DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date APR - 6 1998

From Acting Director, Division of Programs and Enforcement Policy, Office of Special Nutritionals, HFS-455

Subject 75-Day Premarket Notification for New Dietary Ingredients

To Dockets Management Branch, HFS-305

New Dietary Ingredient:	Methyltetrahydrofolate (5-MTHF)
Firm:	General Nutrition Corporation
Date Received by FDA:	February 26, 1998
90-Day Date:	May 27, 1998

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after May 27, 1998.

Sincerely yours,

James Tanner

James Tanner, Ph.D.
Acting Director,
Division of Programs and
Enforcement Policy
Office of Special Nutritionals
Center for Food Safety and
Applied Nutrition

Attachment

cc:
HFS-22, CCO
HFS-450 (r/f, OSN w/control slip:TRAC#57386 & cpy incoming)
HFS-456 (r/f, Latham, Moore)
r/d:HFS-456:JELatham:jel:03/31/98:DocName:#57386.mem:Disc4

3443 '98 APR -9 P2:11

95S-0316

RPT23

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

APR - 6 1998

Dr. John P. Troup
Vice President, Scientific Affairs
General Nutrition Corporation
300 Sixth Avenue
Pittsburgh, Pennsylvania 15222

Dear Dr. Troup:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the act) dated February 19, 1998, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., Methyltetrahydrofolate (5-MTHF)) was received by the Food and Drug Administration (FDA) on February 26, 1997. Your submission will be kept confidential for 90 days from the date of receipt, and after May 27, 1998, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

Sincerely yours,

James Tanner, Ph.D.
Acting Director
Division of Programs and
Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

cc:
HFA-224 (w/incoming)
HFS-22 (CCO)
HFS-456 (r/f, Latham, Moore)
HFS-450 (r/f, w/ control slip OSN#57386 & cpy incoming)
f/t:HFS-456:JELatham:jel:03/31/98:DocName:#57386.OSN:Disc4

57384

GNC LiveWell™

John P. Troup, Ph.D.
Vice President,
Scientific Affairs

February 19, 1998

RECEIVED 2/26/98

Linda S. Kahl, Ph.D.
Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street S.W.
Washington, DC 20204

Dear Dr. Kahl:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, General Nutrition Corporation , located at 300 Sixth Avenue, Pittsburgh, PA 15222 and BASF Corporation located at 3000 Continental Drive, North, Mount Olive, NJ 07828, wish to notify the Food and Drug Administration that it will market a new dietary ingredient, Methyltetrahydrofolate ("5-MTHF"), the active form of folate. Accordingly, enclosed please find two (2) copies of this notification.

The dietary supplement which contains 5-MTHF will consist of four hundred (400) mcg of 5-MTHF in a tablet or capsule which will be suggested to be taken one (1) time per day.

Attached please find clinical studies and other information which establish that this dietary ingredient, when used under the conditions suggested in the labeling of the dietary supplement, is reasonably expected to be safe. These supporting studies include:

(1) Chemical Pharmaceutical Data

(2) Toxicology (acute, subchronic, testology, chronic, cancerogenicity)

(3) Clinical studies

Very truly yours,

John P. Troup, Ph.D.
Vice President, Scientific Affairs

3445 '98 APR -9 P2:11

JPT/jaj

cc: Dan Patriarca

Enclosures

General Nutrition Corporation, 300 Sixth Avenue, Pittsburgh, PA 15222
Tel: (412) 338-8844 Fax: (412) 338-8954

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Role of methyltetrahydrofolate in the Central Nervous System

Transmethylation is an important process taking place in all body cells, but it seems to be of particular importance in brain tissue because of its participation in the process of neurotransmission, principally through the enzyme catechol-*O*-methyltransferase, although other methyltransferases (i.e., hydroxyindole-*O*-methyltransferase, phenylethanolamine-*N*-methyltransferase, etc.) are involved in the synthesis and metabolism of specific nervous system products.

Methyl transfer in the brain may be involved in the etiology and recovery from depression. Substances that increase the brain's capacity to transfer methyl groups may be effective antidepressants. Two compounds are available that may have the potential to increase this capacity: namely ademetionine and methyltetrahydrofolate (5-MTHF). These two physiological compounds are metabolically strictly correlated (Figure 1 page 6).

The first step in the biosynthesis of ademetionine in the nervous system is the reduction of methylene tetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). The next step involves the transfer of a methyl group from 5-MTHF to homocysteine to form methionine. The enzyme for this reaction is 5-MTHF-homocysteine-S-methyltransferase and requires vitamin B_{12} and ademetionine in catalytic amount (Spector et al. 1980).
Finally, methionine is converted to ademetionine by the enzyme methionine adenosyl transferase (MAT).

A major function of folate-ademetionine interrelations is the synthesis of methyl groups in the folate cycle which are subsequently utilized by ademetionine as the methyl donor in many methylation reactions.

The relation between folate and ademetionine metabolism in brain has been demonstrated by in vivo experiments in which rats treated with a commercial folate-deficient diet had significantly lower cerebral levels of ademetionine than those on a standard diet (Ordonez and Wurtman 1974). The administration of *L-dopa*, a methyl acceptor, lowered brain ademetionine to a greater extent in the folate-deficient than control rats.

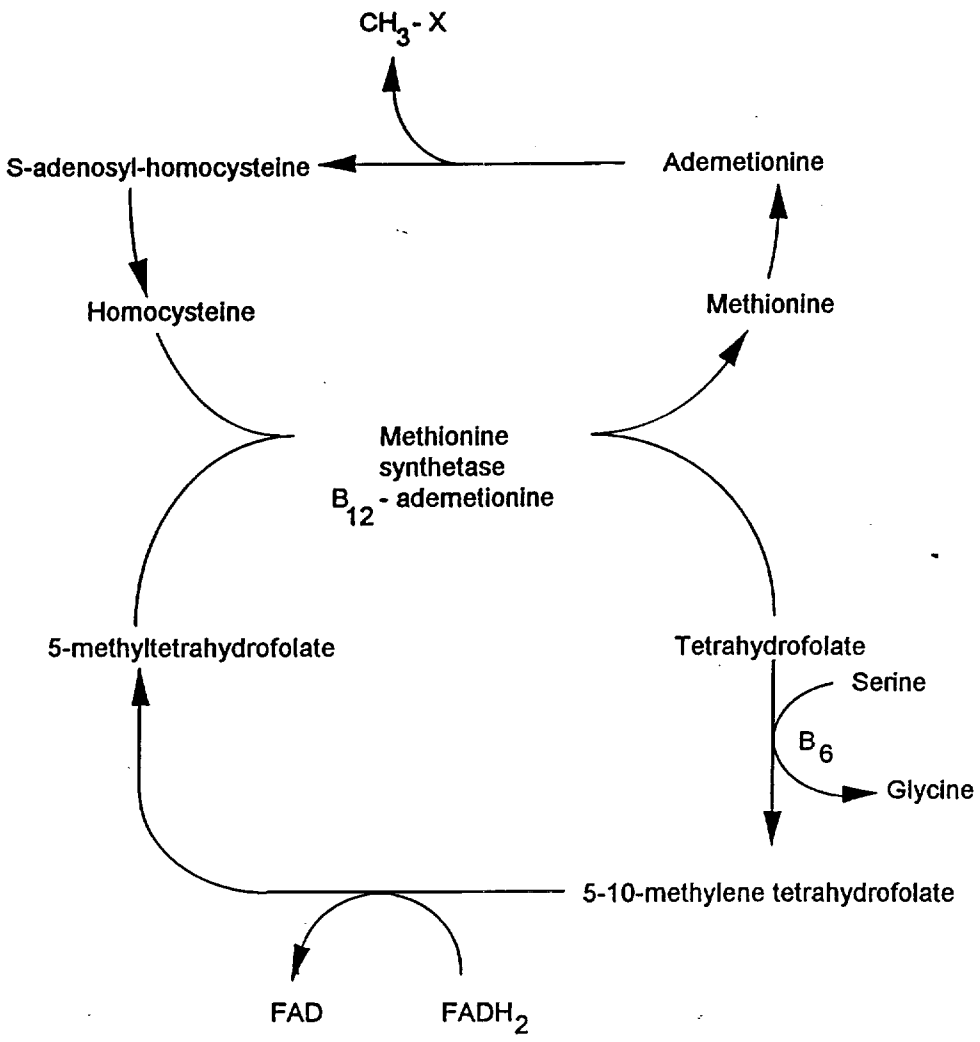


Figure 1: Relationship between folate cycle, ademetionine and transmethylation

This suggests the existence of a homeostatic mechanism in brain capable of regenerating ademetionine by the re-methylation of homocysteine when demand for methyl groups is increased by the presence of methyl acceptors such as *L-dopa*.
Folate is present in brain in high concentrations, especially in the synaptic region (McClain et al. 1975), where it may be involved in neurotransmission (Hommes et al. 1979; Brennan et al. 1981). Methyltetrahydrofolate is the form which is actively transported into the nervous system (Spector and Lorenzo 1975a), where it has been shown in man to be concentrated in the cerebrospinal fluid (CSF) (Reynolds 1979). CSF folate levels decline in the presence of folate deficiency (Reynolds et al. 1972).

In addition, there are many evidences linking depression and folate deficiency: surveys of psychiatric in-patient populations indicate that between 10 and 30% may have low serum folate levels (Carney 1967; Hällström 1969; Källström and Nylöf 1969; Reynolds et al. 1970; Reynolds 1976; Thornton and Thornton 1978). The deficiency may be associated with various diagnostic categories but it is most commonly associated with depression (Carney 1967; Reynolds et al. 1970; Reynolds 1976).
Patients with folate deficiency able to to induce megaloblastic anemia, frequently showed (56%) depression as neuropsychiatric complication (Shorvon 1980).

In a study of 100 consecutive admissions with depression low folate levels were found in 24% (Reynolds et al. 1970). The deficient patients had significantly higher depression scores, lower Marke-Nyman validity (psychiatric energy) scores and responded less well to conventional antidepressant therapy. A recent study of 107 out-patients with affective disorder attending a lithium clinic again confirmed that those with lower serum folate had a higher affective morbidity, both at the time of folate assay and during the previous two years (Coppen and Abou-Saleh 1982).

Therefore, the possibility to use 5-MTHF as a therapeutic agent in the treatment of depression is based on the following considerations:

a) 5-MTHF is the only form of folate actively transported into the CNS;
b) the metabolism of 5-MTHF is intimate correlated with the synthesis of ademetionine that itself exerts antidepressant activity (Kagan et al. 1990; Rosenbaum et al. 1990; Bell et al. 1988; Janicak et al. 1989);
c) relationship between 5-MTHF and serotonin metabolism (Botez et al. 1979);
d) association between low serum folate and depression (Carney 1967; Reynolds et al. 1970; Reynolds 1976; Shorvon 1980).

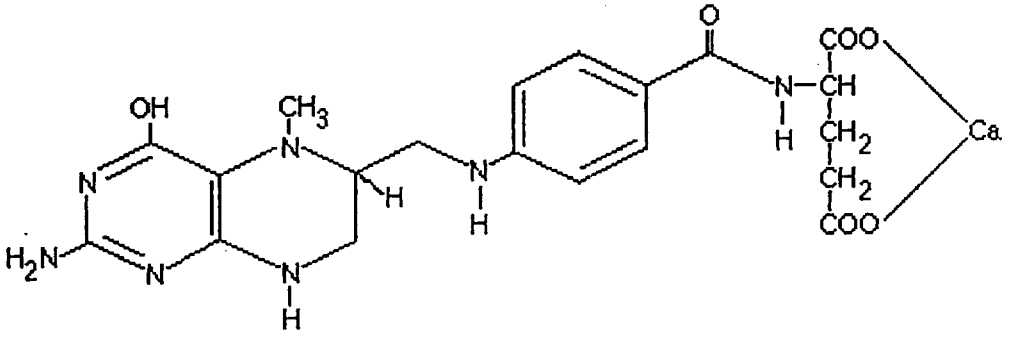
2. PHYSICOCHEMICAL PROPERTIES

5-MTHF is the only active ingredient present in the dosage forms.

2.1 Chemical name

N-[p-[[2-amino-5-methyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino] benzoyl]glutamic acid calcium salt.

2.2 Chemical structure



2.2.1 Empirical formula (as calcium salt pentahydrate)

$C_{20}H_{23}N_7O_6$ Ca. $5H_2O$

Empirical formula (as free acid)

$C_{20}H_{25}N_7O_6$

2.2.2 Molecular weight (as calcium salt pentahydrate): 587.55

Molecular weight (as free acid): 461.50

2.2.3 Synonyms of 5-MTHF

MTHF, N^5 methyltetrahydropteroylglutamic acid, N^5 methyltetrahydrofolic acid, methylfolate.

2.3 Manufacturing (active ingredient)

5-MTHF is produced by chemical synthesis starting from folic acid and recovered as calcium salt after recrystallization from water.

2.4 Properties

2.4.1 Appearance

5-MTHF is a crystalline, odourless white-cream powder.

2.4.2 Stability, pH

5-MTHF is soluble in acid and basic solutions, sparingly soluble in water at pH 7 (about 3 g/l at room temperature) and insoluble in organic solvents.

2.4.3 Purity

Purity (as salt on dry substance) is not less than 98%.
Impurities: the principal impurities are:
- folic acid
- dihydrofolic acid
- tetrahydrofolic acid
- 5, 10 methylentetrahydrofolic acid

2.4.4 Isomerism

5-MTHF is available as racemic mixture containing 50% each of the two 6(R) and 6(S) isomers.

2.5 Dosage forms

2.5.1 5-MTHF racemate: oral form

- 15 mg gastroresistant tablets

2.5.2 Stability

According to the available stability data, the dosage forms containing 5-MTHF are stable for at least three years if stored in the original blister.
We recommend to open the blister only immediately before use.

3. PHARMACOLOGY

3.1 General pharmacology

General pharmacological studies with 5-MTHF were carried out 'in vivo' and 'in vitro' (Data on BioResearch file). In the "in vivo" studies the substance was administered to anaesthetized animals by oral and/or systemic route.

3.1.1 'In Vivo' studies

These studies were carried out to evaluate the influence of 5-MTHF on some neurovegetative functions.

EFFECT ON THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM IN MICE

The following parameters were evaluated:

- Behaviour modification (according to Irvin's method) by oral and intraperitoneal route of 25, 50, 100, 200, 400 and 800 mg/kg.
- Anti-metrazole activity, evaluated against extensor tonic convulsions induced by i.p. metrazole (130 mg/kg) administered after the doses of 50 mg/kg by oral route and 100 mg/kg by s.c. route of 5-MTHF.
- Anti-amphetamine activity, evaluated against d-amphetamine (by i.p. route of 10 mg/kg) administered after 5-MTHF at the doses of 50 and 100 mg/kg by oral and s.c. administration respectively.
- Anti-reserpine activity, evaluated against reserpine (by i.p. route 2.5 mg/kg). 5-MTHF was tested by i.p. and s.c. at the doses of 50 and 100 mg/kg respectively.

The results obtained by the behavioural modification test showed that the oral administration of 5-MTHF was well tolerated up to the highest dose. After i.p. administration, 5-MTHF induced a slight general depression (reduction of spontaneous activity, muscular tone, reflexes, pain reaction) at the doses of 400 and 800 mg/kg.

These symptoms probably were caused by a peritoneal mucosae irritation dose-related.
These symptoms were documented by abdominal pain associated to an increased defecation, and this could also depend to an increase of intestinal peristalsis but with absence of concomitant signs of cholinergic stimulation.
Regarding the pharmacological interaction, 5-MTHF resulted to be free from any anti-metrazole activity. The amphetaminic and reserpinic activities were slightly worsened possibly due to the type of injection adopted (i.p. route).
The s.c. administration of 100 mg/kg of 5-MTHF was also uneffective to counteract symptoms induced by metrazole, amphetamine or reserpine.

EVALUATION OF NEUROVEGETATIVE FUNCTION IN ANAESTHETIZED CATS
Cardiovascular parameters, ganglionic and muscular transmission, tone and motility of the uterus and of the intestinal tract were observed.
The test compound was injected into the femoral vein at the ranging doses of 5 to 20 mg/kg.
From the analysis of the data, the i.v. administration of 5-MTHF up to the dose of 20 mg/kg does not modify any parameters investigated.

3.1.2 'In Vitro' studies

The following isolated organs were prepared in order to find out a specific indication of 5-MTHF activity:
- rabbit jejunum
- rat stomach 'in toto'
- guinea-pig gall-bladder
- rat uterus
- guinea-pig trachea
- rat mesenteric and guinea-pig portal vein
- rat isolated and perfused tail artery
- rabbit and guinea-pig atria.

5-MTHF was added into the bath solution at the concentration ranging from 50 to 200 µg/ml.
The results obtained from the 'in vitro' preparations confirm that 5-MTHF does not exert any specific and selective actions on any particular organs nor interferes in their normal function.

Regarding to the spasmolitic activity "in vitro", two experiments were performed: the guinea-pig trachea was prepared according to the common method of the spiral-shaped cut and to the Coleman's method. 5-MTHF does not exert any relaxant or spasmoltic effects on the muscle preparation whose tone was enhanced by the addition of carbachol into the bath and it does not alter biphasic response of the tracheal smooth muscles to electic stimulation.

These pharmacological investigations, which can also be considered test of tolerance to the substance demonstrated that 5-MTHF has not particular side-effects which might affect the neurovegetative functions.

4. PRECLINICAL PHARMACOKINETICS

4.1 Absorption

Intestinal absorption of 5-MTHF occurs through carrier-mediated, diffusive, and solvent drag processes, mainly occurring in the small intestine (Sehlub et al. 1984; Blair et al. 1975; Said and Strum 1983).

After oral administration of 5 and 25 mg/kg of labelled [methyl-^{14}C] 5-MTHF, only 18-30% of the applied radioactivity was found in faeces, indicating a good intestinal absorption of the compound (Strum and Hanstein 1978).
In the same experiment, radioactivity in urines was found to be mainly associated with the unmodified compound and accounted for 14-23% of the applied dose.

Similar results were found in studies performed in rats orally treated with unlabelled racemic 5-MTHF at a 10 mg/kg dose, in which the urinary excretion of the substance accounted for 23±4% (mean ± S.D.; n=4) of the applied dose (Data on Knoll Framaceutical file)

By comparison of these urinary excretions with those observed after i.v. administration, the systemic availability of racemic 5-MTHF in rats was estimated to be 35%.

Oral administration of high, increasing toxicological doses of the compound (40, 120, 360 mg/kg), resulted in a less than proportional increase of the plasma concentrations: areas under the plasma curves (AUC) at 360 mg/kg resulted to be 270±40 mg.min/l in comparison with 90±18 mg.min/l found at the 40 mg/kg dose (Data on Knoll Farmaceutici file). This finding could be explained with the saturation, at high doses, of the carrier-mediated absorption processes.

The intramuscular absorption of 5-MTHF was studied in dogs by comparison of the area under the plasma curves observed after i.m. and i.v. administration of 1 mg/kg of the drug and a 83% systemic availability was estimated.

4.2 Distribution

Studies performed with the labelled [methyl-^{14}C] 5-MTHF at 1 mM concentration, showed that the compound is 60-80% bound to plasma proteins, mainly albumin (Strum and Hanstein 1978).

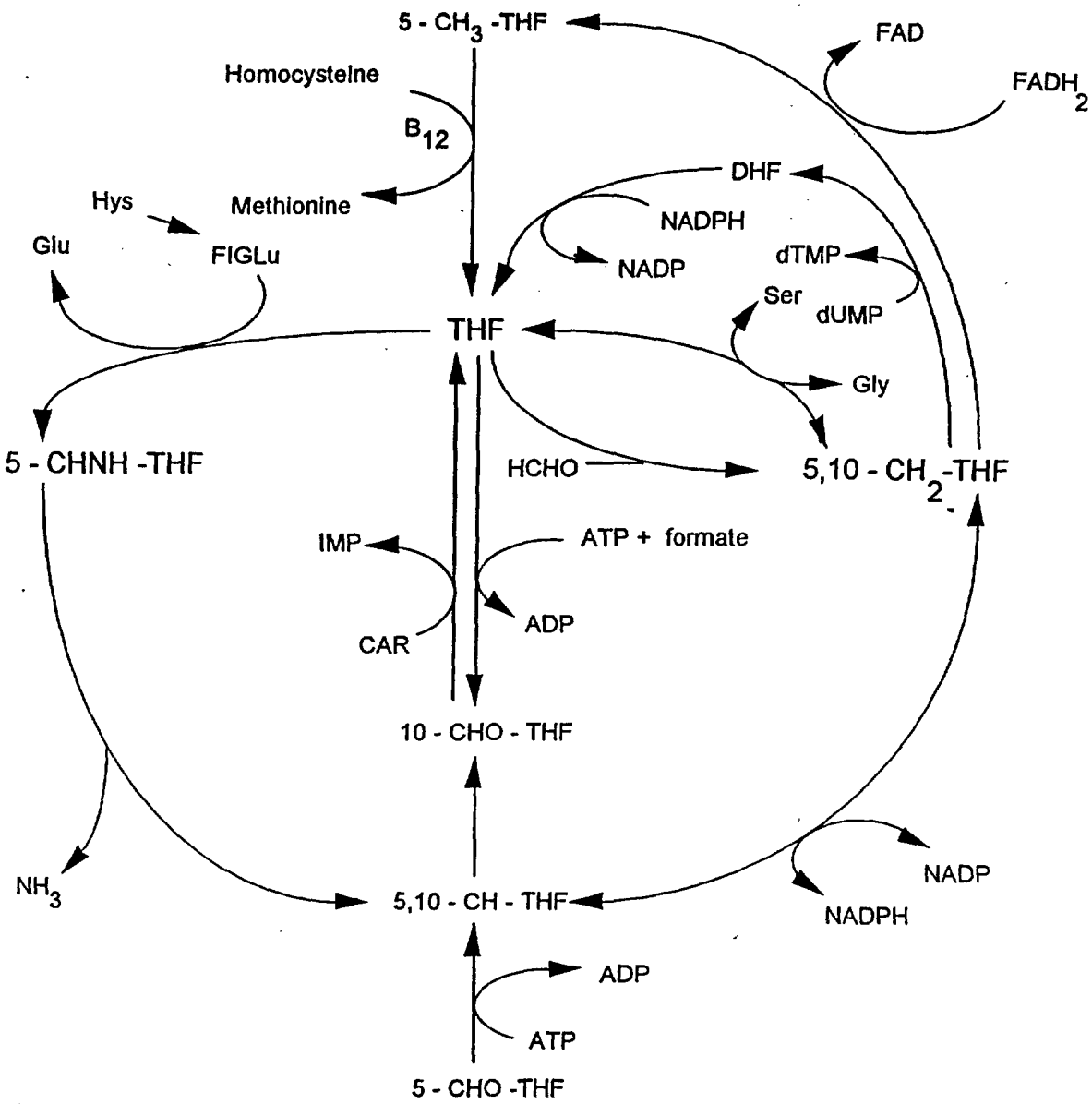
Several "in vitro" studies showed that 5-MTHF is taken up by different cell types. Carrier-mediated transport systems for 5-MTHF have been found in hepatocytes (Horne et al., 1978) erithrocytes (Branda et al. 1978) and proximal tubular cells (McMartin et al. 1992).

With "in vivo" experiments in dogs after intravenous administration of 1.0 and 10 mg/kg of the racemic drug, apparent volumes of distribution of the 5-MTHF diastereomers were estimated to be 564 and 489 ml/kg bw for the 6(S) 5-MTHF, and 463 and 441 ml/kg bw for the 6(R) 5-MTHF (Data on Knoll Farmaceutici file).

Experiments performed in dogs after intravenous administration of 25 and 50 mg/kg of doubly labelled [methyl-^{14}C; 9, 3', 5' ^{3}H] 5-MTHF, showed that 5-MTHF enters the CNS, reaching the equilibrium between plasma and cerebrospinal fluid (CSF) within 3 hours. Unlike 5-MTHF, the administration of folic acid and of formyltetrahydrofolic acid did not give rise to the appearance of these compounds in the CSF, thus suggesting that the relevant transport systems preferentially take up 5-MTHF and that the other folates have to be converted to this compound before uptake (Levitt et al. 1971).

4.3 Metabolism

5-MTHF metabolism is known to occur mainly through the methylation of Vitamin B_{12} as an intermediate step in the synthesis of methionine from homocysteine: this reaction leds to the formation of tetrahydrofolate, a key compound for the synthesis of purine and pyrimidine (Figure 2 page 16).
5-MTHF, as well as the other folate coenzymes, are stored in the cells as polyglutamates.



CH- = methenyl- ; CHO- = formyl; CHNH- = Formimino; THF = tetrahydrofolate; DHF = dihydrofolate

Figure 2: Reduced folates cycle. Adapted from Kisliuk (1984)

4.4 Elimination

5-MTHF is eliminated from the body by metabolism and by renal excretion. Biliary excretion has also been found for the compound (Strum and Liem, 1980).

Faecal elimination of radioactivity after the intravenous administration of 5 and 25 mg/kg of labelled [methyl-^{14}C]5-MTHF, accounted for only the 4-7% of the applied radioactivity, thus indicating an extensive intestinal reabsorption of the compound excreted with the bile (Strum and Hanstein 1978).

Plasma half-times of 5-MTHF in rats intravenously treated with 1mg/kg resulted to be 165 minutes (Data on Knoll Farmaceutici file).

Studies were performed in dogs aiming the plasma kinetics of the 6(S) and 6(R) 5-MTHF diastereomers after the intravenous administration of 1 and 10 mg of the racemic 5-MTHF per kg body weight.
For the 6(S) 5-MTHF half-life resulted to be 93±49 and 92±22 min (mean ± S.D., n=5) at the 1 and 10 mg/kg doses, for the 6(R) 5-MTHF half-life were 105±18 and 106±11 min at lower and higher dose, respectively (Data on Knoll Farmaceutici file).

Urinary excretion of 5-MTHF was studied in rats after intravenous administration of the substance at the dose of 1mg/kg bw and accounted for the 65±5% (mean ± S.D.; n=4) of the applied dose.
of the applied dose.
After i.v. administration of 5 and 25 µg/kg of labelled [methyl-^{14}C] 5-MTHF, 61-71% of the applied radioactivity was found in urines, mainly as unmodified compound (Strum and Hanstein 1978).

5. TOXICOLOGY

The toxicological studies on 5-MTHF were performed in different animal species legally recommended and commonly utilized in preclinical research laboratories and include mutagenicity investigation as well. All the studies were performed in compliance with Good Laboratory Practice (GLP).

The groups and the number of animals per group were sufficient to allow a complete statistical evaluation of the results obtained through the assessment of the toxic effects.

Table I Summary of toxicological studies on 5-MTHF racemate.

Type of study	Species	Route	Duration	Doses (mg/kg)
Acute	Rat	os	1 day (single dose)	5000
Acute	Rat	i.p.	1 day (single dose)	690-985-1400-2000
Subchronic	Rat	os	4 weeks	0-40-120-360
Subchronic	Dog	os	4 weeks	0-40-120-360 (as suspended powder) 120 (as enteric coated tablets)
Chronic	Rat	os	26 weeks	0-40-120-360
Chronic	Dog	os	26 weeks	0-40-120-360
Fertility	Rat	os	males from Day 60 a.c.- females from Day 14 a.c. up to 21 p.p.	0-20-60-180 0-40-120-360
Embryotoxicity	Rat	os	Day 6-15	
Embryotoxicity	Rabbit	os	day 6-18	0-50-150-450
Peri-post natal	Rat	os	From Day 15 p.c. to Day 21 p.p.	0-50-150-450 0-40-120-360
Mutagenicity	- Ames Test - Chromosomal Aberration - Gene mutation - Unscheduled DNA synthesis - Micronucleus test (rat bone marrow i.p. 700 mg/kg)			

os = oral; i.p. = intraperitonal
a.c. = ante coitum; i.p. = intraperitonal; p.c. = post coitum; p.p. = post partum

5.1 Single Dose Toxicity

The single dose toxicity studies were performed in rats, both sexes. The administration routes were: oral and intraperitoneal. Changes related to sex were not observed.

Overview:

Animal species	Route of administration	LD_{50} (mg/kg) (male+female)
Rat	os	> 5000
Rat	i.p.	1049 (917-1303)

5.1.1 Oral administration/male and female rats/single dose (1 day)

RBM Study Code: 890566 (Data on Knoll Farmaceutici file)

No mortality occurred in rats treated at the dosage of 5000 mg/kg; therefore, the LD_{50} was considered to be higher than 5000 mg/kg.

The main clinical signs observed related to a respiratory distress were: shallow breathing, decrease of respiratory rate, nasal discharge and cyanosis.
The onset of the above sign was within 2-4 hours; all the animals achieved a recovery within 24 hours.

No alterations were detected in animals killed at the end of the 14-day observation period.

5.1.2 Intraperitoneal administration/male and female rats/single dose (1 day)

RBM Study Code: 890566 (Data on Knoll Farmaceutici file)

The LD_{50} was calculated to be 1094 mg/kg with 95% confidence limits of 917-1303 mg/kg.

Besides the same signs recorded in rats treated by oral route, sedation, muscular hypotonia, ataxia, kyphosis and abdominal dilatation (with frequency and appearance times varying among groups) were the more frequently observed clinical modifications.

The onset of the overall signs was within 30 minutes-4 hours and the surviving rats were normal within 2-5 days.

No important change in the body weight gain was recorded in surviving rats treated at 985 and 1400 mg/kg.

The gross pathology examination performed on dying animals of all dosage groups, showed high occurrance of changes (mainly congestion) in the gastro-enteric tract, lung and kidney. These changes were partially related to agonal or post-mortal changes.

Changes in the organs of the abdominal cavity found at the autoptic examination performed on died and surviving animals were probably attributable to the irritable property of 5-MTHF given by intraperitoneal route.

5.2 Repeated Dose Toxicity

5.2.1 Subchronic toxicity

5.2.1.1 Oral administration/male and female rats/4 weeks

Knoll Farmaceutici Study Code: 02/88 (Data on Knoll Farmaceutici file)

5-MTHF was administered to Sprague-Dawley rats by oral route at the doses of 0, 40 ,120 and 360 mg/kg/day for 4 consecutive weeks.
From routine examinations (clinical observations, laboratory investigations and post-mortem examinations) no changes were observed in the treated animals.
5-MTHF was considered well tolerated by rats up to and including the highest dose level tested of 360 mg/kg/day.

5.2.1.2 Oral administration/male and female dogs/4 weeks

RBM Study Code: 880317 (Data on Knoll Farmaceutici file)

Beagle dogs were treated by oral route with doses of 0, 40, 120, 360 mg/kg/day of 5-MTHF as suspended powder.
An additional group of animals was treated with 5-MTHF at the dosage of 120 mg/kg as enteric coated tablets.
No animal died during the study.

From the examinations carried out according to the protocol (clinical observation, laboratory investigations and post-mortem examinations) no treatment-related changes were observed with the exception of a decrease in food consumption (more evident during the last week of dosing) noted in the animals treated with the dose of 360 mg/kg/day but this change was not accompained by important modifications in body weight gain.

5.3 Chronic Toxicity

5.3.1 Oral administration/male and female rats/26 weeks

RBM Study Code: 880353 (Data on Knoll Farmaceutici file)

5-MTHF was administered to rats by oral route at the doses of 0, 40, 120 and 360 mg/kg/day.

Neither deaths, no clinical signs, no ophthalmoscopic alterations or haematological changes related to the treatment occurred during the 26-week dosing period.

A slight dosage-related body weight gain decrease was seen in the second half of the treatment period in males at the doses of 120 and 360 mg/kg/day; at the highest dose this was concomitant to a tendency to consume less food.

At laboratoy investigations, the only parameters with slight dosage-related changes were: increase of the serum glucose level at the end of treatment in both sexes treated with 360 mg/kg/day; slight reduction of the serum organic phosphorus at the end of treatment in both sexes dosed with 120 and 360 mg/kg/day; slight decrease at the end of treatment of the GOT serum activity in females treated with 120 or 360 mg/kg/day; slight decrease of mean pH values of urines in males dosed with 360 mg/kg/day at the end of treatment.

The post-mortem examinations do not show any drug-related changes in organ weight, in gross pathology and at the histological level.

5.3.2 Oral administration/male and female dogs/26 weeks

RBM Study Code: 880318 (Data on Knoll Farmaceutici file)

The Beagle dogs were treated with 5-MTHF at the doses of 0, 20, 60 and 180 mg/kg/day over a period of 26 weeks.

No animals died during the course of the study.

The various evaluated parameters (clinical observations, laboratory investigations and post-mortem examinations) did not reveal major differences from controls in any treated groups.
From histological point of view, none of the observed pathological changes are regarded as associated with treatment.
A range of degenerative or inflammatory lesions was observed in the organs examined. They were considered incidental spontaneous pathology, as they were not dose-correlated and all of them are characteristic for control Beagle dogs of this age.

5.4 Reproduction Toxicity

5.4.1 Fertility and reproduction

5.4.1.1 Oral administration/male and female rats

RBM Study Code: 880322 (Data on Knoll Farmaceutici file)

Male and female rats were treated with 0, 40, 120 and 360 mg/kg/day by oral route.
5-MTHF was administered daily to the F0 males from Day 60 prior to the mating phase until the end of this phase and to the F0 females for 14 days before the start of the mating period and throughout the same. Treatment continued during gestation up until Day 19 for the females killed on Day 20 of gestation and until the end of lactation (day 21 of lactation) for the remaining females.

No clinical signs, behavioural changes, effects on parturition and deaths were seen at any dosage.
The mating or fertility indices were not affected by the 5-MTHF administration.
A higher body weight gain was observed in females treated with 120 and 360 mg/kg/day during the second week of pre-mating period; a slight increases in the body weight gain was observed in the lactation period at 360 mg/kg/day.

No embryotoxic effects were found at any dosage.
No variations in morphological and physical development were observed in the treated groups.
No effects were found in motor coordination after the lactation period.

The post-natal survival of the F1 generation was similar in all experimental groups; only a slightly lower probability of survival was observed in the F2 generation belonging to the 360 mg/kg/day group.
Nevertheless, these values are not infrequent in this strain of rat.

No important effects were noted on the body weight gain of F1 and F2 generations.

The gross pathology observation carried out on all animals and the organ weight (testes and ovaries) did not show any findings which could be attributed to the administration of 5-MTHF.

5.4.2 Embryotoxicity

5.4.2.1 Oral administration/female rats

RBM Study Code: 880319 (Data on Knoll Farmaceutici file)

Female rats were treated with 5-MTHF from Day 6 up to Day 15 of pregnancy at the dosage of 0, 50, 150 and 450 mg/kg/day by oral route.
No changes were observed in any treated groups.

No differences were observed between control and treated groups in post-implantation losses, gravid uterus weight, mean number of viable foetuses per litter, foetal weight, litter weight or placental weight.

In conclusion, under the experimental conditions adopted and in the species used, no toxic effects on the dams and their conceptuses or teratogenic effects were observed at the dosages used.

5.4.2.2 Oral administration/female rabbits

RBM Study Code: 880320 (Data on Knoll Farmaceutici file)

New Zealand White gravid female rabbits were treated with 5-MTHF by oral route from Day 6 up to Day 18 of pregnancy with the doses of 0, 50, 150 and 450 mg/kg/day.
No clinical signs or behavioural changes were observed in any experimental group during the study.

Some accidental deaths were found in all experimental groups.

A lower body weight gain was observed in the 450 mg/kg/day groups during the treatment period.

Two does treated with 150 mg/kg/day aborted on day 28 of gestation; these abortions could not be definitely related to 5-MTHF, since no abortions were found at the maximum dosage (450 mg/kg/day).

The skeletal anomalies and variants were similar in all experimental groups.
The visceral examination did not show any malformation, anomaly or variant.
The increase of the post-implantation losses in the 150 mg/kg/day group was related exclusively to the two abortions found in this group.
The malformations at the level of limbs were found only in one litter of the 150 mg/kg/day group, consequently they could not be related to the substance.

5.4.3 Peri/Postnatal Toxicity

5.4.3.1 Oral administration/female rats

RBM Study Code: 880321 (Data on Knoll Farmaceutici file)

5-MTHF was administered by oral route to female rats from Day 15 of pregnancy to Day 21 of lactation at the dosage of 0, 40, 120 and 360 mg/kg/day.
In the dams, no important effect on the body weight of the treated groups was observed during the gestation period. During the lactation period, a slight lower body weight gain was observed in the group treated with 360 mg/kg/day.

In the pups, there were no effects on the body weight during lactation and during the post-lactation period.

No effects on morphological, physical and behavioural development were observed.

5.5 Mutagenic Toxicity

5.5.1 'In Vitro' Studies

5.5.1.1 Ames Test/Salmonella typhimurium

RBM Study Code: 880323 (Data on Knoll Farmaceutici file)

The Ames test was performed on TA 1535, TA 1537, TA 1538, TA 98 and TA 100 Salmonella typhimurium strains in a duplicate experiment.
The dosage levels expressed as quantities of 5-MTHF per plate were: 1, 10, 100, 1000 and 2000 µg/plate.

5-MTHF did not induce any significant increase in the number of reversions, both in absence and in presence of metabolic activation, up to the concentration of 2000 µg/plate.

5.5.1.2 Chromosomal aberrations/Human lymphocytes culture

RBM Study Code: 880326 (Data on Knoll Farmaceutici file)

Chromosomal aberration in human lymphocytes cultures were tested with 1, 10, 100 and 800 µg/ml both in absence and in presence of metabolic activation.
5-MTHF does not induce a statistically significant increase of chromosome aberrations.

5.5.1.3 Gene mutation/V79 cells

RBM Study Code: 880324 (Data on Knoll Farmaceutici file)

The V79 cells were exposed at the following concentrations in a duplicate experiment: 10, 100, 500 and 1000 µg/ml both in absence and in presence of hepatic microsomal enzymes. 5-MTHF did not induce any significant increase of gene mutation (HGPRT-frequency) in V79 Chinese hamster lung cells up to the dosage level of 1000 µg/ml.

5.5.1.4 Unscheduled DNA synthesis/HeLa cell culture

RBM Study Code: 880325 (Data on Knoll Farmaceutici file)

The unscheduled DNA synthesis in HeLa cell cultures were performed with 1, 10, 100 and 2000 µg/ml of 5-MTHF in absence and in presence of metabolic activation.

5-MTHF did not induce statistically significant increases in incorporation of tritiated thymidine in presence of hydroxyurea in a duplicate experiment.

5.5.2 'In Vivo' Studies

5.5.2.1 Micronucleus Test/intraperitoneal administration/rat bone marrow

RBM Study Code: 880327 (Data on Knoll Farmaceutici file)

The micronucleus induction in bone marrow cells of rats treated intraperitoneally with 700 mg/kg of 5-MTHF was performed using Mitomycin C as the positive control.

The results of this study indicate that 5-MTHF did not induce any statistically significant increase in the frequency of micronucleated cells in the bone marrow after 17, 42 and 65 hours from the administration.

5.5.3 Conclusion

The results of all five test systems indicate that 5-MTHF has not genotoxic effect.

6. CLINICAL PHARMACOLOGY

6.1 Clinical pharmacokinetics

6.1.1 Absorption

Oral bioavailability was studied in 8 healthy volunteers each receiving 15 mg of 5-MTHF racemate.
The plasma concentrations of 6(S) and 6(R) 5-MTHF were determined and compared with those obtained after an equal intravenous dose, in a cross-over experiment.
Plasma concentrations of both diastereomers reached a peak within 2 and 3 hours after the oral administration. Systemic availabilities resulted to be 51 and 54% for the 6(R) and the 6(S) diastereomers (Data on Knoll Farmaceutici file).
At low doses, the transport system seemed to be more stereospecific: Weir et al. (1973) gave in a separate administration 0.3 mg of 6(S) and 6(R) [U-^{3}H] 5-MTHF and, with a "flushing" technique, showed a preferential (twice) intestinal absorption of the natural 6(S) diastereomer.

6.1.2 Distribution

Plasma protein binding studies showed that 5-MTHF is 70% bound to plasma proteins (Spector et al. 1975b). Albumin seems to be the predominant binder, however, α_2-macroglobulin and transferrin have also been reported to bind physiological concentrations of 5-MTHF (Markkanen et al. 1972).

Apparent volumes of distribution of the 6(S) and 6(R) 5-MTHF diastereomers were estimated in healthy volunteers after intravenous administration of 15 mg of the racemic 5-MTHF and resulted to be 13±2.3 and 9.8±1.4 l (mean ± S.D, n=8) for the 6(S) and the 6(R) 5-MTHF, respectively.

6.1.3 Plasma kinetics

Plasma kinetics of 5-MTHF diastereomers was studied in healthy volunteers receiving 15 mg of the racemic drug by intravenous bolus administration.
5-MTHF disappeared from the plasma with a bioexponential decay profile: half-life of the terminal phase resulted to be 1.7±0.3 hours for the 6(S) and 5.5±0.3 hours for

the 6(R) diastereomers (n=8), respectively. In the same study, total body clearances resulted to be 88±12 and 20±4 ml min^{-1} for 6(S) and 6(R)5-MTHF.

The increase of the dose to 50 mg of racemic 5-MTHF did not affect the apparent half-life resulting to be 1.7±0.3 and 5.9±0.6 hour (n=4) for the two diastereomers. (Data on Knoll Farmaceutici file).

6.1.4 Metabolism and excretion

A preliminary study in healthy volunteers receiving 15 mg of 5-MTHF by intramuscular route showed that 48±5% (n=4) of the applied drug was recovered in the 24-hour urines.

6.2 Clinical trials

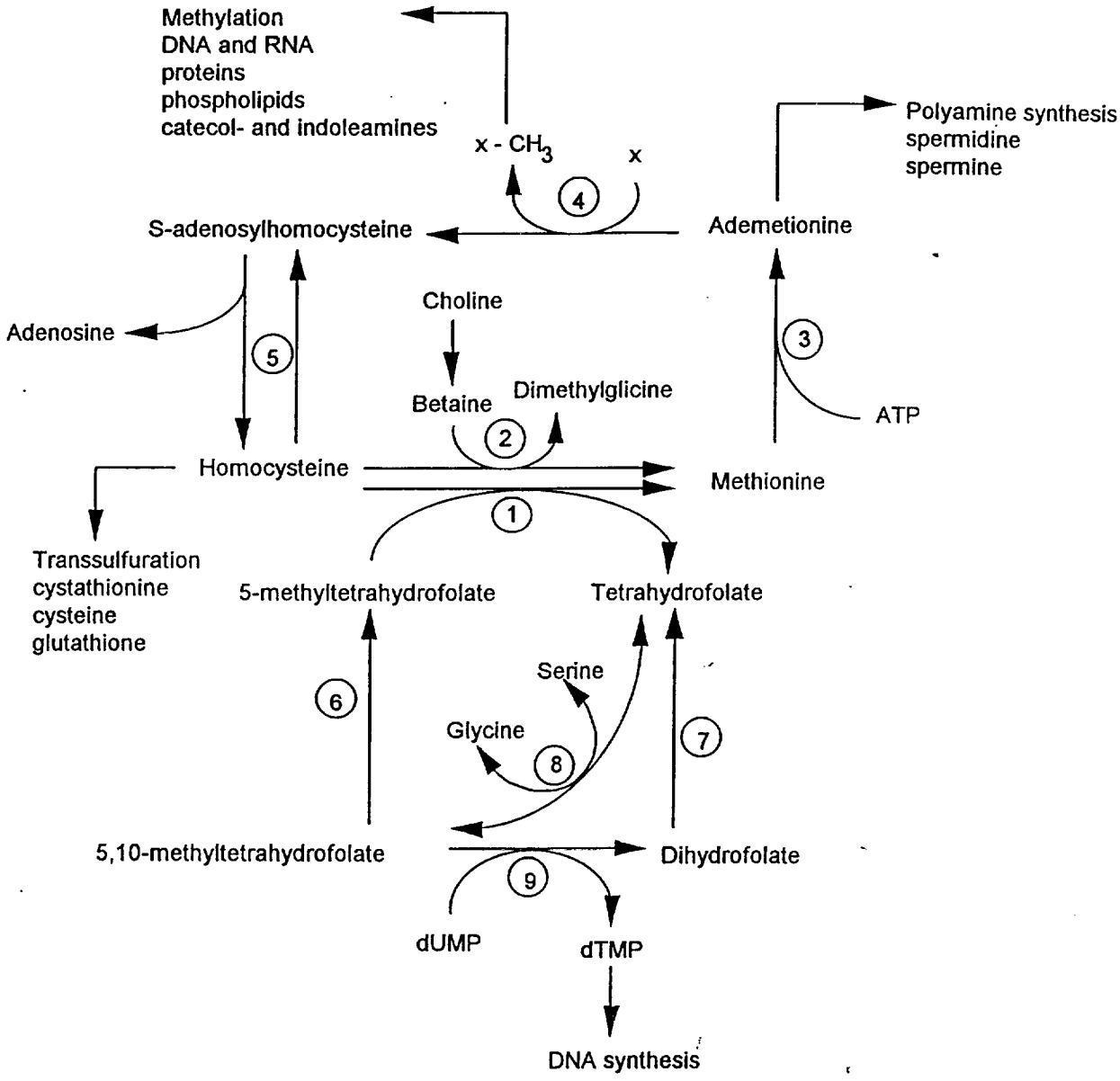
6.2.1 Introduction

Several independent lines of evidence indicate that methylation, a biochemical process required by every living cell, may be an important factor underlying the etiology of various neurological and psychiatric illnesses. The major methyl donor in methylation reactions is ademetionine, the metabolism of which is intimately linked with the folate one-carbon cycle and vitamin B_{12} metabolism. It has been known for some time that deficiencies in either folate or vitamin B_{12} (also known as cobalamin) can result in a number of neurological and psychiatric sequelae, including depression, dementia, and demyelinating myelopathy (Scott 1992).

The pivotal reaction that links ademetionine, folate, and vitamin B_{12} metabolism is catalysed by the enzyme methionine synthetase (Figure 3 page 29). In this reaction, the methyl group from 5-methyltetrahydrofolate is transferred to homocysteine to form methionine and tetrahydrofolate. The "de novo" synthesis of methionine requires vitamin B_{12}, which is involved directly in the transfer of the methyl group to homocysteine. An alternative route for the synthesis of methionine is via the betaine: homocysteine methyl transferase reaction. This reaction does not require vitamin B_{12} and is not a de novo synthesis route, as labile methyl groups supplied in the diet as choline are converted to betaine and then transferred to homocysteine (Figure 3 page 29). There is an essential and important difference between peripheral and

central nervous system (CNS) tissue in which the enzyme betaine: homocysteine methyltransferase is absent in the latter (Reynolds 1985).

5-MTHF is the form which is actively transported in the CNS, where it has been shown in man to be concentrated in the Cerebrospinal Fluid (CSF). CSF folate levels decline in presence of folate deficiency (Korevaar et al. 1973).



1 = methionine synthetase; 2 = betaine: homocysteine-S-methyltransferase; 3 = methionine adenosyltransferase; 4 = x-methyltransferase; 5 = S-adenosylhomocysteine hydrolase; 6 = 5,10-methylenetetrahydrofolate reductase; 7 = dihydrofolate reductase; 8 = serine hydroxy methyltransferase; 9 = thymidylate synthetase

Figure 3: Relationship between the folate one-carbon cycle and ademetionine metabolism.

Folate is present in brain in high concentration, especially in the synaptic region and, in the rat brain, in the regions in which serotoninergic pathways are present (Korevaar et al. 1973).
In animal model there is the evidence that experimental folate deficiency can result in impaired CNS serotonin (5-HT) activity. Furthermore, in children with severe folate deficiencies due to a congenital deficiency of MTHF reductase, CSF 5-hidroxyindole acetic acid (5-HIAA) levels also were reduced (Carney 1979).
Low CSF concentrations of 5-HIAA the metabolite of 5-HT that reflects global CNS tissue levels have been reported in patients with folate deficiency associated with neuropsychiatric disorders and depression.

The mechanism of the influence of folate on serotonergic function is unknown. A possible mechanism may be through a link between folate and tetrahydrobiopterin (BH_4) metabolism. BH_4 is a cofactor required in the synthesis of monoamine neurotransmitters: there are structural similarities between folates and BH_4, as both contain a pteridine moiety. Furthermore, the folate enzymes 5-MTHF folate reductase and dihydrofolate reductase have been postulated to be involved in BH_4 metabolism (Bottiglieri et al. 1992).

6.2.2. Folate deficiency and depression: clinical studies

The first major study of the incidence of folate deficiency in psychiatric patients was described by Carney (1967) who measured serum folate levels in 423 patients admitted to a psychiatric ward.

These authors established that a high incidence of folate deficiency occurs in patients with depression (29% to 35%), organic psychosis (24%), and schizophrenia (20%). Between 1967 and 1990, there have been 21 surveys of folate deficiency in psychiatric patients; 16 studied patients with depression, mania, schizophrenia, or various other diagnoses (including personality disorders), and 5 studied psychogeriatric patients.

These studies have been the subject of an extensive review (Carney 1978). The most consistent and remarkable finding from all of the above studies is a high incidence of folate deficiency, ranging between 25% and 35% in patients with depression and schizophrenia and between 12% and 36% in psychogeriatric patients. In all studies, serum folate was used to determine the status of the patients. While serum folate was used as a measure of deficiency for many years,

fluctuations in daily dietary intake will rapidly affect serum folate levels and may obscure the true status of the patient. Red cell folate is now regarded as a much better indicator of folate status and more accurately reflects tissue stores of the vitamin (Shorvon et al. 1980). The most recent and largest detailed survey of red cell folate status in psychiatric patients reported by Carney (1990) and coworkers showed that folate deficiency was highest in endogenous (20%) and neurotic (11%) depression and in alcoholic patients (13%). The percentage of patients with borderline deficiency was much higher in endogenous (39%) and neurotic (37%) depression, rates similar to that found in alcoholic patients (40%) in the same study. These findings corroborate previous studies of the incidence of folate deficiency in depression based on serum folate determinations.

Anticonvulsants decrease absorption of folate from the gastrointestinal tract, and their use may lead to folate deficiency. Several studies have indicated that anticonvulsant-induced folate deficiency is associated with a much higher than usual incidence of psychiatric symptoms. Depressive symptoms and schizophrenia-like psychosis are the most commonly noted psychiatric symptoms in folate-deficient epileptic patients, although memory loss, irritability, and behavioural disorders also have been reported (Reynolds 1985).

6.2.2.1 Supportive Trial

In a recent supportive study 41 (33%) of 123 patients consecutively admitted, patients with acute psychiatric disorders (DSM III diagnosis of major depression or schizophrenia) had borderline or definite folate deficiency (RBC folate below 200 µmol/l) and took part in a double-blind, placebo-controlled trial of 5-MTHF, 15 mg daily, for 6 months in addition to standard psychotropic treatment. Among both depressed and schizophrenic patients 5-MTHF significantly improved clinical and social recovery. The difference in outcome scores between 5-MTHF and placebo groups became greater with time (Godfrey et al. 1990).

6.2.2.2 Uncontrolled Trial

In an open study (Guaraldi et al. 1993) to assess the efficacy and tolerability of an oral preparation of 5-MTHF, 20 elderly patients with a DSM III-R diagnosis of Depressive Disorder and an HDRS-21 score>18, underwent six-weeks of open-label treatment with 50 mg/day of oral 5-MTHF (Table II page 32).

Table II: Summary of the main quoted trials with 5-MTHF in the treatment of patients with depressive syndromes

Reference	Diagnosis	Study Design	No. of Pts	Daily dosage Route of administration (mg/day)	Clinical evaluation and standard scales for depression
Passeri et al., 1993	Depression and OMD	DB vs Trazodone (TRZ)	96	MTHF 50 p.o. TRZ 100 p.o.	↓ in MTHF from 23±5 to 18±6 ($p<0.05$) in HRDS
Godfrey et al., 1990	Depressive Syndrome	OP (supportive)	41	15 p.o.	Improvement of Clinical Outcome
Guaraldi et al., 1993	Depressive Syndromes and Disthimia	OP	16	50 p.o.	↓ in MTHF from 34.8±5.5 to 9.9±10.8 ($p<0.01$) in HDRS
Crellin et al., 1993	Minor depression	DB vs ANY	31	5-MTHF 50 p.o. ANY 150 p.o.	↓ in both drugs in MADRS

OMD = Organic Mental Disorders; HDRS = Hamilton Depression Scale; MADRS = Montgomery-Asberg Depression Scale.

Sexteen out of 20 patients completed at least 4 weeks of treatment after 1-week placebo run-in period. The mean HAM-D-21 score of these 16 depressed patients was 34.8±5.5 before starting treatment and decreased significantly to 9.9±10.8 ($p<0.001$) at the end of treatment with 5-MTHF 50 mg/day. Similarly, there were significant decreases the in Symptom Questionnaire scores of the scales of anxiety, depression an somatization at the end of treatment. Thirteen out of 16 (81%) evaluable patients were considered responders, with response being defined as a HAM-D-21 score decrease > 50% vs baseline.

There were no clinically relevant changes in the routine laboratory tests during the study, and no adverse events considered to be drug-related were reported. Adverse events were the following: mild temporary tension headaches (n=2), slight worsening of insomnia (n=2), hand tremors (n=1), loss of appetite (n=1), and mild worsening of fatigue (n=1).

6.2.2.3 Comparative trial

In a controlled, double-blind study (Passeri et al. 1993) the effect of 5-MTHF on depressive symptoms and cognitive status was compared to Trazodone (TRZ) in normofolatemic elderly patients with mild to moderate dementia and depression (Table II page 32). Ninety-six patients with dementia, scoring 12-23 at the Mini Mental State Examination (MMSE) and > 18 at the 21-item Hamilton Depression

Rating Scale (HDRS) after a 2-week placebo run-in, were randomised to receive either 5-MTHF (50 mg/day p.o.) (47 patients) or TRZ (100 mg/day p.o.) (49 patients) for 8 weeks. HDRS was assessed before, after 4 weeks and at the end of treatment. Rey's Verbal Memory (RVM) test for immediate and delayed recall was evaluated before, and at the end of treatment. After 4 weeks of treatment HDRS score was reduced from 23±5 to 20±6 in the 5-MTHF group ($p<0.05$ vs baseline), and from 23±3 to 21±4 in the TRZ group ($p< 0.05$ vs baseline). A further decrease to 18±6 and 19±5 respectively was obtained at the end of the treatment period ($p<0.05$ vs week 4) with 5-MTHF and TRZ. HDRS was administered again after a 4-week, drug-free, follow-up period: no change vs the post-treatment scores was observed either in the 5-MTHF or in the TRZ group (18±7 and 19±5 respectively). RVM test for immediate recall was significantly improved ($p<0.05$) at week 8 vs baseline in the 5-MTHF group whereas no significant change was observed in the TRZ group. No significant change was observed in delayed recall in either group. Tolerability was good for both treatment.
The results obtained with 5-MTHF in a normofolatemic population of chronic, progressively deteriorating and poor drug-responder patients suggest that pharmacological doses of 5-MTHF may exert psychotropic effects irrespective of folate status, and offer a preliminary answer to the question raised in previous studies (Passeri et al. 1993).

Crellin et al. (1993) undertook a pilot double-blind comparison of 5-MTHF 50 mg daily versus the standard antidepressant amitriptyline (ANY) 150 mg daily each as monotherapy, for 6 weeks in 31 out patients with moderate depression most of whom had normal red cell folate levels. Any patients showing >25% decrease in Montgomery-Asberg Depression Scores (MADRS) during a 2-week placebo run-in phase were not randomized. In the trial, the MADRS response rate was very similar with amitriptyline and with 5-MTHF.
Interestingly, of 3 amitriptyline nonresponders who were subsequently crossed over to 5-MTHF, 2 improved. A further interesiting observation was that 5-MTHF responders showed striking increase in red cell folate levels in comparison with 5-MTHF non-responders.

6.2.2.4 Tolerability

Adverse effects recorded up to now with 5-MTHF in all clinical trials have generally been mild and transient with no serious adverse reactions observed (Table III page 34).

Table III: 5-MTHF tolerability

Side effects	5-MTHF (85)	Placebo (19)	TRZ (49)
Headaches	2	-	-
Slight insonia	2	-	-
Hand tremors	1	-	-
Loss of appetite	1	-	-
Worsening fatigue	1	-	-
Vertigo and blurred vision	-	-	1
	8 (8.2%)	0 (0%)	1 (2%)

Furthermore, during all these trials, there were no drop-outs due to side effects.

6.2.3. Conclusions and implications

a) Pharmacological doses of 5-MTHF were as effective as an atypical antidepressant and as amitriptyline in improving depressive features accompanying senile Organic Mental Disorders in normofolatemic patients.

b) 5-MTHF also exerted beneficial effects on the loss of short term memory a key symptom of cognitive impairment in aged subjects.

c) Both treatments were well tolerated and safe as no patient withdrew due to side effects nor changes in vital parameters and routine laboratory tests were observed.

d) Considering 285 successive psychiatric admission, borderline folate deficiency was found in 31% and definite folate deficiency in 12%.

e) A concentration of low RBC folate was observed among patients with endogenous depression and alcoholic patients.

f) Up to 40% of endogenously depressed inpatients have low folate levels and may take advantage by 5-MTHF administration.

7. HANDLING AND DISPENSING

7.1 Storage conditions

No particular precautions are requested if the product is stored in the original primary packaging without evident damage.

7.2 Expiry date

According to the available stability data, a preliminary expiry date is fixed in 3 years from the production date for the pharmaceutical dosage forms. In any case, the expiry date and the storage conditions will be clearly stated on the packaging and on the analytical report.

7.3 Spills and waste disposal

According to domestic law on pharmaceutical products disposal.

8. REFERENCES

Bell KM, Plon L, Bunney WE, Potkin SG. S-adenosylmethionine treatment of depression: a controlled clinical trial. American Journal of Psychiatry 145:1110-1114, 1988.

Blair JA, Matty JA, Razzaque A. Uptake of 5-methyltetrahydro- folic acid by the rat jejunum. Journal of Physiology 250: 221-230, 1975.

Botez MI, Young SN, Bachevalier J, et al. Folate deficiency and decreased brain 5-hydroxytryptamine synthesis in man and rat. Nature 278:182-183, 1979.

Bottiglieri T, Hyland K, Laundy M, et al. Folate deficiency, biopterin and monoamine metabolism in depression. Psychological Medicine 2:871-876, 1992.

Branda RF, Anthony BK, Jacob HS. The mechanism of 5-methyl-tetrahydrofolate transport by human erythrocytes. Journal of Clinical Investigation 61:1270-1275, 1978.

Brennan MJW, Fernandes-Costa F, Metz J, Kramer S, Scriven DRL. Depolarization-induced release of folates from slices of rat cerebellum. Neuroscience Letters 27:347-350, 1981.

Carney MWP. Psychiatric aspects of folate deficiency. In: Folic Acid in Neurology, Psychiatry, and Internal Medicine; Botez MI and Reynolds EH (Eds). Raven Press, New York:475-482, 1979.

Carney MWP. Serum folate values in 423 psychiatric patients. British Medical Journal IV:512-516, 1967.

Carney MWP. Vitamin deficiency and mental symptoms. British Journal of Psychiatry 156:878-882, 1990.

Carney MWP, Sheffield BF. Serum folic acid and B12 in 272 psychiatric in-patients. Psychological Medicine 8:139-144, 1978.

Coppen A, Abou-Saleh MT. Plasma folate and affective morbidity during long-term lithium therapy. British Journal of Psychiatry 141:87-89, 1982.

Crellin R, Bottiglieri T et al. Folates and Psychiatric Disorders. Drugs 45(5):623-636, 1993.

Godfrey PSA, Toone BK, Carney MWP, et al. Enhancement of recovery from psychiatric illness by methylfolate. Lancet 336:392-395, 1990.

Guaraldi GP, Fava M, Mazzi F, et al. An open trial of methyltetrahydrofolate (MTHF) in elderly depressed patients. Annals of Clinical Psychiatry 5: 101-105, 1993.

Hällström T. Serum B_{12} and folate concentrations in mental patients. Acta Psychiatrica Scandinavica 45:19-36, 1969.

Hommes OR, Hollinger JL, Jansen MJT, Schoofs M, Vander Weil T, Kok JCN. Convulsant properties of folate compounds: some considerations and speculations. In Folate Acid in Neurology, Psychiatry and Internal Medicine (Ed. MI Botez and EH Reynolds), pp. 285-316. Raven Press, New York, 1979.

Horne DW, Briggs WT, Wagner C. Transport of 5-methyltetra-hydrofolic acid and folic acid in freshly isolated hepatocytes. Journal of Biological Chemistry 258: 3529-3535, 1978.

Janicak PG, Lipinski J, Davis JM, Altman E, Sharma RP. Parenteral S-adenosylmethionine in depression: literature review and preliminary data. Psychopharmacology Bulletin 25:238-242, 1989.

Kagan BL, Sultzer DI, Rosenlicht N, Gerner RH. Oral S-adenosylmethionine in depression: a randomized, double-blind, placebo-controlled trial. American Journal of Psychiatry 147:591-595, 1990.

Källström B, Nylöf R. Vitamin B12 and folic acid in psychiatric patients. Acta Psychiatrica Scandinavica 45:137-152, 1969.

Kisliuk RL. The biochemistry of folates. In: Sirotnak FM, Burchall JJ, Esminger WD, Montgomery JA (Eds) Folate antagonist as therapeutic agents, Academic Press,2-68, 1984.

Korevaar WC, Geyer MA, Knapp S, et al. Regional distribution of 5-Methyl-tetrahydrofolic acid in brain. Nature New Biology 245:244-245, 1973.

Levitt M, Nixon PF, Pincus JH, et al. Transport characteristics of folates in cerebrospinal fluid; a study utilizing doubly labeled. 5-Methyltetrahydrofolate and 5-Formyltetrahydrofolate. Journal of Clinical Investigation 50:1301-1308, 1971.

Markkanen T, Virtanen S, Himanen P, Pajula RL. Transferrin, the third carrier protein of folic acid activity in human serum. Acta Haematologica 48: 213-217, 1972.

McClain LD, Carl GF, Bridgers WF. Distribution of folic acid coenzymes and folate dependent enzymes in mouse brain. Journal of Neurochemistry 24:719-722, 1975.

McMartin KE, Morshed KM, Hazen-Martin DJ, Sens DA. Folate transport and binding by cultured human proximal tubule cells. American Journal of Physiology 263: F841-F848, 1992.

Ordonez LA, Wurtman RJ. Folic acid deficiency and methyl group metabolism in rat brain: effect of L-dopa. Reversal effect of S-adenosyl-L-methionine. Archives of Biochemistry and Biophysics 160:372-376, 1974.

Passeri M, Cucinotta D, Abate G. et al. Oral 5' - Methyltetrahydrofolic acid in senile organic mental disorders with depression: results of a double-blind multicenter study. Aging Clinical Experimental Research 5:63-71, 1993.

Reynolds EH. Cerebrospinal fluid folate: clinical studies. In Folic Acid in Neurology, Psychiatry and Internal Medicine (Ed. M.I. Botez and E.H. Reynolds), pp. 195-203. Raven Press: New York, 1979.

Reynolds EH. Folic acid and neuropsychiatry. Farmaci & Terapia II:163-168, 1985.

Reynolds EH. Neurological aspects of folate and vitamin B12 metabolism. In Clinics in Haematology (Ed. A.V. Hoffbrand), pp. 661-696. Saunders: London, 1976.

Reynolds EH, Gallagher BB, Mattson RH, Bowers M, Johnson AL. Relationship between serum and cerebrospinal fluid folate. Nature 240:155-157, 1972.

Reynolds EH, Preece JM, Bailey J, Coppen A. Folate deficiency in depressive illness. British Journal of Psychiatry 117:287-292, 1970.

Rosenbaum JF, Fava M, Falk WE et al.The antidepressant potential of oral S-adenosylmethionine. Acta Psychiatrica Scandinavica 81:432-436, 1990.

Said HM, Strum WB. A pH-dependent, carrier-mediate system for transport of 5-methyltetrahydrofolate in rat jejunum. Journal of Pharmacological Experimental Therapy 226:95-99, 1983.

Scott JM. Folate-vitamin B12 interrelationships in the central nervuos system. Proceedings of the Nutrition Society 51:219-224, 1992.

Sehlub J, Powell GM, Rosenberg IH. Intestinal transport of 5-methyltetrahydrofolate American Journal of Physiology 246:G515-G520, 1984.

Shorvon SD, Carney MWP, Chanarin I, Reynolds EH. The neuropsychiatry of megaloblastic anaemia. British Medical Journal 281:1036-1042, 1980.

Spector R, Coakley G, Blakely R. Methionine recycling in brain: a role for folates and vitamin B12. Journal of Neurochemistry 34:132-137, 1980.

Spector R, Lorenzo AV. Folate transport in the central nervous system. American Journal of Physiology 229:777-782, 1975a.

Spector R, Lorenzo AV, Drum DE. Serum binding of methyltetrahydrofolic acid. Biochemical Pharmacology 24: 542-544, 1975b.

Strum WB, Hanstein WG. Pharmacokinetics of 5methyltetrahydro-folate. Research Communication Chemistry Pathology Pharmacology 20:449-456, 1978.

Strum WB, Liem HH. Hepatic uptake, intracellular accumulation and biliary secretion of 5-methyltetrahydro- folate. Research Communication Chemistry Pathology Pharmacology 30:493-507, 1980.

Thornton WE, Thornton BP. Folic acid, mental function and dietary habits. Journal of Clinical Psychiatry 39:315-322, 1978.

Weir DG, Brown JP, Freedman DS, Scott JM. The absorption of the diastereoisomers of 5-methyltetrahydropteroylglutamate in man: a carrier mediate process. Clinical Science Molecular Medicine 45:625-631, 1973.