studies this, in general, is no more than an indication that controlled studies are warranted. However, open studies have the potential for revealing serious side effects. The most serious side effect which was seen in several studies was hypomania, an effect seen with a variety of other antidepressants.

B. Double-blind comparison of SAM with tricyclic antidepressants

The most common tricyclic antidepressants (TCA) used to compare with the antidepressant effect of SAM are imipramine, amitriptyline and chlorimipramine (Table 1b). Janicak et al (1988) reviewed the literature examining the use of SAM (given i.v. or i.m.) as an antidepressant. They performed a meta-analysis on several of the controlled trials comparing SAM to either placebo or a standard TCA. Results of their inspection revealed a chi-square of 6.7 ($p<0.01$) with 109 of 142 SAM-treated patients and 80 of 124 TCA-treated patients classified as responders, indicating a 12% difference between SAM and the comparison antidepressant.

Several of the controlled studies concluded that SAM has a comparable efficacy to TCAs, and in some studies a more rapid onset of action than TCAs was found (Mantero et al 1975; Barberi and Pusateri, 1978; Del Vecchio et al 1978; Miccoli et al 1978; Scarzella and Appiotti, 1978; Küfferle and Grünberger,

1982). Doses of TCAs used in these studies were in the range 75-150 mg/day for imipramine, 100 mg/day for amitriptyline, and 50-150 mg/day for chlorimipramine all of which are in accordance with the recommended dosage for these drugs. Doses of SAM used were in the range of 75-400 mg/day. In all these studies SAM and TCAs were injected i.v. or i.m.. In a single study the effect SAM, given i.v., was compared with oral nomifensine (200 mg/day), and SAM was found to be an effective antidepressant (Scaggion et al 1982).

Janicak et al (1988) performed a study comparing SAM to either placebo or imipramine (150 mg/day) for a period of 2 weeks. In terms of percent improvement on the Hamilton Depression Rating Scale score, the SAM treated patients had a significantly greater percent improvement than did the placebo treated patients. Imipramine and SAM were similar in efficacy.

C. Double-blind placebo-controlled trials

There are 8 controlled trials comparing SAM to placebo (Table 1c). In the first trial of SAM in depression, Fazio et al (1973) treated patients with SAM (45 mg/day i.v.) or placebo for a period of one week. Significant improvement on Hamilton Depression Rating Scale "core" depressive items (e.g. suicidal tendencies, psychomotor retardation, work and interests) occurred in the SAM group but not in the placebo group.

In a study of 30 randomly selected depressed patients, Agnoli et al (1976) looked at the effect of 45 mg/day of SAM given i.m. (two thirds of the

Table 1a - Clinical Effects of SAM
Open Trials

Reference	Type of patients	N	Design of experiment	Length of study	Dose mg/day	Clinical effects	Side effects
Agnoli et al 1978	Endogenous, reactive, involutional, neurotic depression (Kilholz's classification)	51	Open trial	2 weeks	45 i.m.	significant improvement on HDRS on mood, anxiety, work, insomnia, suicide, somatic, hypochondria	mild manic rebound in 3 pateints
Salvadorini et al 1976	Major depression (DSMIII), non-responsive to antidepressants	39	Open trial	21 days	45-70 i.v. or i.m.	Significant effect after one week, mainly for reactive depression: least effect on circular depression	1 rebound mania
Lipinski et al 1984	Major depression (DSMIII)	9	Open trial	14 days	200 i.v.	3 complete remission, 3 much improvement, 1 slight improvement, 2 drop outs	2 with mania or hypomania needed lithium therapy
De Leo 1985	Minor depression (DSM III)	75	Open trial	30 days	100 i.m.	Significant improvement in Zung SRDS and Global Impressions (CGI, PGI)	No side effects
Rosenbaum et al 1990	Major depression with and without prior history of resistance to antidepressants	20	Open trial, placebo for one week in a single blind phase	6 weeks	up to 1600 orally	significant improvement with oral SAM specially for non-treatment resistant	1 case of hypomania: other side effects were transient and mild including thirst, nausea, headache, diarrhea, in-crease urination and salivation

Table 1b - Clinical Effects of SAM
Double-Blind Comparison with TCA

Reference	Type of patients	N	Design of experiment	Length of study	Dose mg/day	Clinical effects	Side effects
Mantero et al 1975	Endogenous, involutional, neurotic, endoreactive	31	Double-blind, SAM vs imipramine	3 weeks	SAM:75 i.m. imip :75 i.m.	No significant differences between SAM and imipramine	
Barberi et al 1978	Endogenous depression	20	Double-blind SAM vs amitrptyline	20 days	SAM: 200 i.v. amit: 100 i.v.	8 out of 10 patients in SAM and 6 out of 10 in amit group improved > 70%(no sig difference between groups)	
Del Vecchio 1978	Neurotic, involutional, endoreactive, endogenous	28	Single-blind SAM vs Chlorimipramine	3 weeks	SAM: 150 i.v. chlorimi: 100 i.v.	SAM was similar to chlorimi	Anxitety only with SAM
Miccoli et al 1978	Endogenous, neurotic, reactive, involutional	86	Double-blind SAM vs Chlorimipramine and Amiuriptyline	21 days	SAM: 200 i.v. chlori: 100 i.v. amitri: 100 i.v.	No significant difference between groups	
Scarzella et al 1978	Endogenous, Neurotic, Reactive	20	Double-blind SAM vs Chlorimipramine	15 days	SAM: 200 i.v. chlori: 100 i.v.	SAM>chlori after 7 days, but similar results after 14 days	

Scaggion et al 1982	Endogenous, reactive, involutional	40	Double-blind SAM vs Nomifensine	2 weeks	SAM: 300 i.v. nomif: 200 orally	Significant improvement >30% on HDRS in SAM group	
Kufferle et al 1982	Manic depressive	20	Double-blind SAM vs Chlorimipramine	18 days	SAM:150 i.v. chlori: 50 i.v.	SAM was similar to chlori on HDRS, Zung(SDS), Global response	No side effects with SAM
Bell et al 1988	Major depression (DSMIII)	22	Double-blind, SAM vs imipramine	16 days	400 i.v.	Significant effect after 1st week with SAM: on the 2nd week 66% had a clinical improvement as compared to 22% with imipramine	No significant side effects

Table 1c - Clinical Effects of SAM
Double-Blind Comparison with Placebo

Reference	Type of patients	N	Design of experiment	Length of study	Dose mg/day	Clinical effects	Side effects
Fazio et al 1973	Depression: bipolar, unipolar, reactive, neurotic, females	14	Double-blind, comparison with placebo	5-11 days	45 i.v.	Significant improvement in certain symptoms: retardation, work & interest, suicidal ideation, insight, somatic	-
Agnoli et al 1976	Depression: endogenous, neurotic, involutional, endoreactive, reactive	30	Double-blind, comparison with placebo	2-15 days average 6.6 days	45 i.m.	Significant effects on certain symptoms: depressed mood, suicidal ideation, work and interest, somatic	worsening of some already existing symptoms associated with anxiety
Muscettola et al 1982	Major depression: bipolar, unipolar	20	Double-blind, comparison with placebo	14 days	150 i.m.	Significant improvement in depressive mood, work and activity, blunted affect, somatic concern and anxiety	No side effect except an increase of free anxiety, insomnia and hostility during the first 5 days of therapy in one patient
Caruso et al 1984	Rheumatoid arthritis with moderate to severe depression	49	Double-blind, comparison with placebo	21 days	200 i.m.	Rheumatoid arthritis unchanged, decreased HDRS score for SAM relative to placebo	

Carney et al 1986	Severely depressed (Feighner criteria)	32	Double-blind, comparison with placebo	14 days	200 i.v.	SAM had greater effect than placebo, endogenous patients more responsive than neurotic	Free of side effects with possible exception of mania and agitation
De Leo 1987	Major depression, atypical depression and dysthymia (DSMIII)	40	Double-blind, comparison with placebo	5 weeks	200 i.v.	SAM exerted an action superior to placebo	Increased anxiety in 5 cases
Janicak et al 1988	Major depression, bipolar	20	Double-blind, comparison with TCA and placebo	14 days	SAM:400 i.v. imipramine: 150 i.v.	SAM comparable to TCAs and superior to placebo	
Carrieri et al 1990	Parkinsonian, depressed	21	Double-blind, placebo controlled crossover	30 days for each treatment	200 i.m. and 800 orally	Parkinson disease unchanged but improvement in depression in SAM groups in males	5 in SAM treated group and 1 in placebo treated; insomnia and heartburn
Kagan et al 1990	Major depression (DSMIII), unipolar, males	18	Double-blind, comparison with placebo	21 days	1600 orally	significant difference between treatment and placebo observed at day 14 and 21	5 on SAM: 1 with mania, 6 on placebo

patients) or placebo (one third of the patients) for an average period of 7 days. Results obtained using the Hamilton Depression Rating Scale indicated a significant effect of SAM on specific depressive symptoms (depressed mood, work and interests, suicidal tendencies). No side effects was reported in this study.

Muscettola et al (1982) looked at the clinical efficacy of SAM using more restricted diagnostic criteria than Fazio et al (1973) and Agnoli et al (1976). Twenty patients diagnosed as having a major depressive disorder were randomly assigned to either SAM (150 mg/day i.m.) or placebo. The mean Hamilton Depression Rating Scale total score at the end of the trial was significantly lower than the mean pretreatment score in the SAM group, but not in the placebo group. No side effects were reported by patients treated with SAM, with the exception of one patient who showed a sharp increase in anxiety, insomnia and hostility during the first five days of therapy.

Caruso et al (1984) gave SAM (200 mg/day i.m.) or placebo to patients with rheumatoid arthritis who were also depressed. SAM, but not placebo, caused a significant decline in Hamilton Depression Rating Scale scores. There was no mention of whether arthritis pain was influenced by SAM.

In a preliminary report of a double-blind trial of 32 patients, Carney et al (1986) investigated the effect of an intravenous injection of 200 mg/day SAM or placebo for 2 weeks. Three of the SAM treated patients became very

anxious during the second week and were withdrawn from the study. There was a significant reduction in the Hamilton Depression Rating Scale score for both SAM and placebo patients, and there was no significant difference between the groups on this measure. However, more patients in the SAM group than in the placebo group achieved a 50% reduction in Hamilton and Beck Depression Scale scores.

In a trial of one month, De Leo (1987) looked at the effect of SAM (200 mg/day) and placebo administered intramuscularly. Direct comparison of the response in the placebo and SAM groups revealed a significant superiority of the SAM group, both in Zung Depression Scale scores and in Clinical Global Impression scores, even though placebo produced a significant improvement in the Clinical Global Impression scores.

A double-blind cross-over study by Carrieri et al (1990) compared 600 mg/day SAM with placebo over 30 days in depressed patients with Parkinson's disease. In the SAM-placebo treatment group (first 2 weeks SAM and last 2 weeks placebo), patients showed a progressive and significant score reduction in Hamilton and Beck Depression scores compared with baseline values while on SAM. During the drug-free wash out period scores increased and remained virtually unchanged throughout the placebo period. A reduction of the above scores were also observed in the placebo-SAM group in the placebo period ($p<0.05$), as well as in the SAM period ($p<0.01$), compared with baseline.

However, when data were pooled together, that is between all patients receiving SAM and those receiving placebo, the therapeutic effect of SAM was significantly superior to that of placebo. Side effects were reported by six patients, five in the SAM-placebo group, and one in the placebo-SAM group, only during SAM treatment.

The effect of SAM given orally was first observed by Kagan et al (1990), who gave SAM (1600 mg/day) for a period of 21 days. Hamilton Depression Rating Scale scores for the SAM group were significantly lower than for the placebo group at day 14 and 21. Manic symptoms were experienced by one patient in the SAM group.

D. Conclusions concerning the antidepressant effects of SAM

The studies performed to date, taken together, suggest strongly that SAM is an effective antidepressant with few side effects. The most serious side effect commonly noted is mania, an effect SAM has in common with other antidepressants. However, in spite of the relatively large number of studies investigating the antidepressant effect of SAM, there are still a number of important questions that remain unanswered. First, is SAM really more rapid in its action than standard antidepressants? This question can only be answered definitively by studies with larger groups than those carried out so far. Second, will the therapeutic effect of SAM be maintained over period of longer than three weeks? Most of the studies so far have lasted only three weeks or

even less. Third, what is the most advantageous route of administration of SAM, oral, i.m. or i.v.? Fourth, what is the optimum dose of SAM? Doses used so far have ranged widely and there is an urgent need for a dose response study. One of the major factors that will limit how quickly these questions are answered is the high cost of SAM.

4. Behavioral studies of methionine

Studies on behavioral effect of methionine, the precursor of SAM, have been conducted only on schizophrenic patients. Results of the ten studies reviewed by Cohen et al (1974) indicate that administration of methionine in combination with a monoamine oxidase inhibitor produced functional psychosis in 62 of 107 chronic schizophrenic patients. In these studies, daily doses of L- or DL-methionine ranged from 2 to 40 g and were given for periods of 1 week to 2 months. The adverse effects observed in animal studies together with the metabolic changes observed in healthy individuals after single doses of methionine as low as 3 g (Anderson and Raiten, 1992) suggest that doses of methionine given to the patients of up to 40 times the normal daily dietary intake may have been in the toxic range. The authors of this review concluded that there is a possibility that an abnormal transmethylation of biogenic amine precursors plays some role in the pathogenesis of schizophrenia. This may be due to the fact that a major effect of large doses of methionine is to increase

the available pool of methyl groups and thereby enhance the possibility of abnormal transmethylation. Although methionine is known to be psychoactive, as it exacerbates schizophrenia (Cohen et al 1974), little is known about its psychopharmacological profile and its effect on mood and behavior.

5. Metabolic interrelation of SAM, methionine and folic acid

Common interest in the observations on folate deficiency and depression, and on the antidepressant effect of SAM is reinforced by consideration of the intimate metabolic interrelations of folate and SAM (Fig. 1). The first step in the biosynthesis of SAM in the nervous system is the reduction of methylene THF to 5-methyl-THF which is the form of folate that is transported to the brain. The next step involves the transfer of a methyl group from 5-methyl-THF to homocysteine to form methionine the precursor of SAM. A major function of folate, methionine and SAM interrelations is the transport of methyl groups in the folate cycle, which are subsequently utilized by SAM as the methyl donor in many methylation reactions.

A. Folic acid deficiency, S-adenosylmethionine and 5-HT

Clinical studies have demonstrated effects of folate deficiency on 5-HT. It has been shown that in patients who were folate deficient due to inadequate diet or malabsorption, and who exhibited folate-responsive mental symptoms, levels of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, in the

cerebrospinal fluid (CSF) were significantly lower than in patients without folate deficiency, or than in patients who were folate deficient but who did not show any folate-responsive mental symptoms. When the patients who had low CSF 5-HIAA and were folate deficient were given supplements of folic acid (15 mg/day), their mental symptoms resolved and their CSF 5-HIAA returned to normal (Botez et al 1982). In another study, on depressed patients one third of whom were folate deficient, there was a significant correlation between red cell folate and CSF 5-HIAA (Bottiglieri et al 1986). Studies on experimental animals are also in accordance with clinical studies. A study on the effect of folate deficiency on biogenic amines showed that in folate deficient animals brain 5-HT, but not brain dopamine or noradrenaline, was lowered (Botez et al 1979).

Folate deficiency lowers brain SAM (Ordonez and Wurtman, 1974) and 5-HT. SAM supplementation raises brain 5-HT in rats (Curcio et al 1978), and CSF 5-HIAA in depressed patients (Bottiglieri et al 1984). This suggests that the effect of folate deficiency on brain 5-HT is mediated through lowered brain SAM. How SAM affects brain 5-HT is unknown.

B. Folate, methionine, SAM and diet

Folate deficiency can result from a variety of causes (Young and Ghadirian, 1989). One is malabsorption which can result from drugs such as anticonvulsants or from gastrointestinal pathology. Another possibility is

inadequate dietary intake of folate. Diet could potentially influence methylation capacity in the brain through a variety of other mechanisms.

Methionine supplementation at a dose of 100 mg/kg can increase rat brain SAM by up to 50% (Rubin et al 1974), but dietary intake is also capable of influencing SAM. Methionine is a large neutral amino acid and is transported into brain by the transport system active towards all the LNAA (Oldendorf and Szabo, 1976). Thus, the brain level of methionine will tend to follow the plasma ratio of methionine to the sum of the other LNAAs (Rubin et al 1974). Acute changes of SAM in relation to methionine intake have been demonstrated in a study in which rat brain SAM exhibited a diurnal variation, with highest levels at night, when rats ingest the most food (Rubin et al 1974).

Overall, the animal data suggests that the relationship between dietary intake of methionine on one hand, and brain methionine and SAM on the other hand, is far from a direct linear one. Nonetheless, it does indicate that, in some circumstances increases in dietary methionine may be associated with increases in brain methionine and SAM.

C. Human requirement for methionine and folate

The Recommended Nutrient Intake (RNI) for folate is 3.1 µg /kg body weight. This equals a daily intake of 217 µg for a 70 kg man and 170 µg for a 55 kg woman (Murray et al 1990). Folate is widely distributed in foods. Liver, yeast, leafy vegetables, legumes and some fruits are especially rich sources.

Although the mean daily folate intake of Canadians is estimated to be above the RNI requirements, as mentioned earlier studies on the adequacy and variation of folate intake of psychiatric patients are not conclusive.

The human adult requirement for methionine is about 13 mg/kg/day (National Research Council, 1989). Approximately 30% of the human adult requirement of methionine can be replaced by cysteine (National Research Council, 1989) . The concentration of methionine and cysteine may be limiting in some but not all the plant protein sources. In general, legumes are low in methionine and most nuts and grains are high in methionine. Thus even a vegetarian diet which contains these food groups is likely to provide the adequate amounts of methionine and cysteine. However, variations of methionine intake within the adequate range may influence brain SAM. Although consumption of essential amino acids in the general population has been studied, little has been done in relation to amino acids intake of psychiatric patients. In one study intake of tryptophan in institutionalized patients was studied: none of the patients were taking tryptophan below the minimum requirements (Payne and Hudson, 1972).

6. Methylation and Affective Disorders

There are several possible areas to consider when looking at the methylation function of folate and SAM in brain and their influence on the

expression of mood. SAM is used in many important methylation pathways in brain, involving amines, proteins, nucleoproteins, neurotransmitters and membrane phospholipids. There is both clinical and experimental evidence of an effect of SAM and folate on brain monoamines which suggests a link between methylation and the amine hypotheses of depression. SAM increased CSF 5-HIAA in depressed patients (Agnoli et al 1978; Bottiglieri et al 1984). Similarly, administration of SAM increased the turnover of brain 5-HT in rats (Curcio et al 1978). Both folate deficiency or excess decreased brain 5-HT turnover in the rat and decreased levels of 5-HIAA in the CSF of folate deficient patients (Botez et al 1979). While folate deficiency lowered CSF 5-HIAA as described above, folate supplements have been reported to lower the level of the dopamine metabolite homovanillic acid in CSF (Hunter et al 1971). The mechanisms of these effects of SAM and folate on monoamine metabolism are unknown.

Another possible way in which SAM could influence mood arises from the discovery by Axelrod and his colleagues (Crews et al 1980) of two methyltransferase enzymes in brain and other tissues which methylate membrane phospholipids, using SAM as a methyl donor. Hirata et al (1989) suggest that phospholipid methylation is an initial common pathway for the transduction of many receptor-mediated biological signals through membranes. It is thus possible that changes in neurotransmitter function, receptor

sensitivity or endocrine function in depression may be mediated by disturbances in membrane function and may be modified by the administration of SAM. Cimino et al (1984) have shown that 3 months treatment with SAM in senescent rats restored membrane fluidity to control values for younger animals and significantly increased ß-noradrenergic receptor activity in striatum.

SAM is also required for the methylation of proteins and nucleoproteins. There are no data linking this specifically to mood or depression, but it is interesting that in bacteria carboxyl-methylation of proteins is essential for behavioral control mechanisms and signal transduction (Reynolds and Stramentinoli, 1983). It is possible that the ubiquitous influence of methylation in so many different essential metabolic reactions in the nervous system is more important than any single postulated mechanism in influencing mood and related functions.

7. Rationale and hypotheses

A. Methionine, SAM and behavior

One of the most marked effects of SAM on a neurotransmitter described so far is on 5-HT. If SAM alters 5-HT levels we need to know whether it alters 5-HT function. Thus we looked at the behavioral profile of SAM by studying its effect in an animal pain model, the tail-flick test. In this test the time the animal takes to flick its tail away from a thermal stimulus is

measured. The reason for this choice was that (a) 5-HT modulates the response to pain (Le Bars, 1988), (b) antidepressants often have therapeutic effects on pain and SAM is an antidepressant, and (c) the work may have clinical implications. In the previous studies SAM was given by injection in more than one dose in both animal and human studies. In the studies described in this thesis it was given orally in a single dose. The oral route of administration is a more practical way of giving drugs to patients. A single dose of SAM may be as effective as its multiple administration and results of experiments with a single dose are easier to interpret.Since SAM showed some effect in the tail-flick test we examined whether methionine can have the same effect. Although methionine is known to be psychoactive, as it exacerbates schizophrenia (Cohen et al 1974), little is known about its psychopharmacological profile.

B. Effects of SAM and methionine on brain 5-HT

As mentioned above both animal and human studies suggest that SAM administration increases brain 5-HT. In order to address the possibility that SAM exerts its psychopharmacological action by increasing 5-HT levels in the brain, doses of SAM used in the behavioral studies were tested in a biochemical study. As methionine can influence SAM, we also examined whether methionine can influence brain 5-HT. In these experiments we measured brain SAM, S-adenosylhomocysteine (SAH), tryptophan, 5-HT and 5-HIAA. Blood levels of SAM and 5-HT and liver levels of SAH and SAM were also determined.

C. Folic acid and methionine intake

One important unanswered question related to folic acid deficiency concerns the inconsistent effect of folate deficiency on the brain. One study failed to find an effect of folate deficiency on brain 5-HT in the rat (Butler and Rothenberg, 1987), and not all folate deficient patients show a lowering of mood and CSF 5-HIAA (Botez et al 1982). It may be that other factors can compensate for low folate levels in some patients. Folic acid supplies the methyl groups which methylate homocysteine to form methionine, the precursor of SAM. SAM gives up its methyl group to form S-adenosylhomocysteine (SAH), which is converted to homocysteine, thus completing the cycle. The only alternative mechanism for regeneration of methionine is via a reaction catalyzed by betaine:homocysteine methyltransferase, in which betaine, a metabolite of choline, serves as the methyl donor (Zeisel, 1981). If brain methionine levels are in the high normal range, then it may not be necessary to recycle all the methionine. Some of the methionine may contribute methyl groups, and the SAH formed may pass through an irreversible catabolic pathway instead of being recycled. If this hypothesis is correct, then animals or humans with folate deficiency should show effects on mood and brain 5-HT when brain methionine is in the low normal range, but not when methionine is in the high normal range.

The idea that high brain methionine may partially protect the brain from

the adverse effects of folate deficiency is so far unexplored. Brain methionine levels seem to be related in part to dietary methionine, as discussed in a previous section. If brain methionine levels are, in part, controlled by dietary methionine, then a wide variation of dietary methionine would suggest a wide range of brain methionine. Little is known about dietary intake of methionine, and whether it is related to intake of folic acid. If low dietary intake of folic acid is always accompanied by low intake of methionine, then methionine intake is not able to substitute for low intakes of folate. But if the intake of these two nutrients are not related to each other, then a low intake of folate may be accompanied by a high methionine intake and hence methionine may overcome the effects of a low folate diet. Therefore, we decided to look at dietary intakes of folate and methionine in a type of patient which responds to folic acid. Affective disorder patients fit this description (Godfrey et al 1990; Coppen et al 1986).

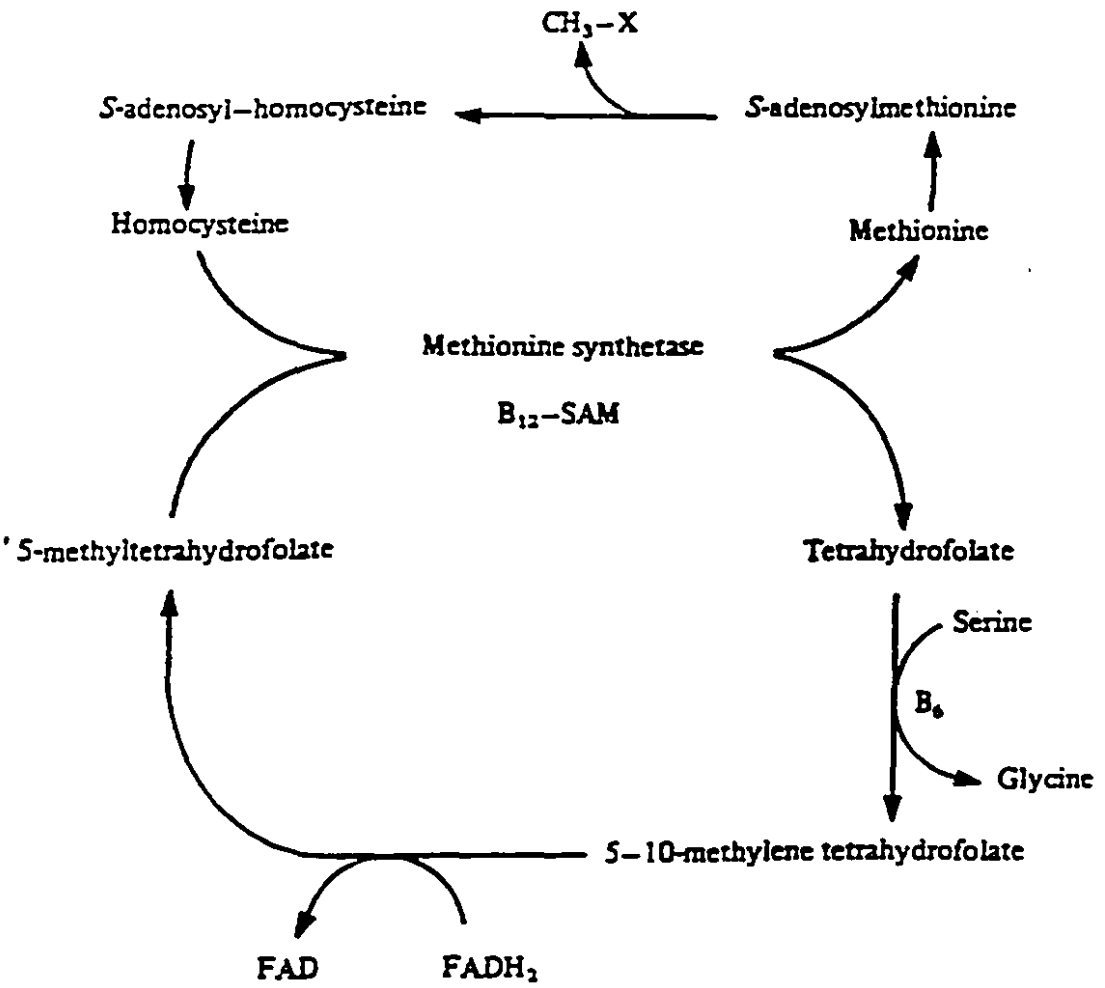


FIG. 1. Relationships between folate cycle, SAM and transmethylation.

II. EXPERIMENTAL

1. Materials and methods in the animal studies

A. Animals

Male Long Evans rats, obtained from Charles River Canada, Inc., St. Constant, Québec, were housed in groups of 4 for the duration of the experiments. Rats for all the acute experiments weighed 150-175 grams at the time of testing; in the chronic experiment rats weighed initially 125-150 grams (day 0) and 325-350 grams at day 21, the last day of the experiment. Rats were maintained in a room with 12 hour light/dark cycle (lights on at 7:00 a.m and off at 7:00 p.m) and food and water were ad libitum. In the chronic experiment, rats were kept in wire mesh cages in groups of two in order to monitor their food intake.

B. Chemicals

S-Adenosylmethionine and L-methionine (Sigma Chemicals, St. Louise, MO) were dissolved immediately before use in distilled deionized water. In the acute experiments rats were given S-adenosylmethionine, methionine or water orally by gavage at a volume of 1 ml/kg body weight. In the chronic experiment methionine was added to powdered rat chow to raise the concentration of methionine in the diet from 0.45% (usual % of methionine in the rat chow) to 0.6%. The slight increase in methionine concentration was within its

physiological range and did not change weight gain or food intake of rats (Appendix B).

C. Radiant heat tail-flick test: apparatus and procedures

The apparatus to present thermal stimulation of the tail and automatically record tail-flick latencies of rats was obtained from Omnitech Electronics (Columbus, Ohio). Rats were habituated to the laboratory environment by bringing them to the laboratory, weighing them and holding them for 1-2 minutes for 3 days prior to an experiment. During testing the animals were placed on a platform so that a portion of their tail was placed over a V-shaped groove under which is a heat source. The animals were restrained in a towel with their tail protruding for the period of the test. A switch on the apparatus turned on a light source and the heat source, and activated a timer. The time it took for the rat to move its tail away from the heat source (the tail-flick latency) was measured automatically. Movement of the tail allowed the light beam to reach a detector that automatically stopped the clock and turned off the heat source. The intensity of the heat source was adjusted to produce a baseline response time of 7-9 seconds. To prevent tissue injury, a cutoff time of 18 seconds was chosen.

D. Biochemical analysis

i. Measurement of SAM and SAH

SAM and SAH were measured by high performance liquid

chromatography with absorbance detection (Guttari, 1991). A 501 solvent delivery system with a 710B automatic injector (both from Waters Assoc., Milford, MA, U.S.A), a Waters Nova-Pak C18 steel column (300 mm X 3.9 mm I.D.), a Waters model 441 UV detector and an Omniscribe recorder (OmniScribe, Austin, TX, U.S.A.) were used. The mobile phase consisted of 40 mM ammonium dihydrogenphosphate ($NH_4H_2PO_4$), 6 mM heptanesulphonic acid sodium salt monohydrate (from Sigma, St. Louis, MO, U.S.A.) and 6% (v/v) methanol. The pH was adjusted to 4.2 by addition of hydrochloric acid, and then the mobile phase was filtered through a 0.45-µm Millipore membrane filter. The flow rate was set at 0.8 ml/min. The standard compounds, SAM and SAH from Sigma (St.Louis, MO, U.S.A), were dissolved in water at a concentration of 1 mM and then diluted in 0.4 M perchloric acid to the final concentration of 5 µM used during HPLC analysis. The injection volume was 30 µl. The detector was set at 254 nm. Chromatograms were similar in appearance to those in the paper of Guttari (1991).

ii. HPLC system for tryptophan, 5-HT and 5-HIAA analysis

Tryptophan, 5-HT and 5-HIAA were measured by high performance liquid chromatography with fluorometric detection (Anderson et al 1981). A 6000 solvent delivery system with a 710B injector, a µBondapak C18 reversed-phase column (300 mm X 3.9 mm I.D., 4 micron), all from Waters Assoc., a Shimadzu fluorescence detector (Kyoto, Japan) and an OmniScribe recorder were used.

The mobile phase consisted of 0.01 M sodium acetate and 5% (v/v) methanol; the pH was adjusted to 4.25 with glacial acetic acid. Stock solutions of standards (10 mg/100 ml) were made up in distilled water with 0.1% ascorbic acid added and were stored at -70°C for up to 2 months. Diluted standards (1 ng/µl) were prepared every two days in distilled water. The detector was set at an excitation wavelength of 285 nm and an emission wavelength of 340 nm. Chromatograms were similar in appearance to those in the paper of Anderson et al (1981).

iii. Tissue preparation

Tissues were weighed, placed in polycarbonate centrifuge tubes and homogenized in 4 volume (v/wet weight) of 0.4 M perchloric acid (containing 0.1% (w/v) EDTA and 0.1% (w/v) sodium metabisulphite to prevent oxidation of the compounds being measured) with a Polytron Sonicator (Branson Sonic Power, Danbury, CT, U.S.A). Samples were then centrifuged at 10,000 g for 20 minutes at 4°C. The supernatants were divided into two parts; one was injected directly into the chromatograph for 5-HT, tryptophan and 5-HIAA analysis; the other was filtered through a 0.2-µm membrane and then injected into the chromatograph for SAM and SAH analysis.

Whole blood levels of 5-HT and SAM were determined by collecting blood in a tube containing 50 µl of 25% w/v heparin (Sigma Chemicals, St.Louis, MO). To each ml of blood 250µl of ascorbic acid and 250µl of $HClO_4$ was added and

the tube was vortex mixed for 10 sec. Precipitated protein was then removed by centrifuging the mixture for 10 minutes at 10,000 g. The clear supernatant was poured off into a storage tube and stored at -70 °C until analysis.

2. Procedures

A. Time course of the effects of SAM and methionine on tail-flick latency

i. SAM administration

Rats (n= 8) were given, by gavage, water or 200 mg/kg SAM. This dose was chosen because a dose of 100 mg/kg has been shown to raise brain SAM when given intravenously (Stramentinoli et al 1977). Tail-flick latency was done at baseline, 1.5, 3, 6 and 24 hours after SAM or water administration.

ii. Methionine administration

In this experiment rats (n=10/group) were given 50 mg/kg methionine or water orally by gavage. Tail-flick latency was measured at baseline, 2, 4 and 6 hours after methionine or water administration. The initial time points were later than in the study on the effects of SAM administration to allow time for the synthesis of methionine into SAM (Rubin et al 1974).

B. Dose-response study of the effects of SAM and methionine on tail-flick latency

i. Acute administration of SAM

Rats (n=10/group) were given a dose of 100, 200, 400 mg/kg SAM or

water orally by gavage. Tail-flick latency was measured at 2 hours, the time of the peak effect observed in the time-course study.

ii. Acute administration of methionine

In the methionine experiment rats (n=10/group) were given 25, 50, 100 mg/kg methionine or water. Tail-flick latency was measured at 4 hours, the time of peak effect of methionine seen in the time-course study.

C. Effect of chronic administration of methionine on tail flick latency

The effect of methionine added to the diet was studied in a 21 day experiment. In this experiment, rats were kept in wire mesh cages in groups of two in order to monitor their food consumption. Rats (n=18) were given Powdered Purina Rat Chow with a methionine content of 0.45% or the same diet supplemented with methionine to increase its methionine content to 0.6%. Food consumption and weight gain were monitored every 3 days throughout the experiment. Tail-flick test was done at day 0, 3, 7, 14 and 21.

D. Biochemical effects of SAM and methionine

i. Acute effect of SAM and methionine

Rats were given the same doses of SAM, methionine or water as in the dose-response studies. Rats were decapitated after 2 or 4 hours, the times of peak effect in the time effect study for SAM and methionine respectively. A 10 mm section of the spinal cord at the level of the lumbar enlargement and the brain were rapidly removed. The brain was separated into brainstem,

cerebellum and rest of the brain. Tissues were stored at -70°C until HPLC analysis.

ii. Chronic effect of methionine

On day 22 of this experiment, after the last tail-flick measurement, the rats were decapitated and the brain and spinal cord of the animals were removed as described above and stored at -70°C until HPLC analysis.

E. Statistical analyses

Analysis of variance (repeated measurements) was used in the time-course and two-way ANOVA was used in the dose-response studies of SAM and methionine to determine the significance of the difference between the treatment and the control groups. Values of $p<0.05$ were considered significant. In the dose-response studies and the biochemical study when ANOVA was significant, Scheffé's F test is used to examine which means were different from each other. The data were analyzed using STATVIEW, version 4.0 (Abacus Concepts, Inc., Berkeley, CA, 1992).

3. Clinical study

A. Subjects and recruitment

Subjects were patients aged 18 to 65 with bipolar affective disorder according to the criteria of the Diagnostic and Statistical Manual, Third Edition, Revised of the American Psychiatric Association (American Psychiatric

Association, 1987). All subjects were attending the Affective Disorders Clinic at the Allan Memorial Institute. To avoid disruptions in normal diet associated with hospitalization only outpatients who had been living at home for at least three months were studied. The nature of the study was explained to the patients by their treating psychiatrist during their normal visit to the clinic. Those who agreed to participate in the study met with the dietitian who performed the actual dietary assessments. The study was approved by the Research Ethics Board of the Department of Psychiatry, McGill University. A signed consent was obtained by the dietitian.

B. Dietary assessments

Results of a comparison of the methods used for determining the intake of nutrients in humans show that no method is ideal (Bingham, 1991; Barrett-Connor, 1991). Direct observation or weighed food records are expensive to obtain, involve a serious time commitment on the part of the patient, and may not reflect usual intake. A food record or diary is less accurate, although it can be improved if the participants are trained by a dietitian. Twenty four hour recall can be reasonably accurate when done for an estimate of the mean intake of a group of roughly fifty persons or more (Young et al 1952) if it is done under the supervision of a trained dietitian, but food intake can vary greatly from one day to the next. The food frequency questionnaire is convenient and relatively cheap, but is culture specific and

limited by the foods that are listed. Also, it is at best only semi-quantitative.

While weighed food records would have some advantages, this procedure involves a greater time commitment on the part of the patients and is likely to result in low participation. A seven day food record done after training by the dietitian is easier for the participants, and is likely to produce good compliance and acceptable data. Thus, in a recent study intake of folic acid obtained from a seven day food was correlated significantly with serum and erythrocyte folic levels (Payette and Gray-Donald, 1991). The procedure used was the same as described in this study.

At the first visit (in the clinic), a questionnaire on the medication use, food supplement use, weight, height and current health status was administered along with a brief medical history. A copy of the form is included in Appendix A. Much of this information was also available in the patient's chart, which was used to ensure accuracy of the data. Patients were trained by the dietitian on how to record both quality and quantity of foods and beverages ingested over the next seven consecutive days. Plastic food models were used to help participants better estimate quantities. Food intake were recorded in household units, by volume or by weight. The record include brand names, method of food preparation, and recipes of home-prepared dishes. Information were also obtained on the day, date, and location of each meal and snack as well as on the use of medication and nutrient supplements.

Intake of folic acid in vitamin supplements was not included in the assessment of intake, as the purpose of the study was to determine dietary intake. Patients returned their dietary records after completion and the dietitian checked the diet record for accuracy, completeness and clarity. Participants were contacted to provide additional information where necessary.

C. Calculation of dietary intakes and statistical analyses

Average daily dietary intakes of folic acid, methionine, cysteine were calculated by using a computerized database (ESHA Research, Salem, OR, U.S.A.). The methionine content of some of the ready to eat dishes, such as spaghetti and meat balls, veal parmigiana etc., were not available on the data base. For these dishes the estimated intakes of the various components of the dish were used. As mentioned before, 30% of the human adult requirement of methionine can be replaced by cysteine (National Research Council, 1989). Therefore in estimating the total methionine intake of patients, the proportion of methionine which can be substituted by dietary cysteine was calculated and added to the dietary intake of methionine. Caloric consumption as well as energy derived from fat, carbohydrates and protein were also calculated using this database.

Descriptive statistics included the mean and standard deviation of the dietary intake averaged over 7 days. By entering patients' weight, height, sex and the level of activity, nutrient intake of individuals as a percentage of their

recommended level was calculated. Student's t test was used for the comparison of data between males and females. To determine whether dietary intakes of methionine and folic acid were related, Pearson product-moment correlations were calculated between intakes of methionine plus cysteine and folic acid. The interrelationships of these nutrients with energy and protein intakes were also evaluated using Pearson correlation coefficients.

III. RESULTS

1. Time course of the effects of SAM and methionine on tail-flick latency

A. SAM administration

Fig. 2 shows tail-flick latencies of rats (% of control) given 200 mg/kg SAM or water. There was no significant difference in tail-flick latency between control and treatment group at the baseline. SAM increased tail-flick latencies at 1.5 and 3 hr ($p=0.003$ at 1.5 hr and $p=0.013$ at 3 hr). There were no significant differences between the control and the treatment group at 6, 12 and 24 hours after SAM administration.

B. Methionine administration

Fig. 3 represents the effect of 50 mg/kg methionine or water on the tail-flick latency (% of control) at baseline, 2, 4 and 6 hours after methionine or water administration. Tail-flick latencies were significantly longer in the treatment group at 4 and 6 hours ($p=0.0005$ at 4 hr, $p=0.004$ at 6 hr) than in the control group.

Results of these studies suggest that SAM exerts its effect at 1.5 hours and lasts up to 3 hours after its oral administration. In the case of methionine, the time of maximum effect was found to be at 4 hours after its administration.

2. Dose response study

A. Acute SAM administration

Four groups of rats were given SAM at doses of 100, 200 and 400 mg/kg or water orally by gavage. Tail-flick latency was measured at 2 hours, the time of peak effect seen in the time course study. Results of this study show a significant difference between tail-flick latency means ($p=0.02$, Fig. 4) and the Scheffé post-hoc test showed a significant difference between the control and the 400 mg/kg group ($p=0.05$) and between 100 and 400 mg/kg SAM ($p=0.04$).

B. Acute methionine administration

Methionine at doses of 25, 50 and 100 mg/kg or water was given to the rats and tail-flick latency was measured 4 hours later, the time of peak effect of methionine seen in the time course study. The dose response curve was an inverted U shape. There was a significant difference between tail-flick latency means ($p=0.02$, Fig. 5) and the Scheffé post-hoc test showed a significant difference between the control and 50 mg/kg methionine ($p=0.02$).

Results of the dose-response studies suggest that both SAM and methionine increase tail-flick latency and that methionine exerts its effect at a lower dose than SAM does. The effect of methionine disappears at higher doses.

3. Chronic administration of methionine

Results of the acute administration of methionine led us to look at the effect of dietary changes of methionine on tail-flick latency over a period of three weeks. Methionine is the most toxic essential amino acid in terms of depressed food intake and weight gain and tissue damage in animals (Aguilar et al 1974; Harper et al 1970). It has been shown that a diet which contains 0.4-0.45% methionine has the minimum level of methionine to produce maximum growth and a diet which contains up to 0.75% methionine, as well as all other required amino acids, has the highest level of methionine which does not produce growth retardation (Aguilar et al 1974; Byington et al 1972; Rotruck and Boggs, 1977; Harper et al 1970). Thus, in our experiment we chose the powdered Purina Rat Chow which contains 0.45% methionine as our control diet and a diet containing 0.6% methionine as the experimental diet. Food consumption and weight gain was monitored throughout the experiment. No significant difference was observed in terms of weight gain and food consumption between the groups (Appendix B).

Fig. 6 shows the effect of dietary changes of methionine on the tail-flick latency in rats. Adding methionine to a diet of Purina Rat Chow, to increase the methionine content of the diet from 0.45% to 0.6%, caused an increase in tail-flick latency over time that reached significance at days 7 and 21 ($p=0.005$ for both). Results of this experiment show that dietary changes of methionine

within its physiological range result in significant increases in tail-flick latency in rats.

4. Biochemical studies

A. Acute administration of SAM

In this experiment we looked at the effect of different doses of SAM, which were used in the dose-response study, on the concentration of SAH, SAM, 5-HT, tryptophan and 5-HIAA in different sections of brain, liver and blood. ANOVA followed by pairwise comparison using the Scheffé test was done to analyze the data. Table 2a shows results of the oral administration of SAM on the SAM and SAH concentrations in different brain regions, liver and blood. SAM at doses of 100, 200 and 400 mg/kg significantly increased SAM levels in the brain stem as compared to the control group. The level of SAM after the dose of 100 mg/kg was significantly higher than the control group in the rest of brain. There was a significant increase in SAM concentration in the blood at doses of 200 and 400 mg/kg SAM. Liver levels of SAM increased significantly at 400 mg/kg SAM. SAM at a dose of 400 mg/kg lowered SAH level in cerebellum. There was a significant increase of tryptophan level in cerebellum when SAM was given at the dose of 400 mg/kg (Table 2b). 5-HT levels were raised significantly in rest of brain at a dose of 100 mg/kg as compared to all other groups (Table 2c). 5-HIAA levels were raised

significantly in rest of brain and brain stem (positive trend p=0.06) at a dose of 100 mg/kg (Table 2c).

In summary, acute administration of SAM raised SAM levels in the brain stem and rest of brain but in general had no significant effect on SAH levels. Levels of 5-HT and 5-HIAA were significantly increased in rest of brain at a dose of 100 mg/kg SAM. This increase in 5HT was not explained by an increase in brain tryptophan. SAM raised both liver and blood levels of SAM (at doses of 200 and 400 mg/kg) as compared to the control group. The effects on blood and liver were much greater than those on the brain.

B. Acute administration of methionine

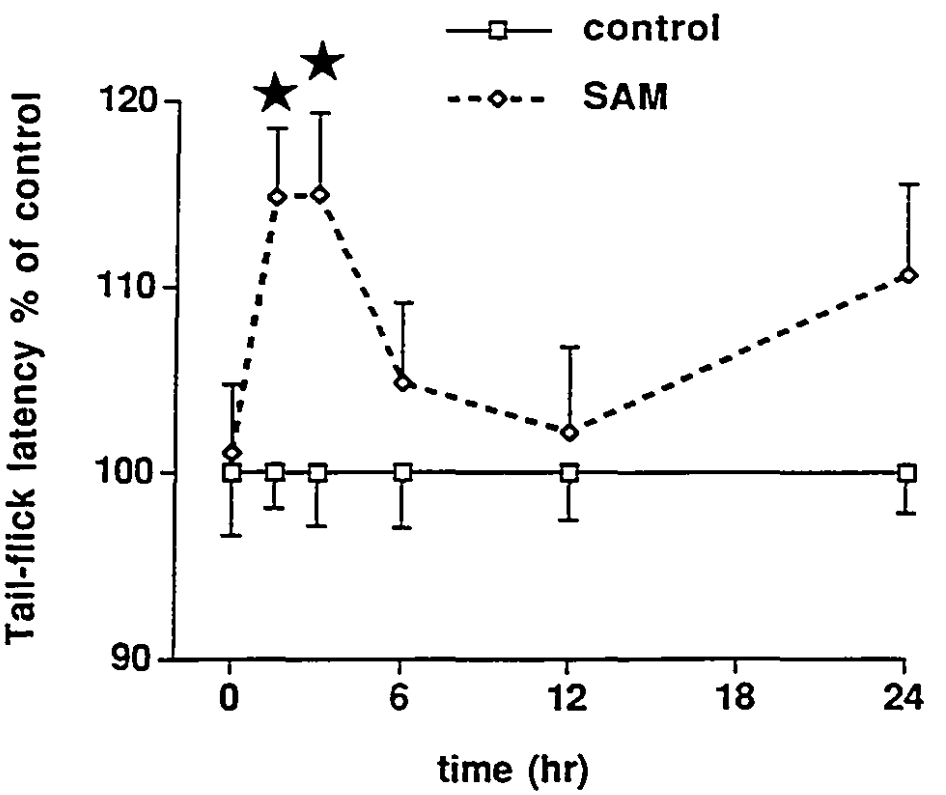
Methionine was given at doses of 25, 50 and 100 mg/kg. In general the effect of methionine on brain SAM, like its effect on tail-flick latency, was an inverted U shape. SAM levels were significantly increased at all doses of methionine in cerebellum and at a dose of 50 mg/kg in spinal cord and brain stem (Table 3a). SAM levels were lowered in liver at doses of 25 and 50 mg/kg methionine (Table 3a). Although there was an increase in SAH concentration in cerebellum and spinal cord at all doses of methionine as compared to the control group, it only reached significance at a dose of 25 mg/kg methionine in cerebellum and spinal cord (Table 3a). SAH levels were significantly lowered at all doses of methionine in liver (Table 3a). Tryptophan and 5-HT levels did not change significantly in different brain regions but 5-HIAA

levels were significantly increased in rest of brain at a dose of 50 mg/kg methionine and in the cerebellum at 25 and 50 mg/kg (Table 3b, 3c).

C. Dietary changes of methionine

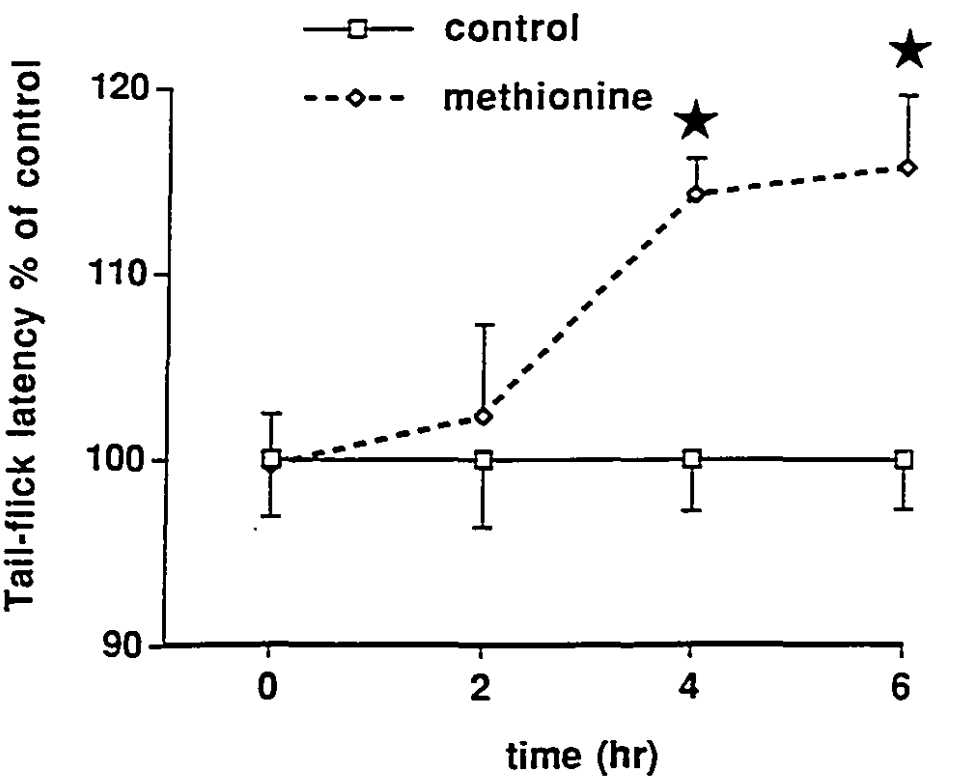
Rats in both the control (0.45% methionine) and the experimental group (0.6% methionine) were decapitated a day after the last day of experiment (i.e. on day 22). SAM levels were significantly higher in cerebellum, spinal cord and in blood and significantly lower in brain stem of rats on 0.6% methionine diet than the control group (Table 4a). SAH levels were higher in rest of brain and brain stem and lower in the liver of rats in the experimental group (Table 4a). While an increase in methionine in the diet increased tryptophan in the rest of brain, 5-HT levels were lowered in rest of brain and spinal cord (Table 4b).

Fig 2.Time-course study of SAM



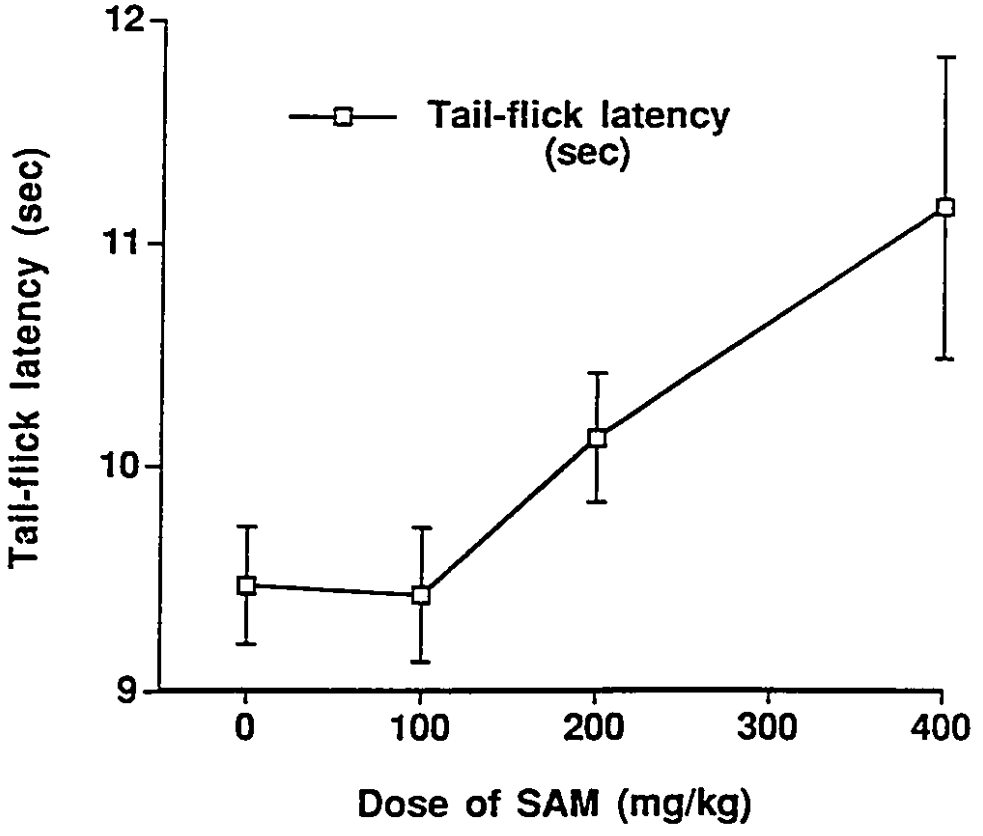
Values are mean±SEM for 8 animals. ANOVA (main effect) is $F_{1,14}$=9.3. p=0.009. Scheffé's test shows a significant difference between two means at 1.5 and 3 hours, p=0.003 and p=0.013 respectively.

Fig 3.Time-course study of methionine



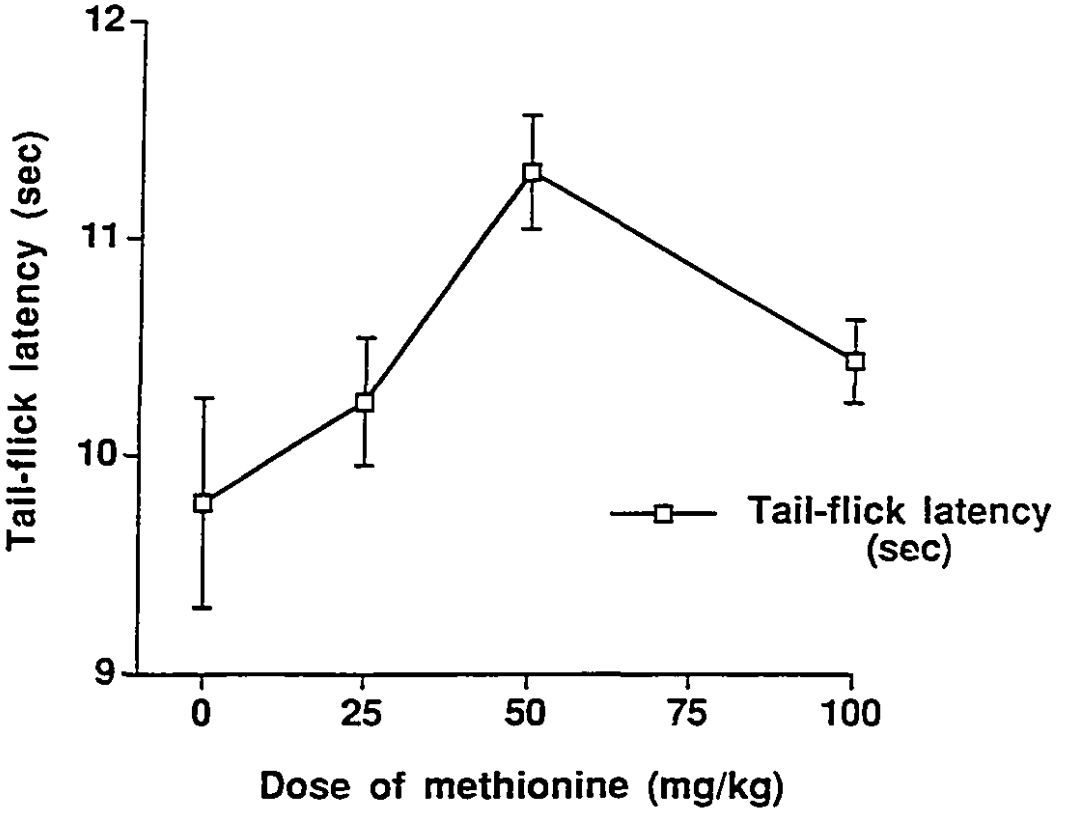
Values are mean±SEM for 10 animals.
ANOVA (main effect) is $F_{1,18}$=13.3, p=0.002.
Scheffé's test shows a significant difference between two means at 4 and 6 hours, p=0.0005 and p=0.004 respectively.

Fig 4. Effect of SAM on tail-flick latency



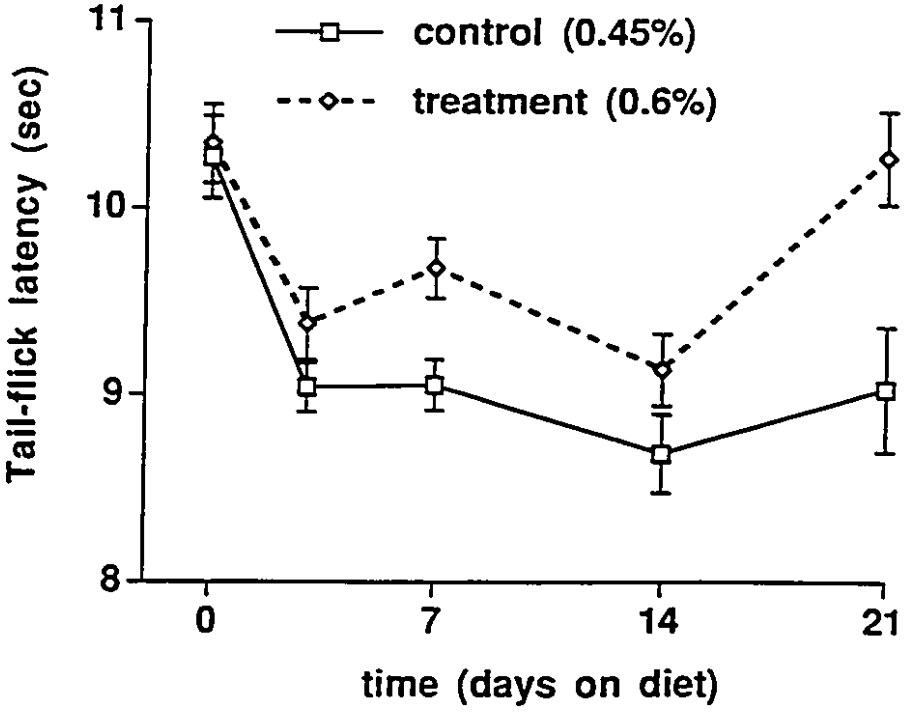
Values are mean±SEM for 10 animals.
ANOVA (main effect) is $F_{3,36}=3.9$, $p=0.02$.
Scheffé's test for multiple comparison shows a significant difference between control and 400 mg/kg SAM ($p=0.05$) and between 100 and 400 mg/kg SAM ($p=0.04$).

Fig 5. Effect of methionine on tail-flick latency



Values are mean±SEM for 10 animals.
ANOVA (main effect) is $F_{3,36}=3.8$, $p=0.02$.
Scheffé's test for multiple comparison shows a significant difference between control and 50 mg/kg methionine, $p=0.02$.

Fig 6. Effect of methionine on tail-flick latency



Values are mean±SEM for 18 animals.
ANOVA (main effect) is $F_{1,34}=8.9$, $p=0.005$.
Scheffé's test shows a significant difference between control (0.45% methionine) and treatment group (0.6% methionine) at 7 and 21 days. $p=0.005$ for both days.

Table 2a. Effect of oral SAM administration on the concentrations of SAH and SAM in different brain regions, liver and blood

	0 mg/kg		100 mg/kg		200 mg/kg		400 mg/kg	
Tissue	SAH	SAM	SAH	SAM	SAH	SAM	SAH	SAM
Brain Stem	1.30±0.15	10.3±0.3[a]	1.24±0.17	12.3±0.5[b]	0.93±0.08	12.3±0.1[b]	1.10±0.06	13.0±0.2[b]
Cerebellum	2.16±0.14[a]	12.9±0.4	2.23±0.16[a]	13.5±0.6	1.73±0.19	14.2±0.2	1.36±0.08[b]	14.5±0.6
Rest of brain	1.32±0.11	10.7±0.3[a]	1.49±0.13	12.0±0.2[b]	1.26±0.09	11.1±0.2[a]	1.30±0.06	11.6±0.2
Spinal Cord	1.37±0.06	11.2±0.8	1.40±0.06	12.1±1.2	1.50±0.08	9.78±0.50	1.71±0.17	12.4±0.9
Liver	9.19±0.23	25.4±2.0[a]	9.54±0.30	30.8±1.8	9.57±0.32	33.4±0.9	10.3±0.4	37.8±2.3[b]
Blood	-	1.83±0.07[a]	-	1.86±0.05[a]	-	2.46±0.08[b]	-	2.5±0.2[b]

Values are means of 8 rats/group ±SEM and are expressed as µg/g tissue and µg/ml blood. Results of the ANOVA (main effect) are as follows: Brain stem (SAM), $F_{3,28}$=12.5, p<0.0001; Cerebellum (SAH), $F_{3,22}$=7.6, p=0.0011; Liver (SAM), $F_{3,22}$=7.2, p=0.002; Blood(SAM), $F_{3,28}$=10.2, p<0.0001. Means for each variable having different letter superscripts differ significanlty ($p < 0.05$) using the Scheffé's test. Abbreviations used: SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

Table 2b. Effect of oral SAM administration on the concentration of tryptophan in different brain regions

	0 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Tissue	tryptophan	tryptophan	tryptophan	tryptophan
Brain stem	4.28±0.13	4.00±0.21	3.70±0.12	4.24±0.09
Cerebellum	3.91±0.08[a]	4.21±0.13	4.26±0.14	4.46±0.13[b]
Rest of brain	3.87±0.07	4.31±0.14[a]	3.77±0.11[b]	4.10±0.08
Spinal Cord	4.07±0.12	4.15±0.19	4.09±0.19	4.36±0.15

Values are means of 8 rats/group ±SEM and are expressed as µg/g tissue. Results of the ANOVA (main effect) are as follows: Cerebellum (tryptophan), $F_{3,27}$=3.7, p=0.025; Rest of brain (tryptophan), $F_{3,27}$=5.2, p=0.006. Means for each variable having different letter superscripts differ significanlty ($p < 0.05$) using the Scheffé's test.

Table 2c. Effect of oral SAM administration on the concentrations of 5-HT and 5-HIAA in different brain regions and blood

	0 mg/kg		100 mg/kg		200 mg/kg		400 mg/kg	
Tissue	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Brain stem	643±15[a]	351±23	539±22[b]	385±23	553±17	386±13	574±30	427±12
Cerebellum	59.3±1.7	77.6±2.5	64.2±5.4	81.7±2.4	58.7±3.2	83.8±2.9	64.6±4.6	83.3±4
Rest of brain	370±5[b]	267±5[a]	419±13[a]	309±7[b]	358±4[b]	260±4[a]	380±9[b]	273±5[a]
Spinal Cord	521±23	248±14	602±86	273±16	556±26	295±24	578±24	300±16
Blood	429±31	-	394±37	-	400±24	-	351±19	-

Values are means of 8 rats/group ±SEM and are expressed as ng/g tissue and ng/ml blood. Results of the ANOVA (main effect) are as follows: brain stem (5-HT), $F_{3,25}=4.2$, $p=0.02$; Rest of brain (5-HT), $F_{3,27}=9.3$, $p=0.0002$; Rest of brain (5-HIAA), $F_{3,24}=15$, $p<0.0001$. Means for each variable having different letter superscripts differ significanlty ($p < 0.05$) using the Scheffé's test. Abbreviations used: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid.

Table 3a. Effect of oral methionine administration on the concentrations of SAH and SAM in different brain regions, liver and blood

	0 mg/kg		25 mg/kg		50 mg/kg		100 mg/kg	
Tissue	SAH	SAM	SAH	SAM	SAH	SAM	SAH	SAM
Brain Stem	1.12±0.05	11.0±0.2[a]	0.91±0.05	11.9±0.2[a]	1.09±0.11	15.6±0.5[b]	1.07±0.04	11.0±0.2[a]
Cerebellum	0.73±0.04[a]	11.6±0.2[a]	0.96±0.04[b]	16.2±0.3[c]	0.86±0.05	15.8±0.4[b]	0.91±0.03[b]	14.7±0.3[b]
Rest of brain	0.86±0.04	9.54±0.16	0.99±0.07	9.40±0.18	0.96±0.08	10.2±0.3[a]	0.82±0.02	9.0±0.2[b]
Spinal Cord	0.93±0.08[a]	7.14±0.66[a]	1.76±0.20[b]	10.8±1.1	1.23±0.05[a]	11.5±1.1[b]	0.78±0.10[a]	10.5±0.6
Liver	18.8±1.0[a]	43.6±2.1[a]	10.3±0.24[b]	30.4±2.7[c]	12.4±0.26[b]	39.9±1.4[b]	10.4±0.5[b]	34.7±1.4[bc]
Blood	-	1.70±0.05	-	1.74±0.11	-	1.85±0.07	-	1.87±0.12

Values are means of 8 rats/group ±SEM and are expressed as µg/g tissue and ug/ml blood. Results of the ANOVA (main effect) are as follows: Brain stem (SAM), $F_{3,28}$=45.6, p<0.0001; Cerebellum (SAH), $F_{3,28}$=5.5, p=0.0041; Cerebellum (SAM), $F_{3,28}$=48.1, p<0.0001; Rest of brain (SAM), $F_{3,28}$=5.4, p=0.005; Spinal cord(SAH), $F_{3,24}$=11.5, p<0.0001, Spinal cord(SAM), $F_{3,24}$=4.2, p=0.016, Liver (SAH), $F_{3,28}$=45.8, p<0.0001, Liver (SAM), $F_{3,28}$=8.8, p=0.003. Means for each variable having different letter superscripts differ significanlty ($p < 0.05$) using the Scheffé's test. Abbreviations used: SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

Table 3b. Effect of oral methionine administration on the concentration of tryptophan in different brain regions

	0 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Tissue	tryptophan	tryptophan	tryptophan	tryptophan
Brain stem	4.18±0.12	4.16±0.13	3.96±0.11	4.38±0.10
Cerebellum	3.75±0.09	3.85±0.06	3.76±0.13	3.89±0.15
Rest of brain	3.47±0.09	3.56±0.71	3.83±0.08[a]	3.41±0.11[b]
Spinal Cord	4.20±0.16	4.14±0.18	4.44±0.21	4.74±0.21

Values are means of 8 rats/group ±SEM and are expressed as µg/g tissue. Results of the ANOVA (main effect) are as follows: Rest of brain (tryptophan), $F_{3,28}$=4.4, p=0.012. Means for each variable having different letter superscripts differ significanlty ($p < 0.05$) using the Scheffé test.

Table 3c. Effect of oral methionine administration on the concentrations of 5-HT and 5-HIAA in different brain regions and blood

	0 mg/kg		25 mg/kg		50 mg/kg		100 mg/kg	
Tissue	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Brain Stem	605±32	522±25	631±25	569±11[a]	655±17	522±19	698±18	475±16[b]
Cerebellum	49.2±3.9	75.0±3.4[a]	44.6±1.9	87.1±2.3[b]	49.7±1.7	75.0±1.9[a]	53.3±4.2	85.2±3.4
Rest of brain	325±11	243±3[a]	345±13	272±9[b]	324±10	273±7[b]	357±7	256±8
Spinal Cord	455±48	347±17	554±23	339±14	446±40	361±11	542±37	369±22

Values are means of 8 rats/group ±SEM and are expressed as ng/g tissue and ng/ml blood. Results of the ANOVA (main effect) are as follows: Brain stem (5-HT), $F_{3,25}$=4.2, p=0.015; Brain stem (5-HIAA), $F_{3,25}$=2.7, p=0.06; Cerebellum (5-HIAA), $F_{3,28}$=5.1, p=0.006; Rest of brain (5-HIAA), $F_{3,28}$=4.6, p=0.009; Blood (5-HT), $F_{3,28}$=4.1, p=0.015. Means for each variable having different letter superscripts differ significanlty ($p < 0.05$) using the Scheffé's test. Abbreviations used: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid.

Table 4a. Effect of diet (0.45% and 0.6% methionine) on the concentrations of SAH and SAM in different brain regions, liver and blood.

	0.45% methionine		0.6% methionine	
Tissue	SAH	SAM	SAH	SAM
Brain stem	1.57±0.24	4.81±0.36	2.70±0.22**	3.78±0.29*
Cerebellum	3.19±0.09	4.09±0.12	3.41±0.23	4.78±0.32*
Rest of brain	1.63±0.25	3.91±0.19	2.12±0.17	3.78±0.26
Spinal Cord	2.42±0.14	2.76±0.19	2.07±0.12	3.99±0.32**
Liver	11.7±0.52	5.38±0.36	9.76±0.66*	4.98±0.42
Blood	-	1.48±0.05	-	1.72±0.05***

Groups of 18 animals were on 0.45% or 0.6% Powdered Purina Rat Chow for 21 days. The values are means ±S.E.and expressed as µg/g tissue and µg/ml for blood. Significant group differences were determined using ANOVA. brain stem (SAM), $F_{1,32}$=4.8; brain stem (SAH), $F_{1,28}$=12.2 Cerebellum (SAM), $F_{1,33}$=4.6, p=0.04; Spinal cord(SAM), $F_{1,29}$=11.8, Liver (SAH), $F_{1,31}$=4.8, p. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$. Abbreviations used: SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

Table 4b. Effect of diet (0.45% and 0.6% methionine) on the concentrations of tryptophan, 5-HT and 5-HIAA in different brain regions and blood

	0.45% methionine			0.6% methionine		
Tissue	tryptophan	5-HT	5-HIAA	tryptophan	5-HT	5-HIAA
Brain stem	5.91±0.15	576±11.8	485±11.82	5.78±0.18	555±12.3	509±16.5
Cerebellum	5.57±0.11	55.6±2.29	80.9±2.45	5.93±0.18	52.4±3.93	82.5±4.45
Rest of brain	4.39±0.18	238±7.30	166±9.20	5.52±0.22**	180±12.0**	198±12.2
Spinal Cord	4.79±0.14	520±17.1	334±14.4	4.59±0.14	454±24.3*	315±14.8
Blood	-	919±24.5	-	-	907±24.8	-

Groups of 18 animals were on 0.45% or 0.6% Powdered Purina Rat Chow for 21 days. The values are means ±S.E.and expressed as ng/g tissue for 5-HT and 5-HIAA values, µg/g tissue for tryptophan values and µg/ml blood. Significant group differences were determined using ANOVA. Rest of brain (5-HT), $F_{1,22}$=13.9, p=0.0012; Rest of brain (tryptophan), $F_{1,22}$=13.7, p=0.0012; Spinal cord(5-HT), $F_{1,33}$=5, p=0.032. *p < 0.05; **p < 0.005. Abbreviations used: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid.

5. Clinical study

Thirty eligible patients were chosen randomly from the Allan Memorial Institute Affective Disorders Clinic and the nature of the study was explained to them in the initial interview. Three patients refused to participate and one patient dropped out after recruitment. The final sample (n=26) consisted of 13 women and 13 men. The age distribution of the study population is described in Table 5. The mean dietary intake, averaged over the 7-day period, and the percentage of energy from protein, carbohydrate and fat are reported in Table 6. Fig. 7 gives the nutrient intakes expressed as a percentage of their recommended allowances, taking into account the recommended intakes for males and females, the weight and age of the subject, and, for energy intake, the level of activity. The averaged caloric intake over 7 days (Table 6) was lower for women than for men. However, when calculated as a percentage of their recommended intake, women's caloric intake was significantly higher than men ($t=2.33$; $p<0.03$) and the average intakes for both men and women were below the recommendations for their ages. The percentage of energy from macronutrients was close to recommended amounts (Murray et al 1990), eg, 32% and 33% of energy for males and females, respectively, was provided by fat compared with the recommended 30%. The percentage of energy derived from protein was 17% and 19% for men and women, respectively, which is close to 15%

recommended intake and that of carbohydrate was 51% and 48% (55% recommended intake) for men and women respectively. The mean folate intake from the diet was 273±77 µg for men, 249±54 for women aged 25-49 and 241±94 for women aged 50-65 all of which are above the Canadian dietary recommendations. In Canada, the mean daily dietary intake is estimated to be 205 µg/day for men and 149 µg/day for women (Murray et al 1990). This compares with RNIs of 217 µg/day for a 70 kg man and 170 µg/day for a 55kg women. Thus, means for the general population are below the RNI. In the patients folate intakes were below the RNI for 5 women and 4 men (35% of the study population) (Fig 7). While this group of patients might be doing better than the general population as far as the proportion above the RNI is concerned, as discussed in section I.2.A, folate deficiency may contribute to depression in some susceptible individuals. Thus, in a vulnerable populations such as bipolar affective disorder patients ideally none should have intakes of folate below the RNI. Eight of the 26 patients were taking vitamin supplements that included folic acid. (The folate ingested in supplements was not included in the dietary intake numbers). However, none of the nine patients with inadequate folate intake were taking vitamin supplements. The dietary intake of patients taking folate supplements (mean±SEM, 140±9% of RNI) was significantly greater ($p<0.05$) than the dietary intake of patients who were not taking supplements (105±9% of RNI). The interrelation of folate and

methionine intake is shown in Fig. 8 ($r = 0.559$; $p < 0.003$). Figs. 9 and 10 present the relationship of folate intake with energy and protein intakes. Correlation coefficients and their probability values were as follows: folate vs energy, $r = 0.488$; $p < 0.01$ and folate vs protein, $r = 0.59$; $p < 0.001$). Methionine intake was positively correlated with intakes of energy ($r = 0.51$; $p < 0.008$) and protein ($r = 0.9$; $p < 0.0001$) (Figs. 11 and 12). Fig. 13 shows the relation of energy and protein intakes ($r = 0.553$; $p < 0.003$).

Daily intakes of methionine were calculated for each patient as the percentage of the recommended allowance. The highest and lowest daily intake for each patient is given in Table 7. The daily variation was in general considerable. The ratio of maximum to minimum intake for individual patients ranged from 1.5 to 16.2 and was less than 2 for only three patients. For all the patients the daily intake of methionine ranged from 13 to 304% of the recommended intake.

Table 5. Age and sex distribution of participants

Age	Male (n=13)	Female (n=13)
25-35 y	3	2
36-45 y	4	2
46-55 y	2	4
56-65 y	4	5

Table 6. Dietary intake of energy and macronutrients

Nutrient	Male (n=13)	Female (n=13)
Energy (kcal)	1958±485*	1471±423
Protein (g)	86±22	82±23
Carbohydrate (g)	248±75	216±68
Total fat (g)	71±23	64±16
Protein (% of energy)	17	19
Carbohydrate (% of energy)	51	48
Total fat (% of energy)	32	33

* Mean daily intake calculated from 7-day records. $x \pm$ SD.

Fig 7. Variation of nutrient intakes of the study population

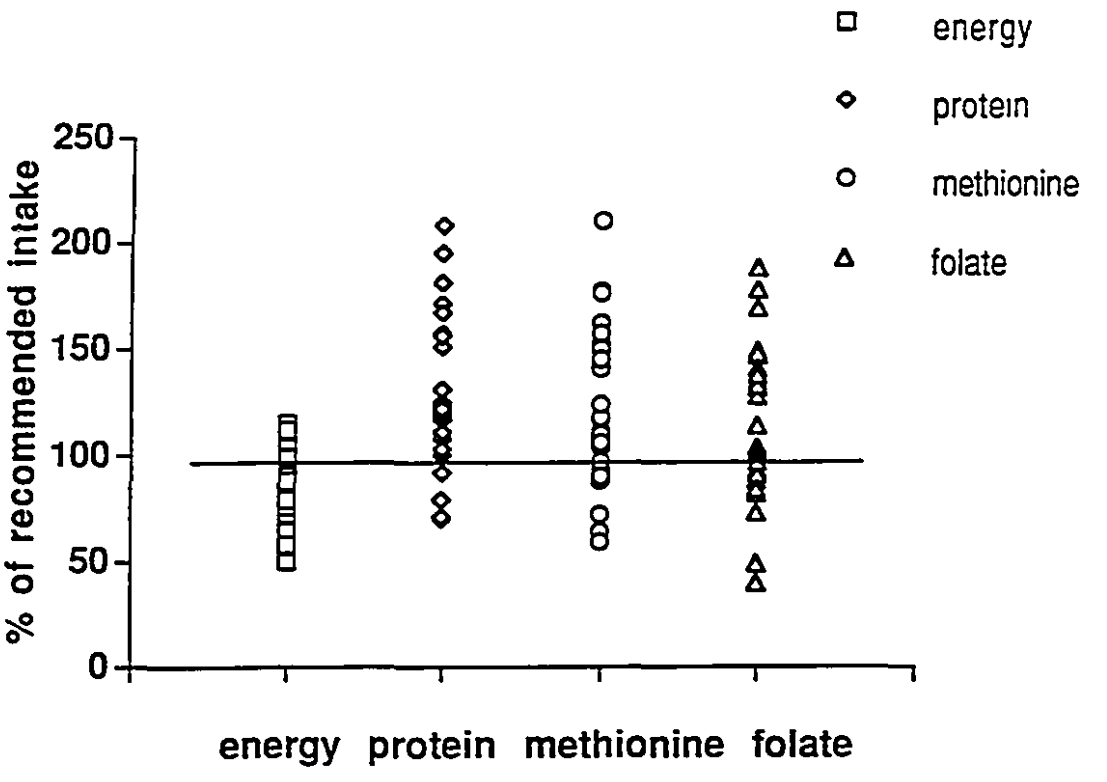
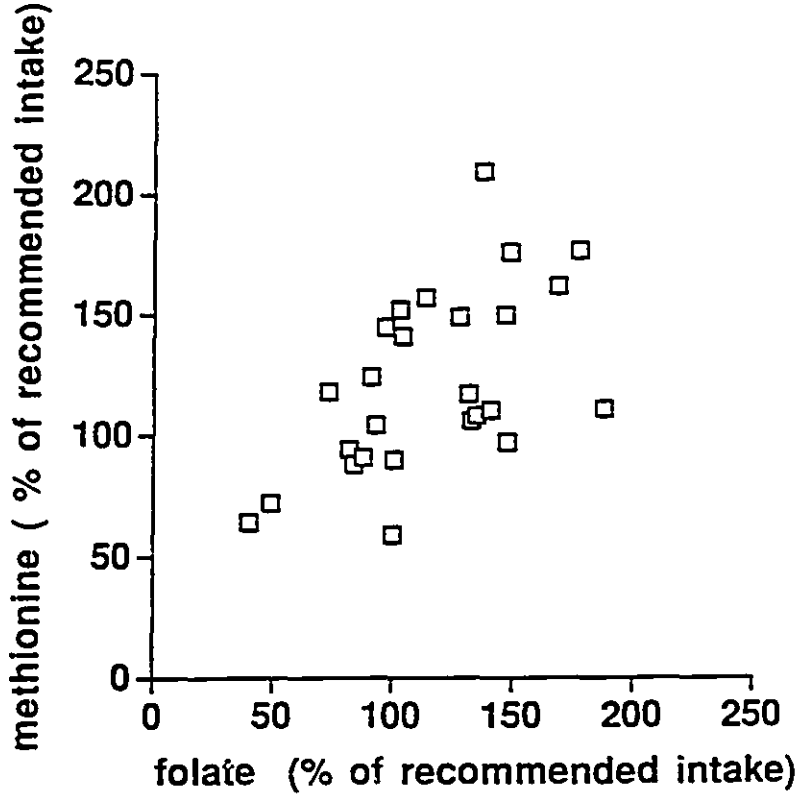
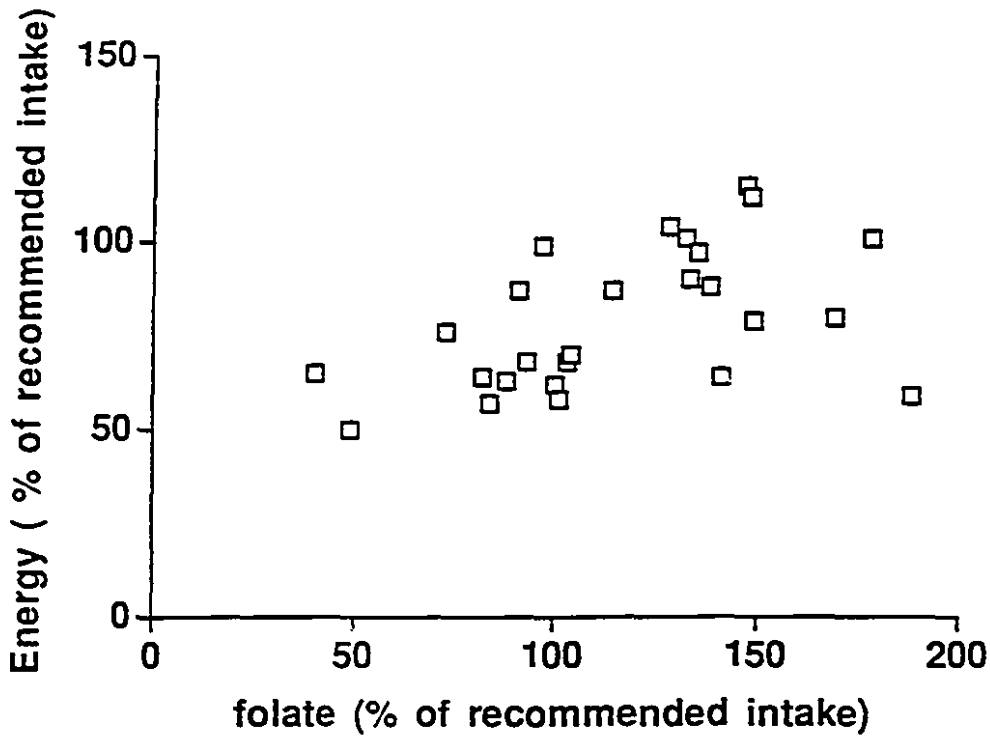


Fig 8. Correlation of folate and methionine intake



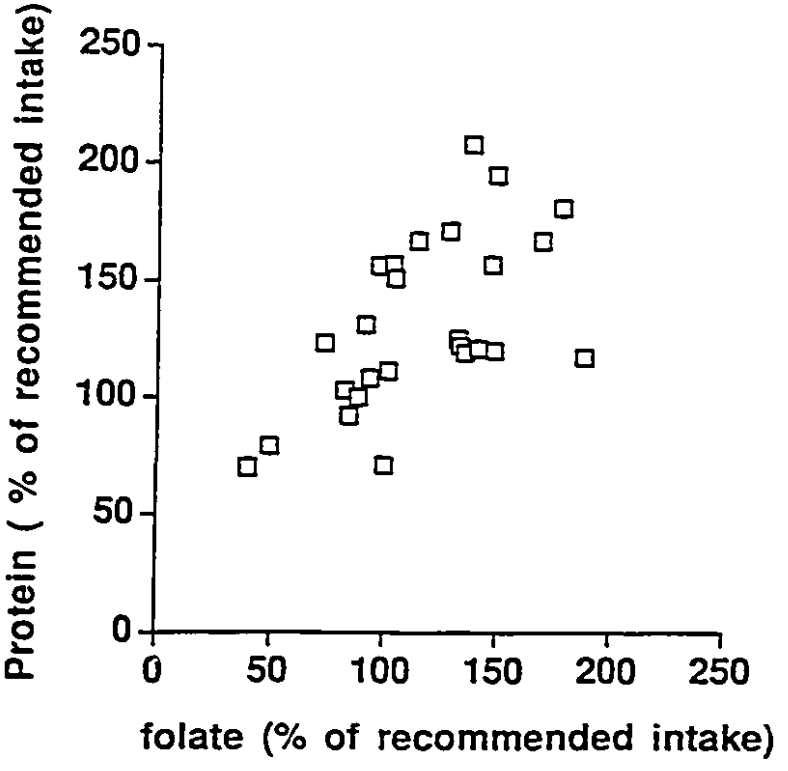
Values are the average of folate and methionine intake over 7 days for 26 patients. Pearson correlation coefficient for the interrelationships between dietary intakes of folate and methionine and the Fisher's r to z for statistical significance are as follows: r=0.559, p<0.003.

Fig 9. Correlation of energy and folate intake



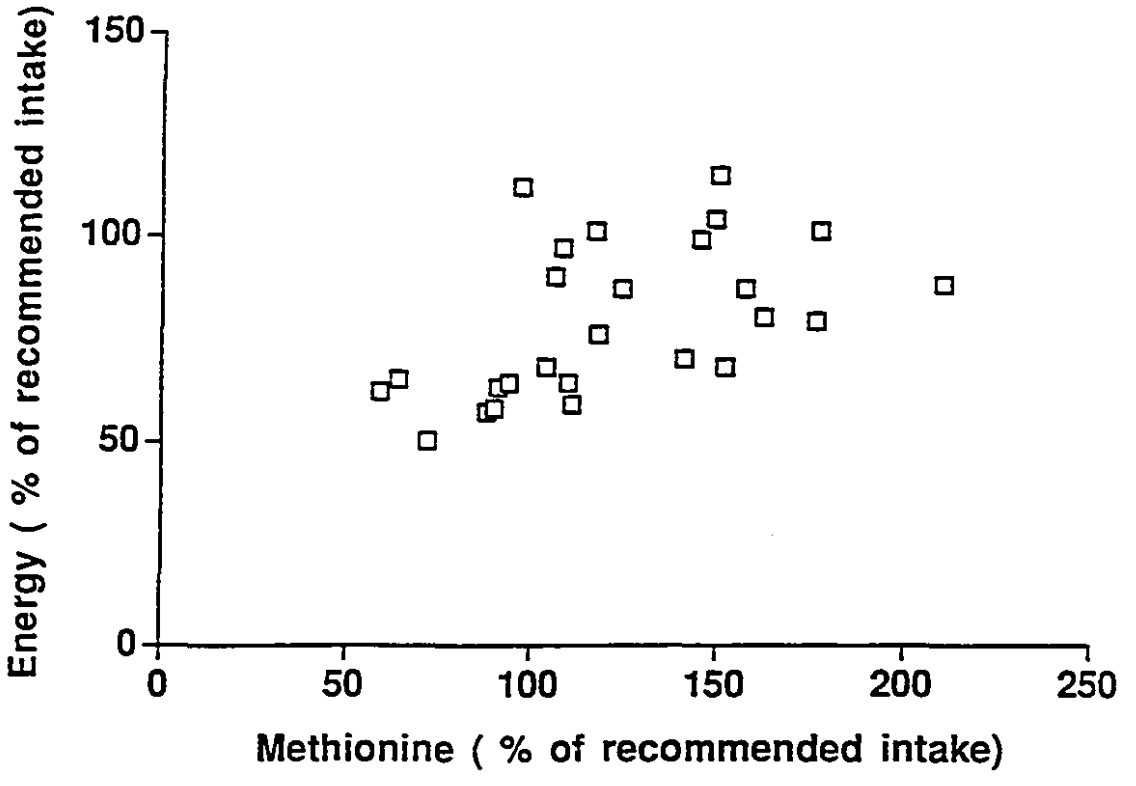
Values are the average of total caloric and folate intakes over 7 days for 26 patients. Pearson correlation coefficient for the interrelationships between total intake of calories and folate and the Fisher's r to z for statistical significance are as follows: $r=0.488$, $p<0.01$.

Fig 10. Correlation of protein and folate intake



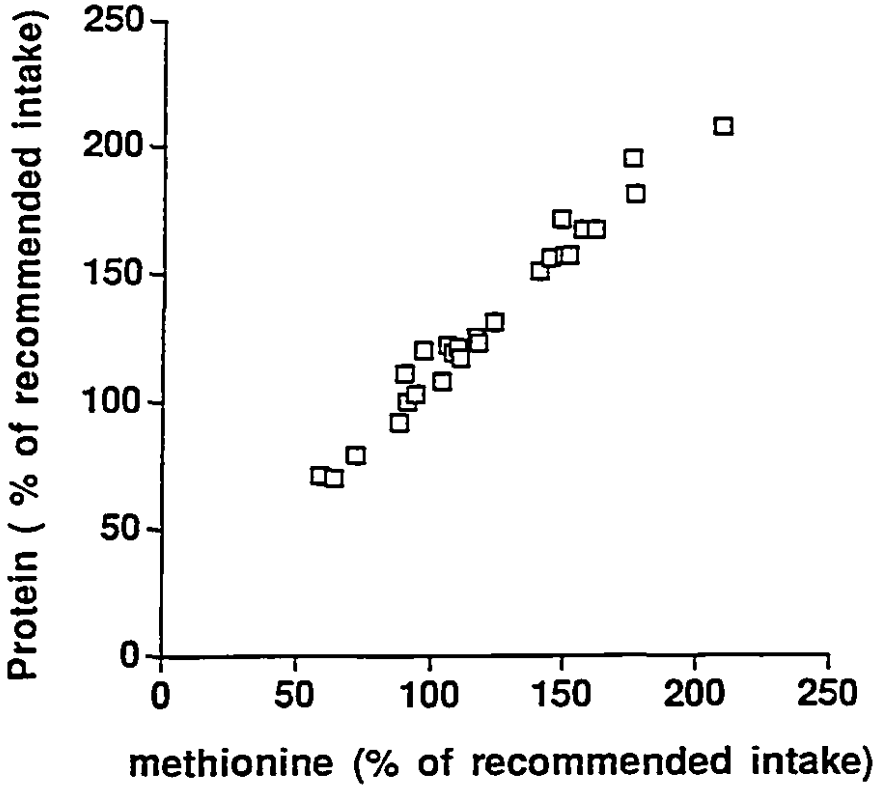
Values are the average of protein and folate intakes over 7 days for 26 patients. Pearson correlation coefficient for the interrelationships between protein and folate intakes and the Fisher's r to z for statistical significance are as follows: r=0.59, p<0.001.

Fig 11. Correlation of energy and methionine intake



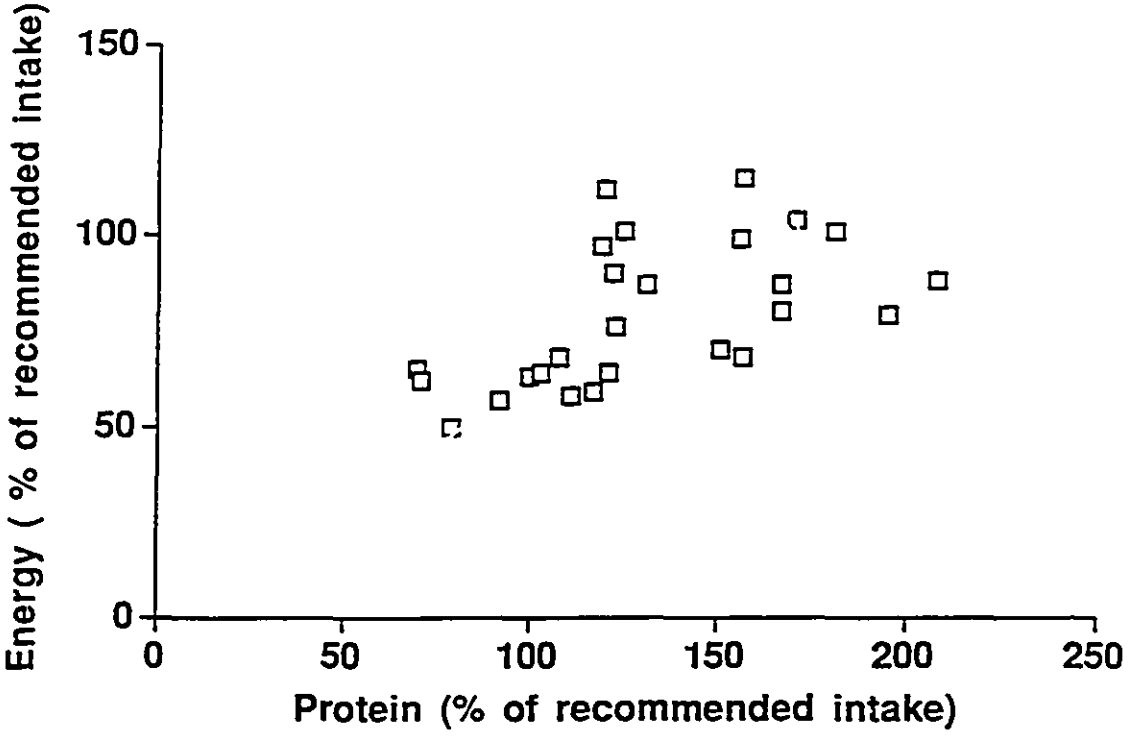
Values are the average of total caloric and methionine intakes over 7 days for 26 patients. Pearson correlation coefficient for the interrelationships between total intake of calories and methionine and the Fisher's r to z for statistical significance are as follows: r=0.51, p<0.008.

Fig 12. Correlation of protein and methionine intake



Values are the average of protein and methionine intakes over 7 days for 26 patients. Pearson correlation coefficient for the interrelationships between intakes of protein and methionine and the Fisher's r to z for statistical significance are as follows: r=0.9, p<0.0001.

Fig 13. Correlation of energy and protein intake



Values are the average of total caloric and protein intakes over 7 days for 26 patients. Pearson correlation coefficient for the interrelationships between total intake of calories and protein and the Fisher's r to z for statistical significance are as follows: r=0.55, p<0.003.

Table7. The highest and lowest values of methionine (% of recommended allowance) over 7-days

Patients	Highest	Lowest	Ratio
1	211	13	16.2
2	188	35	5.4
3	223	57	3.9
4	161	52	3.1
5	123	60	2.1
6	204	69	3.0
7	268	103	2.6
8	286	92	3.1
9	93	36	2.5
10	142	33	4.3
11	212	20	10.6
12	134	35	3.8
13	148	63	3.4
14	283	119	2.4
15	101	30	3.4
16	233	85	2.7
17	221	99	2.2
18	143	77	1.9
19	121	40	3.0
20	157	57	2.8
21	304	125	2.4
22	135	49	2.8
23	222	105	2.1
24	160	73	2.2
25	216	114	1.9
26	170	112	1.5

IV. DISCUSSION

1. Tail-flick test - SAM and methionine

In these experiments the time course of action of SAM and methionine given orally on tail-flick latency was studied. Results show an antinociceptive effect of SAM at 1.5 hour which lasted up to 3 hours (Fig. 2). Methionine exerted its effect at 4 hours and lasted up to 6 hours after its oral administration (Fig. 3).

Although in early clinical trials SAM was given intravenously, recently it has been given more often by the oral route. Success with the oral route depends on the absorption of SAM from the gastrointestinal tract and its metabolic fate in the liver after absorption. The pharmacokinetics of SAM given to rats either i.v. or i.m. has been studied (Stramentinoli, 1987). A bioavailability of 80 to 90% was determined for the i.m. dose. In relation to the absorbability of oral SAM, doses of 10, 20 and 100 mg/kg SAM were administered orally to rats (Stramentinoli et al 1979). A significant dose-response increase in plasma levels of SAM was observed. However a low bioavailability of the drug resulted from the oral administration as evidenced by a comparison of the plasma concentration after an oral or i.v. administration. Three possible explanations of the low bioavailability were considered and examined in this study: 1) SAM is not absorbed from the gastrointestinal tract

owing to the highly ionized sulfur atom of the molecule which prevents its diffusion across the lipid membrane of the intestine, 2) degradation of the molecule occurs at the alkaline pH of the intestine, and 3) extensive extraction of the drug by the liver occurs during the first-pass from the portal flow. It was shown that the poor bioavailability of SAM is associated with a first-pass effect resulting in the extraction of a relevant amount of the drug by the liver, where it appears to be metabolized rapidly. In another study the rats were treated either orally or intraduodenally with 32 mg/kg of SAM, and portal blood was collected at different time intervals. SAM levels were raised by both methods, but the concentrations appeared much higher when it was administered by the intraduodenal route (Stramentinoli, 1987). Although the oral bioavailability of SAM is low, some absorption into the blood has been demonstrated leading to increased interest in its clinical study in this convenient dosage form.

So far, the largest experience with SAM is in the treatment of depression, although some investigators have looked at the anti-inflammatory and analgesic activities of SAM. In one study the analgesic effect of intravenous and intraperitoneal administration of SAM was evaluated in experimental models used to study peripheral analgesic activity, namely the phenylquinone writhing tests in mice and the Randall-Selitto test in rats (Seigmud et al 1957; Randall and Selitto, 1957). There was a 71% reduction in

writhing in mice given 100 mg/kg SAM intravenously and a notable analgesic effect of 75 mg/kg SAM, given i.p., in the Randall-Selitto test (Stramentinoli, 1987). The analgesic effect of SAM given intraduodenally was investigated in the tail-flick test by Stramentinoli (1987). Results of this experiment showed an increase of tail-flick latency in rats given 100 mg/kg SAM. In our experiment rats were given SAM orally at a dose of 200 mg/kg and results obtained confirm the analgesic effect of SAM. A single clinical study also suggest that SAM may have an analgesic effect. In patients with fibromyalgia SAM was better than transcutaneous electrical nerve stimulation on subjective symptoms of pain and fatigue (Di Benedetto et al 1993). Given its relative freedom from side effects SAM should be tested in a variety of types of clinical pain.

An antinociceptive effect of methionine was evident 4 hours after its oral administration in the tail-flick test. As methionine is the precursor of SAM and increases SAM levels in the brain its analgesic effect may be related to its effect on SAM. Previous studies have shown that parenteral administrations of methionine resulted in the accumulation of SAM in the brain (Pubin et al 1974; Baldessarini and Kopin, 1966; Stramentinoli et al 1977). However, while methionine is transported actively across the blood brain barrier (Oldendorf and Szabo, 1976), the ease with which SAM passes from blood to brain is controversial. In the studies done by Baldessarini and Kopin (1966) radioactive SAM appeared to pass from blood to brain much more poorly than did

methionine. On the other hand, Stramentinoli (1977) has shown that 0.25 mmole/kg of SAM injected intravenously increased brain levels of this substance to 150% of its basal levels. Equimolar doses of methionine were not able to increase brain SAM levels as much as SAM did. However, methionine must be converted to SAM in the brain before it can raise brain SAM levels. Our results show that methionine exerted its analgesic effect at a lower dose than SAM (0.34 vs. 0.5 mmole/kg respectively in the time course studies). Methionine administration also increased brain levels of SAM at a lower dose than SAM as discussed later in the section on biochemical studies.

2. Dose-response studies of SAM and methionine

Oral administration of graded doses of SAM caused a clear dose-dependent increase in tail-flick latency (Fig. 4). Our results are in accordance with the findings of Stramentinoli (1987) in which a dose-dependent reduction in writhing in mice receiving phenylquinone as well as a dose-dependent increase in the tail-flick latency was observed. Although the maximum dose used in their studies was 100 mg/kg, the route of administration was intravenously or intraduodenally which is more effective than oral administration.

The analgesic effect of methionine did not exhibit a normal dose-response relationship; an inverted U shaped relationship was found. A peak

effect was seen at a dose of 50 mg/kg of methionine while at a dose of 100 mg/kg methionine tail-flick latency moved back towards control values (Fig. 5). Although the reason behind this is not known, we found a similar pattern in our biochemical studies, discussed below. The most likely explanations for this phenomenon are that methionine inhibits SAM formation at higher doses, or that higher levels of methionine enhance SAM degradation. A search of the literature provided no evidence either for or against both these possibilities.

A rat of 256 g (example taken from actual data)consumed 28 g of food in one day. Purina rat chow is 0.45% methionine. Thus, this rat ingested 128 mg of methionine in one day. This is a dose of 500 mg/kg methionine. Thus, a pharmacological effect is seen with a dose of methionine that is one fourth of the daily dietary intake of methionine.

3. Chronic administration of methionine

When methionine was added to the diet we found an increase of tail-flick latency in rats fed 0.6% methionine (Powdered Rat Chow supplemented with methionine) over the 3 week study period. There was an increase of tail-flick latency after day 3 which reached significance on days 7 and 21. Although a few behavioral studies have been done on the effect of methionine on behavior, we were unable to find any previous study in relation to its effect on pain. In a study of the mechanism of action of L-methionine in inducing acute psychotic

reactions in some schizophrenic patients, Beaton et al. (1975) assessed the effects of methionine on behavior (Discriminated Sidman Avoidance performance and slow wave and rapid eye movement sleep) of rats and mice injected subcutaneously with 250 mg/kg body weight of methionine for 21 days. In comparison with saline-injected controls, methionine induced avoidance behavior and REM sleep disturbances. In a study done by Sprince et al. (1969) homocysteine induced convulsions and death when injected intraperitoneally into rats. However, equimolar doses of methionine (1100 mg/kg) did not produce convulsions or result in any marked behavioral change. It is important to note that the dose of methionine used in these studies was much higher than the doses we used in our study.

Results of our study indicated an analgesic effect of 0.6% methionine in rats. This level of methionine which is in its physiological range, like its acute dose, may increase brain levels of SAM and hence increase the tail-flick latency in rats. The reason for the delayed response (after 7 days) during dietary administration of methionine is unclear. Initially metabolic adaptation to the elevated methionine intake may prevent any effect, but continued high intake may eventually overcome the effects of metabolic adaptation.

4. Biochemical studies

There are two possible disadvantages in the use of SAM to influence

brain function (Baldessarini, 1987). First, it may be too labile and too polar to reach the central nervous system in appreciable quantities after systemic administration. Second, it may be too labile to accumulate in the brain. However, this is not supported by experiments in rats given large doses of SAM (100 mg/kg i.v.) (Stramentinoli et al 1977). Within 5 minutes, brain tissue levels of SAM doubled, and they decayed with a half-life of about one to two hours. When the same dose (100 mg/kg) was given intramuscularly, there was a smaller increase in levels of SAM in the brain (46% maximum increase), but the peak appeared later (one hour) and disappeared more slowly. Although these studies indicate that some SAM can reach the central nervous system of an animal after a large parenteral dose, it is not proved that oral doses of SAM can affect the levels of this substance in the brain. To test whether SAM can cross the blood brain barrier and to confirm the assumption that SAM is absorbed when given orally, blood, liver and brain levels of this substance and its metabolite SAH were examined. In order to address the possibility that SAM exerts its psychopharmacological action by increasing 5-HT levels in the brain, doses of SAM used in the behavioral studies were tested in a biochemical study in which brain 5-HT and its metabolite 5-HIAA were measured. As methionine can increase SAM levels and showed an analgesic effect after both acute and chronic administration, the levels of the above substances were also examined after methionine administration.

A. Effect of SAM and methionine on SAM and SAH levels

Results of our experiments suggest that the oral administration of SAM, like its parenteral administration, can result in it crossing the blood brain barrier and accumulating in the brain. This was shown from an increase of SAM in brain stem, cerebellum and rest of brain after SAM administration (Table 2a). However, levels of SAM were significantly higher in all of the brain regions upon administration of 50 mg/kg methionine (Table 3a) than after 400 mg/kg SAM. Comparison of these results suggest that methionine at the dose of 50 mg/kg, which is equivalent to about 130 mg/kg of SAM on the basis of their molecular weight, appears to pass from the blood brain barrier much more efficiently than does SAM. Our results are therefore consistent with those of Baldessarini and Kopin (1966). In fact, the transport mechanism of methionine into the brain (a saturable, energy dependent mechanism in which there is competition between methionine and other large neutral amino acids for transport) is well known (Oldendorf and Szabo, 1976) whereas there is no known mechanism for SAM transport into the brain. The fact that liver SAM levels increased much more than brain levels after SAM administration suggests that any system for transporting SAM into brain is not very active.

The chronic dietary effect of methionine on brain levels of SAM was not as evident as its acute administration. SAM levels increased significantly in cerebellum and spinal cord (Table 4a). Although brain stem SAM levels were

lowered in this group, levels of its metabolite SAH were increased. One explanation might be that the rate of SAM utilization in this brain region was increased and hence the level of its metabolite increased.

In the acute SAM experiment there was a steady increase in SAM levels which reached significance at a dose of 400 mg/kg in liver and 200 mg/kg in blood (Table 2a). In the case of acute methionine administration, however, liver SAM levels were significantly higher in the control group (Table 3a). The reason for this is not known. Liver levels of SAM were not significantly different in the 0.45% and 0.6% group (Table 4a). Since animals were on this diet for 21 days perhaps there was a metabolic adaptation in the livers of the supplemented methionine group.

B. Effect of SAM and methionine on tryptophan, 5-HT and 5-HIAA

Tables 2b and 2c clearly show that SAM did not result in consistent increases in the levels of tryptophan, 5-HT and 5-HIAA in the brain. The same was true for methionine supplementation. The lack of any decline in brain tryptophan indicates that the doses of methionine used were too small to inhibit uptake of tryptophan across the blood-brain barrier. Thus, methionine can be given at a dose sufficient to raise brain SAM without affecting brain tryptophan.

Dietary changes of methionine did not have an affect on 5-HT, its precursor and its metabolite. The biochemical data suggest that the

mechanism of the analgesic action of SAM in the tail-flick test is not mediated through altered 5-HT levels, or in so far as 5-HIAA is an indication of 5-HT release, through altered 5-HT function. Pain modulation is a behaviorally significant physiological process, using a discrete central nervous system network involving release of opioid peptides, biogenic amines, and other neurotransmitters including substance P, somatostatin, and thyroid releasing hormone (Basbaum and Fields, 1984). SAM could be influencing any of these neurotransmitters. Alternatively SAM could be influencing neuronal excitability directly through its effect on membranes, described in the introduction. Whatever the mechanism it is unlikely that 5-HT plays any important role in mediating the effects of SAM or methionine, even though a possible effect on 5-HT was one of the reasons for choosing to look at the tail-flick test.

5. Clinical study

In our study and as stressed by other investigators, the conclusions that can or should be drawn from total intakes of nutrients are not very revealing (Payne and Hudson, 1972). Presenting the data as a percentage of the recommended intake rather than mean intakes will give us more insight into individual nutritional health.

The purpose of the clinical study was to look at the variation of intakes of folate and methionine and to determine whether some subjects have a high

methionine intake with a low folate intake. In addition, the nutritional status of this population (affective disorder patients) by means of a 7-day dietary record was examined. Since our main purpose was the study of folate and methionine interrelations, the results reported are limited to these nutrients and their relation with dietary intakes of macronutrients. Our study included an outpatient population of males and females who had been living at home for at least three months. The patients were not on any special diet and were not taking any medication except lithium.

Despite a low mean reported caloric intake (Table 6), 38% of the study population had a BMI > 27 (obese) and 31% had BMI between 25-27 (over weight). One of the common side effects of lithium is weight gain (Gray and Gray, 1989). As many as 42% of patients on this medication may gain weight. Twenty percent gain more than 10 kg over a 5 year period (Jefferson et al 1983). Although the mechanism behind the weight gain is not known, one possibility is that lithium influences carbohydrate metabolism. Another possibility, always present when high values of BMI exist in conjunction with low caloric consumption, is that energy intake was underestimated (Livingstone et al 1990), particularly in obese people (Prentice et al 1986).

In a study done in the United States (Payne and Hudson, 1972) a survey was made of the dietary histories of 49 patients entering a psychiatric hospital for the first time. Although their results, similar to our findings, indicated that

females did not meet the recommended daily intake for calories no information was provided regarding patients' BMI. Surprisingly, there are very few dietary studies done in psychiatric outpatient populations where the diet is not controlled in the same way as it is in hospital. In the few studies on psychiatric outpatients available results are not conclusive because of poor methodology (Kruesi and Rapoport, 1986). For example, only two studies looked at the overall adequacy of the diet of a group of depressed patients. In addition to using a 24 hr recall, which is not a very reliable method for these patients, both of these studies gave no clear definition of how the patients' diets were categorized into poor, moderate and excellent (Reynolds et al 1970; Thornton and Thornton, 1978). Intakes of both protein and energy were below recommended levels for some patients.

In general, in examining Canadian consumption patterns, it is found that there is a remarkable consistency in protein to energy ratios, ranging from 13%-15% (Murray et al 1990), so if caloric consumption increases, so will protein in most instances. However, in the present study while caloric consumption of 73% of the patients was below their recommended intake, protein intake was below recommended levels for only 15% of the patients. Our results are similar in some respects to those from a group of psychiatric inpatients whose diets were studied using a three day food record. Almost all men and 60% of the women were below the recommended intake for caloric

consumption but only 30% of the men and 45% of the women consumed protein below the recommended intake (Payne and Hudson, 1972).

In the present study intakes of protein, methionine and folate (as a percentage of the recommended intake) are spread over a wide range (Fig. 7). One of the main reasons for looking at dietary intake of the patients was to look at the relationship between intakes of folate and methionine. The r value for this association of 0.559 (Fig. 8) indicates that 31% of the variation in folate intake can be explained by the variation in methionine intake. Thus, there is not a tight relationship between intakes of folate and methionine. As many as 44% of the patients who were not meeting their recommended intakes for folate had intakes of methionine above their recommended intake (range of 100%-145%). For the whole group of patients with folate intakes below their recommended levels methionine intake varied from 64 to 145% of the recommended intake. These results are consistent with the idea that, in some patients with inadequate intake of folate, methionine intakes above the requirement may overcome the metabolic effects of low folate. Further work will be necessary to test this hypothesis.

Intakes of methionine varied greatly from day to day (Table 7) with intake on some days being well below the recommended intake. Although several studies examined the adverse effects of excess dietary methionine intake in both animals and humans (Anderson and Raiten, 1992), the

consequences of the acute effect of very low intakes of this substance in individuals who may need quantities above the normal intake (e.g. folate deficient subjects) have not been explored. Intake of methionine is related strongly to protein intake (Fig. 12) indicating that inadequate intake of methionine is due to inadequate overall protein intake. Since methionine intake was strongly correlated with intakes of protein, the correlation of energy and protein ($r=0.553$, $p=0.003$) was similar to that of energy and methionine ($r=0.51$, $p=0.008$). These results suggest that 31% and 26% of the variabilities of the protein and methionine intakes respectively could be explained by the caloric intake. Intake of folate was positively related to that of energy and protein (Figs. 9 & 10). Variability in the intake of energy and protein account for 24% and 35% of the variability in folate intake respectively. Thus, the relationship between folate and energy or protein is much less strong than that between methionine and protein. This is because the principle sources of folate are in a variety of food groups such as meat (high in protein and high in calories) and fruits and vegetables (low in protein and low in calories).

Although 31% of the patients were taking vitamin supplements, none of the patients who could have benefited from supplements, because of inadequate intake, were taking them. A possible reason for this is that subjects who are more concerned about their diet, and are therefore ingesting their recommended amounts of nutrients, are also those who are most likely to

take supplements. Further work will be necessary to determine whether psychiatric outpatients are more likely than other potentially vulnerable groups to have poor diets. It would also be important to know whether the vulnerability of psychiatric patients to poor diets is related to diagnosis. The degree of morbidity related to poor diet is unclear, but the studies, mentioned in the introduction, in which folate supplementation had a beneficial effect on psychiatric patients suggest that poor diet could have significant adverse effects on mental health in susceptible populations. Obviously interventions to improve the diet of psychiatric patients would be desirable. However, to maximize the possible benefits of such studies it would be important to know to what extent the poor diet was due to lack of nutritional knowledge, or other factors related to the psychopathology of the patients such as disorganized life style or lack of motivation.

V. GENERAL DISCUSSION AND CONCLUSIONS

The results in this thesis add to the evidence suggesting that methionine might have a similar psychopharmacological action to SAM. Thus, (i) methionine raises brain SAM, (ii) both SAM and methionine increase tail flick latency in rats, and (iii) methionine only increases tail flick latency at doses that raise brain SAM. The antidepressant effect of SAM is now well established. However, SAM is not suitable for general use because of its instability in solid form at room temperature and its high cost (sometimes even more than $500 per patient per day). Methionine on the other hand is stable at room temperature and no more expensive than many antidepressants on the market. Obviously, methionine should be tested both as an antidepressant and for the treatment of pain. Although methionine is one of the more toxic amino acids (Gullino et al 1956), it has been used enough clinically to suggest that it is not toxic at the doses that might be used in humans. Until less than 15 years ago methionine was available as a prescription drug in Canada in 250 mg tablets. It was given to infants in that amount as a urinary acidifier, for the treatment of diaper rash. Although amino acids are treated as drugs in Canada, in the U.S.A. methionine, together with other amino acids, is available in health food stores, pharmacies and even supermarkets. The author of this thesis has in her possession a bottle of methionine which was sold illegally in a Montréal

health food store. Given the possibility of widespread use of methionine in the general population it would seem to be important to obtain definite information about its psychopharmacological action in humans.

The dietary study on major affective disorder outpatients was designed primarily to look at intakes of methionine and folate, but revealed a surprisingly high incidence of calorie and protein intake below recommended levels. Obviously more work is needed on the dietary intake of psychiatric outpatients.

Results of the clinical study indicated that only 31% of the variation in folate intake can be explained by the variation in methionine intake. Thus, in some patients with inadequate folate intake and adequate methionine intake, methionine may compensate for the possible effects of low folate intake. However, we did not study the effects of diet on mood and behavior, because of the impact of confounding factors such as medication on mood. This suggests that further work investigating the possibility that the level of methionine (i.e. protein) intake will influence the detrimental mental effects of folate deficiency is worthwhile.

The animal studies demonstrated that methionine has a psychopharmacological effect in rats at a dose that is considerably less than the daily dietary intake of that amino acid, while the clinical study showed that methionine intake varied greatly from day to day in the patients. This raises the possibility that a high intake of methionine on a day following one with a low

intake of methionine might result in alterations in the brain levels of methionine and SAM and subtle changes in mood and behavior. In rats protein meals raise brain methionine and SAM (Rubin et al 1974). Testing the idea that ingestion of high amounts of protein at a single meal or during a day could alter mental state would be difficult, but the implications of possible diet induced alterations of brain function associated with protein intake in humans are great.

REFERENCES

1. Agnoli A, Andreoli V, Casacchia M and Cerbo R (1976) Effect of S-adenosyl-L-methionine (SAMe) upon depressive symptoms. *J Psychiat Res* **13**: 43-54.

2. Agnoli A, Andreoli VM, Casacchia M, Maffei F and Fazio C (1978) Results and possible developments of the clinical use of S-adenosyl-L-methionine (SAMe) in psychiatry. In: Transmethylations and the Central Nervous System. Andreoli VM, Agnoli A, Fazio C. (eds). New York: Springer-Verlag, pp 170-182.

3. Aguilar TS, Benevenga NJ and Harper AE (1974) Effect of dietary methionine level on its metabolism in rats. *J Nutr* **104**: 761-771.

4. American Psychiatric Association (1987) Diagnostic and Stastical Manual of Mental Disorders, Third Edition, Revised. Washington, DC: American Psychiatric Association, pp 1-567.

5. Anderson GM, Young JG, Batter DK, Young SN, Cohen DJ and Shaywitz BA (1981) Determination of indoles and catechols in rat brain and pineal using liquid chromatography with fluorometric and amperometric detection. *J Chromatogr* **223**: 315-320.

6. Anderson SA and Raiten DJ (1992) Safety of amino acids used as dietary supplements. Bethesda, MD: Federation of American Societies for Experimental Biology, pp 62-75.

7. Baldessarini RJ and Kopin IJ (1966) S-Adenosylmethionine in brain and other tissues. *J Neurochem* **13**: 769-777.

8. Baldessarini RJ (1987) Neuropharmacoiogy of S-adenosyl-L-methionine. *Am J Med* **83**: 95-103.

9. Barberi A and Pusateri C (1978) Sugli effetti clinici della S-adenosil-L-metionina nelle sindromi depressive. *Minerva Psichiatr* **19**: 235-243.

10. Barrett-Connor E (1991) Nutrition epidemiology: how do we know what they ate? *Am J Clin Nutr* **54**: S182-S187.

11. Basbaum AI and Fields HL (1984) Endogenous pain control mechanisms: brainstem pathways and endorphin circuitry. *Ann Rev Neurosci* **7**: 309-338.

12. Beaton JM (1975) Methylation and schizophrenia. *Ala J Med Sci* **12**: 193-202.

13. Bingham SA (1991) Limitations of the various methods for collecting dietary intake data. *Ann Nutr Metab* **35**: 117-127.

14. Botez MI, Young SN, Bachevalier J and Gauthier S (1979) Folate deficiency and decreased brain 5-hydroxytryptamine synthesis in man and rat. *Nature* **278**: 182-183.

15. Botez MI, Young SN, Bachevalier J and Gauthier S (1982) Effect of folic acid and vitamin B12 deficiencies on 5-hydroxyindoleacetic acid in human cerebrospinal fluid. *Ann Neurol* **12**: 479-484.

16. Bottiglieri T, Laundy M, Martin R, Carney MWP, Nissenbaum H, Toone BK, Johnson AL and Reynolds EH (1984) S-Adenosylmethionine influences monoamine metabolism. *Lancet* **ii**: 224.

17. Bottiglieri T, Landry M, Carney MWP, Chary TKN, Toone BK and Reynolds EH (1986) Folate status in psychiatric patients: relationship with CSF neurotransmitter metabolites. In: Chemistry and Biology of Pteridines. Berlin: Walter de Gruyter, pp 523-526.

18. Butler IJ and Rothenberg SP (1987) Dietary folate and biogenic amines in the CNS. *J Neurochem* **49**: 268-271.

19. Byington MH, Howe JM and Clark HE (1972) Effect of different levels and proportions of methionine, cysteine, choline, and inorganic sulphur on growth and body composition of young rats. *J Nutr* **102**: 219-228.

20. Carney MWP, Edeh J, Bottiglieri T, Reynolds EH and Toone BK (1986) Affective illness and S-adenosyl methionine: a preliminary report. *Clin Neuropharmacol* 9: 379-385.

21. Carrieri PB, Indaco A, Gentile S, Troisi E and Campanella G (1990) S-Adenosylmethionine treatment of depression in patients with Parkinson's disease: a double-blind, crossover study versus placebo. *Curr Ther Res* **48**: 154-160.

22. Caruso I, Fumagalli M, Boccassini L, Puttini PS, Ciniselli G and Cavallari G (1984) Antidepressant activity of S-adenosylmethionine. *Lancet* **i**: 904-900.

23. Cimino M, Vantini G, Algeri S, Curatola G, Pezzoli C and Stramentinoli G (1984) Age-related modification of dopaminergic and b-adrenergic receptor system: restoration to normal activity by modifying membrane fluidity with S-adenosylmethionine. *Life Sci* **34**: 2029-2039.

24. Cohen SM, Nichols A, Wyatt R and Pollin W (1974) The administration of methionine to chronic schizophrenic patients: a review of ten studies. *Biol Psychiatry* **8**: 209-225.

25. Coppen A, Chaudhry S and Swade C (1986) Folic acid enhances lithium prophylaxis. *J Affective Disord* **10**: 9-13.

26. Crews FT, Hirata F and Axelrod J (1980) Identification and properties of methyltransferases that synthesize phosphatidylcholine in rat brain synaptosomes. *J Neurochem* **34**: 1491-1498.

27. Curcio M, Catto E, Stramentinoli G and Algeri S (1978) Effect of S-adenosyl-L-methionine on serotonin metabolism in rat brain. *Prog Neuropsychopharmacol Biol Psychiatry* **2**: 65-71.

28. De Leo D (1985) S-Adenosylmethionine (SAMe) in clinical practice: preliminary report on 75 minor depressives. *Curr Ther Res* **37**: 658-661.

29. De Leo D (1987) S-Adenosylmethionine as an antidepressant: a double-blind trial versus placebo. *Curr Ther Res* **41**: 865-870.

30. Del Vecchio M, Iorio G and Cocorullo M (1978) Has SAM an antidepressant effect? A preliminary trial versus chlorimipramine. *Riv Sper Freniatria* **102**: 344-358.

31. Di Benedetto P, Iona LG and Zidarich V (1993) Clinical evaluation of S-adenosyl-L-methionine versus transcutaneous electrical nerve stimulation in primary fibromyalgia. *Curr Ther Res* **53**: 222-229.

32. Fazio C, Andreoli V and Agnoli A (1973) Effectti terapeutici e meccanismo d'azione della S-adenosil-L-Methionina nelle sindromi depressive. *Minerva Med* **64**: 1515-1529.

33. Godfrey PSA, Toone BK, Carney MWP, Flynn TG, Bottiglieri T, Laundy M, Chanarin I and Reynolds EH (1990) Enhancement of recovery from psychiatric illness by methylfolate. *Lancet* **336**: 392-395.

34. Gray GE and Gray LK (1989) Nutritional aspects of psychiatric disorders. *J Amer Diet Assoc* **89**: 1492-1498.

35. Gregory JF (1989) Chemical and nutritional aspects of folate research: analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. Life Sciences Research Office. Federation of American Societies for Experimental Biology. Bethesda. Maryland.

36. Gullino P, Winitz M, Birnbaum M, Cornfield J, Otey MC and Greenstein JP (1956) Studies on the metabolism of amino acids and related compounds *in vivo* . I. Toxicity of essential amino acids, individually and in mixtures and the protective effect of L-arginine. *Arch Biochem Biophys* **64**: 319-332.

37. Guttari B (1991) High-performance liquid chromatographic determinatin with ultraviolet detection, of S-adenosyl-L-methionine and S-adenosyl-L-homocysteine in rat tisseus and simulataneously of normetanephrine and metanephrine for phenylethanolamine-N-methyltransferase or catechol-O-methyltransferase activities. *J Chromatogr* **567**: 254-260.

38. Harper AE, Benevenga NJ and Wohlhueter RM (1970) Effects of ingestion of disproportionate amounts of amino acids. *Physiol Rev* **50**: 428-558.

39. Hunter R, Barnes J, Oakley HF and Matthews DM (1970) Toxicity of folic acid given in pharmacological doses to healthy volunteers. *Lancet* **i**: 61-63.

40. Hunter R, Barnes J, Curzon G, Kantamaneni BD and Duncan C (1971) Effect of folic acid by mouth on cerebrospinal fluid homovanillic acid and 5-hydroxyindoleacetic acid concentration. *J Neurol Neurosurg Psychiatry* **34**: 571-575.

41. Janicak PG, Lipinski J, Davis JM, Comaty JE, Waternaux C, Cohen B, Altman E and Sharma RP (1988) S-Adenosylmethionine in depression: A literature review and preliminary report. *Ala J Med Sci* **25**: 306-313.

42. Jefferson JW, Greist JH and Ackerman MS (1983) Lithium: Encyclopedia for Clinical Practice. Washington, DC: American Psychiatric Press, Inc., pp 1-319.

43. Kagan BL, Sultzer DL, Rosenlicht N and Gerner RH (1990) Oral S-adenosylmethionine in depression: a randomized double-blind, placebo-controlled trial. *Am J Psychiatry* **147**: 591-595.

44. Kruesi MJP and Rapoport JL (1986) Diet and human behavior: how much do they affect each other? *Ann Rev Nutr* **6**: 113-130.

45. Küfferle B and Grünberger J (1982) Early clinical double-blind study with S-adenosyl-L-methionine: a new potential antidepressant. In: Typical and Atypical Antidepressants: Clinical Practice. Costa E, Racagni G. (eds). New York: Raven Press, pp 175-180.

46. Le Bars D (1988) Serotonin and pain. In: Neuronal serotonin. Osborne NN, Hamon M. (eds). New York: John Wiley and Sons Ltd., pp 171-229.

47. Lipinski JF, Cohen BM, Frankenburg F, Tohen M, Waternaux C, Altesman R, Jones B and Harris P (1984) Open trial of S-adenosylmethionine for treatment of depression. *Am J Psychiatry* **141**: 448-450.

48. Livingstone MBE, Prentice AM, Strain JJ, Coward WA, Black AE, Barker ME, McKenna PG and Whitehead RG (1990) Accuarcy of weighed dietary records in studies of diet and health. *Br Med J* **300**: 708-712.

49. Mantero M, Pastorino P and Carolei A (1975) Studio controllato indoppio cieco (SAM-Imipramina) nelle sindromi depressive. *Minerva Med* **66**: 4098-4101.

50. Miccoli L, Porro V and Bertolino A (1978) Comparison between the antidepressant activity of S-adenosylmethionine and that of some tricyclic drugs. *Acta Neurol* **33**: 243-255.

51. Murray TK, Carroll KK, Davignon J, Draper HH, Evers S, Greenwood CE, Mongeau E, Zlotkin S, Beare-Rogers J, Fischer P and Palin D (1990) Nutrient Recommendations: The Report of the Scientific Review Committee. Ottawa: Minister of Supply and Services Canada, pp 1-208.

52. Muscettola G, Galzenati M and Balbi A (1982) SAMe versus placebo: a double-blind comparison in major depressive disorders. In: Typical and Atypical Antidepressants: Clincal Practice. Costa E, Racagni G. (eds). New York: Raven Press, pp 151-156.

53. National Research Council (1989) Recommended Daily Allowances. Washington: National Academy Press, pp 1-285.

54. Oldendorf WH and Szabo J (1976) Amino acid assignment to one of three blood-brain barrier amino acid carriers. *Am J Physiol* **230**: 94-98.

55. Ordonez LA and Wurtman RJ (1974) Folic acid deficiency and methyl group metabolism in rat brain: effects of L-dopa. Reversal effect of S-adenosyl-L-methionine. *Arch Biochem Biophys* **160**: 372-376.

56. Payette H and Gray-Donald K (1991) Dietary intake and biochemical indices of nutritional status in an elderly population, with estimates of the precision of the 7-d food record. *Am J Clin Nutr* **54**: 478-488.

57. Payne IR and Hudson MM (1972) Dietary intakes of mental patients. *Am J Public Health* **62**: 1210-1215.

58. Prentice AM, Black AE, Coward WA, Davies HL, Goldberg GR, Murgatroyd PR, Ashford J, Sawyer M and Whitehead RG (1986) High levels of energy expenditure in obese women. *Br Med J* **292**: 983-987.

59. Randall LO and Selitto JJ (1957) A method for measurement of analgesic activity on inflammed tissue. *Arch Int Pharmacodyn* **111**: 409-419.

60. Reynolds EH, Preece JM, Bailey J and Coppen A (1970) Folate deficiency in depressive illness. *Br J Psychiatry* **117**: 287-292.

61. Reynolds EH and Stramentinoli G (1983) Folic acid, S-adenosylmethionine and affective disorders. *Psychol Med* **13**: 705-710.

62. Rosenbaum JF, Fava M, Falk WE, Pollack MH, Cohen LS, Cohen BM and Zubenko GS (1990) The antidepressant potential of oral S-adenosylmethionine. *Acta Psychiatr Scand* **81**: 432-436.

63. Rotruck JT and Boggs RW (1977) Effects of dietary L-methionine and N-acetyl-L-methionine on growing rats. *J Nutr* **357**: 362.

64. Rubin RA, Ordonez LA and Wurtman RJ (1974) Physiological dependence of brain methionine and S-adenosylmethionine concentrations on serum amino acid pattern. *J Neurochem* **23**: 227-231.

65. Salvadorini F, Galeone F, Saba P and Tognetti G (1980) Evaluation of S-adenosylmethionine (SAMe) effectiveness on depression. *Curr Ther Res* **27**: 908-918.

66. Scaggion G, Baldan L and Domanin S (1982) Azione antidepressiva della SAMe a confronto con il nomifensine maleato. *Minerva Psichiatr* **23**: 93-97.

67. Scarzella R and Appiotti A (1978) Confronto clinico in doppio cieco della SAMe versus clorimipramina nelle sindromi depressive. *Riv Sper Freniatria* **102**: 359-365.

68. Sebrell WH (1981) History of pellagra. *Federation Proc* **40**: 1520-1522.

69. Seigmud E, Cadmus R and Go L (1957) A method for evaluating both non-narcotic and narcotic analgesics. *Proc Soc Exp Biol Med* **95**: 729-731.

70. Sprince H, Parker CM, Joseph JA and Magazino J (1969) Convulsant activity of homocysteine and other short chain mercaptoacids: protection therefrom. *Ann N Y Acad Sci* **166**: 323-325.

71. Stramentinoli G, Catto E and Algeri S (1977) The increase in S-adenosyl-L-methionine (SAMe) concentration in rat brain after its systemic administration. *Commun Psychopharmacol* **1**: 89-97.

72. Stramentinoli G, Gualano M and Galli-Kienle M (1979) Intestinal absorption of S-adenosyl-L-methionine. *J Pharmacol Exp Ther* **209**: 323-326.

73. Stramentinoli G (1987) Pharmacological aspects of S-adenosylmethionine: pharmacokinetics and pharmacodynamics. *Am J Med* **83 suppl 5A**: 35-42.

74. Thornton WE and Thornton BP (1978) Folic acid, mental function , and dietary habits. *J Clin Psychiatry* **39**: 315-322.

75. Young CM, Hagan GC, Tucker RE and Foster WD (1952) A comparison of dietary study methods. II. Dietary history vs. seven-day record vs. 24-hr. recall. *J Amer Diet Assoc* **28**: 218-221.

76. Young SN (1986) The clinical psychopharmacology of tryptophan. In: Nutrition and the Brain, Volume 7, Food Constituents Affecting Normal and Abnormal Behaviors. Wurtman RJ, Wurtman JJ. (eds). New York: Raven Press, pp 49-88.

77. Young SN and Ghadirian AM (1989) Folic acid and psychopathology. *Prog Neuropsychopharmacol Biol Psychiatry* **13**: 841-863.

78. Zeisel SH (1981) Dietary choline: biochemistry, physiology, and pharmacology. *Ann Rev Nutr* **1**: 95-121.

APPENDIX A

CHART SUMMARY

Data Base

Patient/Client profile

Chart number --------- Patient Initials ------ Sex ---- Age -----

Height -------- Weight ------ kg. Occupation ----------

Culture ---------- Marital Status ----------

Languages --------, ----------

Family Description ----------

Patient/Client Socio-economic history

Diagnosis ----------

Chief Complaint ----------

Food and Nutrition Information ----------

Medications and dosage ----------

Past Medical History

Patient Name: _____

Age: ____ Sex: ____ Date of visit : _____

Medical Record Number: _____

DIET HISTORY

Height: _____ (cm) Weight: _____ (Kg) IBW: _____ (Kg) Desirable wt: _____ (Kg)

PAST WEIGHT(S): _____

DIET HISTORY: _____

_____

PHYSICAL ACTIVITY / EXERCISE: _____

SOCIOECONOMIC CONSIDERATIONS: _____

I. DAILY MEAL PATTERN

BREAKFAST TIME:	LUNCH TIME:	SUPPER TIME:
SNACK (AM)	SNACK (PM)	SNACK (BS)

III. SUMMARY (from Food Intake Pattern II)

_____

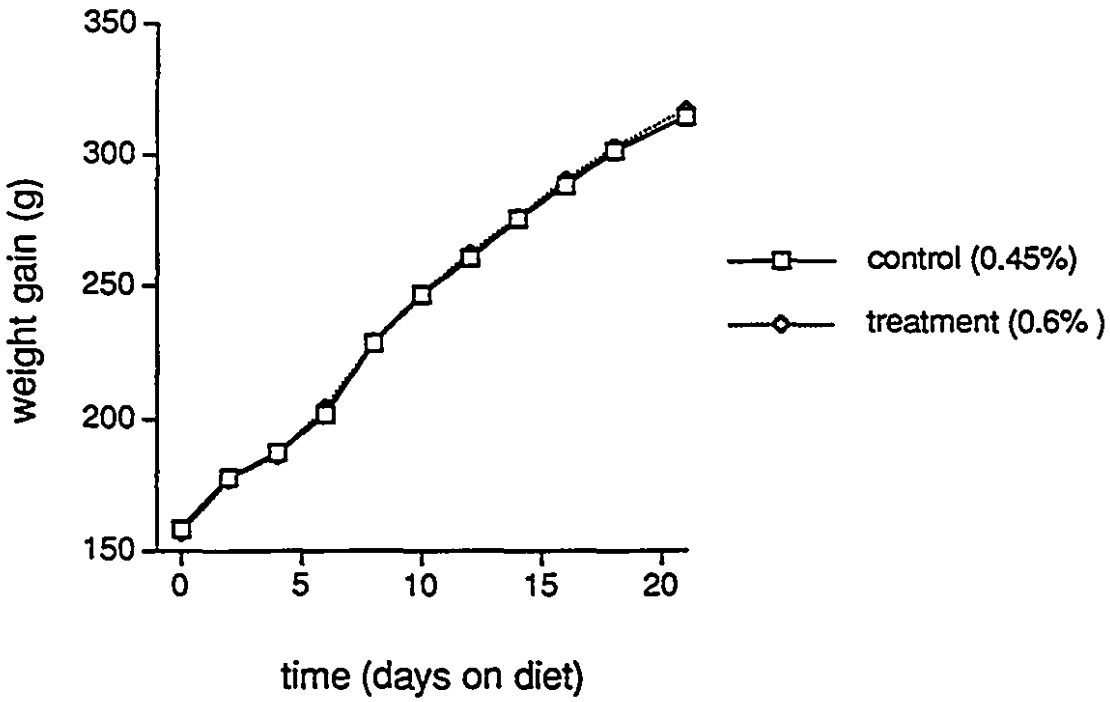
_____

_____

_____

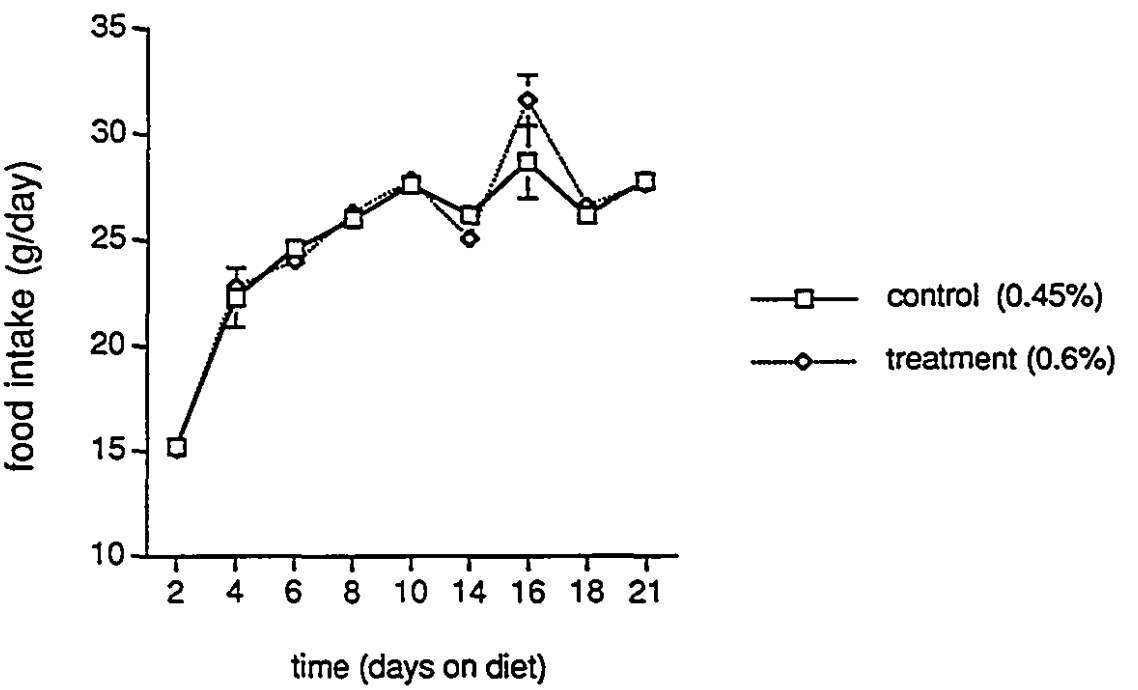
APPENDIX B

Effect of diet on weight gain



Values are mean±SEM for 18 animals. ANOVA (repeated measurement) is $F_{1,34}=0.08$, $p=0.8$

Effect of diet on food intake



Values are means±SEM for 9 animals. ANOVA (repeated measurement) is $F_{1,16}=0.18$, $p=0.7$