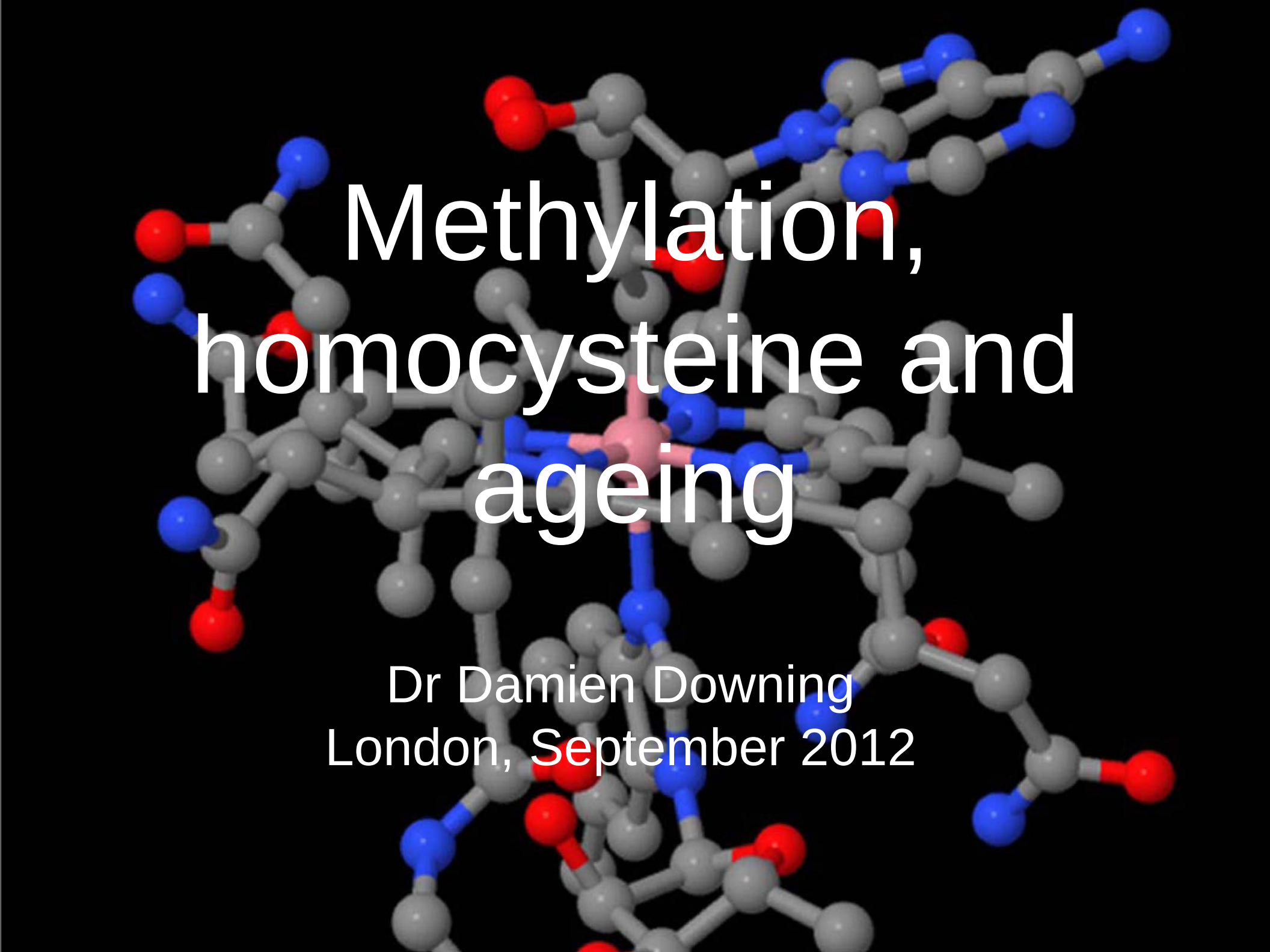


Methylation, homocysteine and ageing

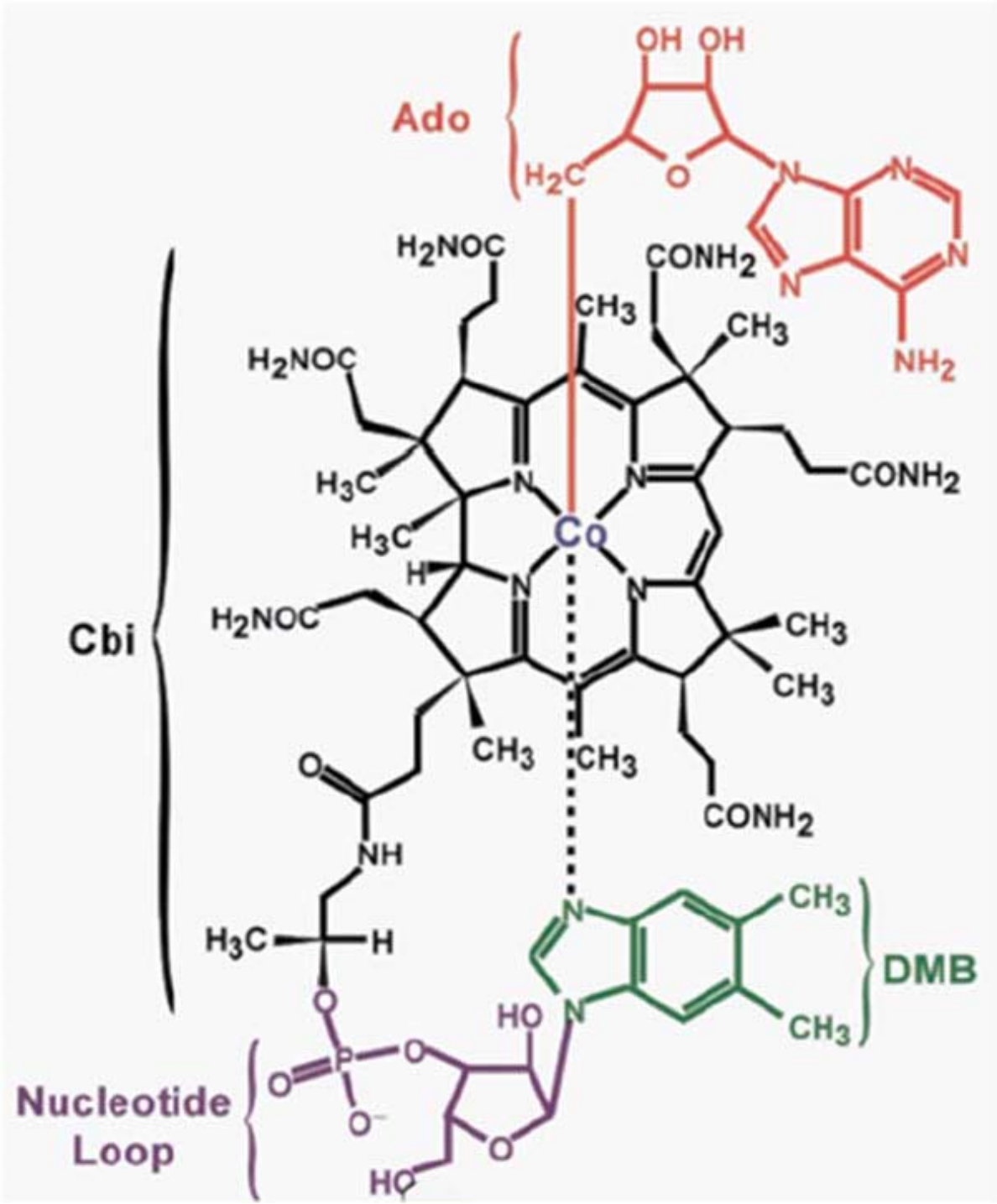
Dr Damien Downing

London, September 2012

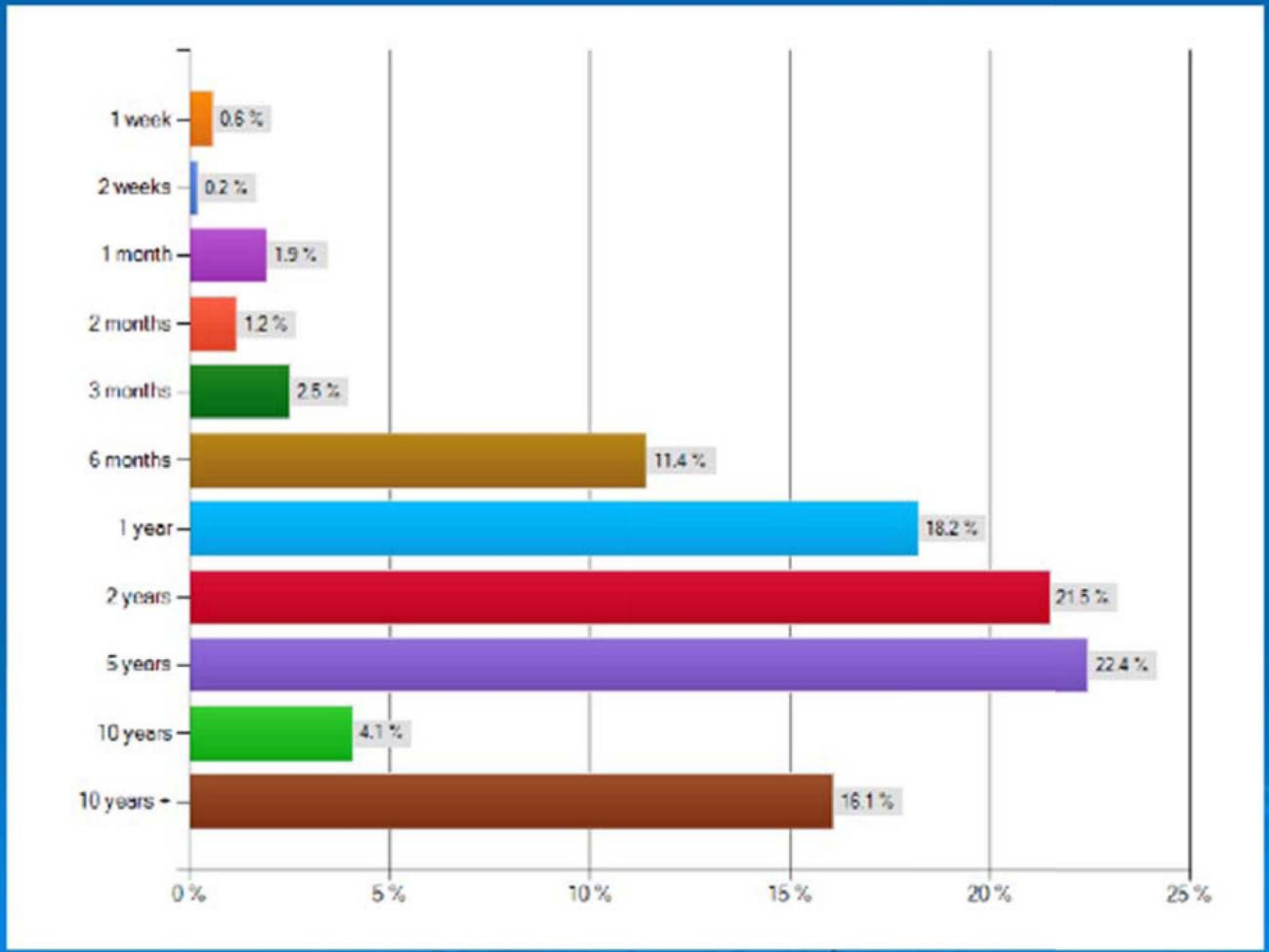
PA Society (UK)

- 6,000+ members
- not all c/o anaemia
- all B12-dependent
- 2010-11 survey, 588 responses

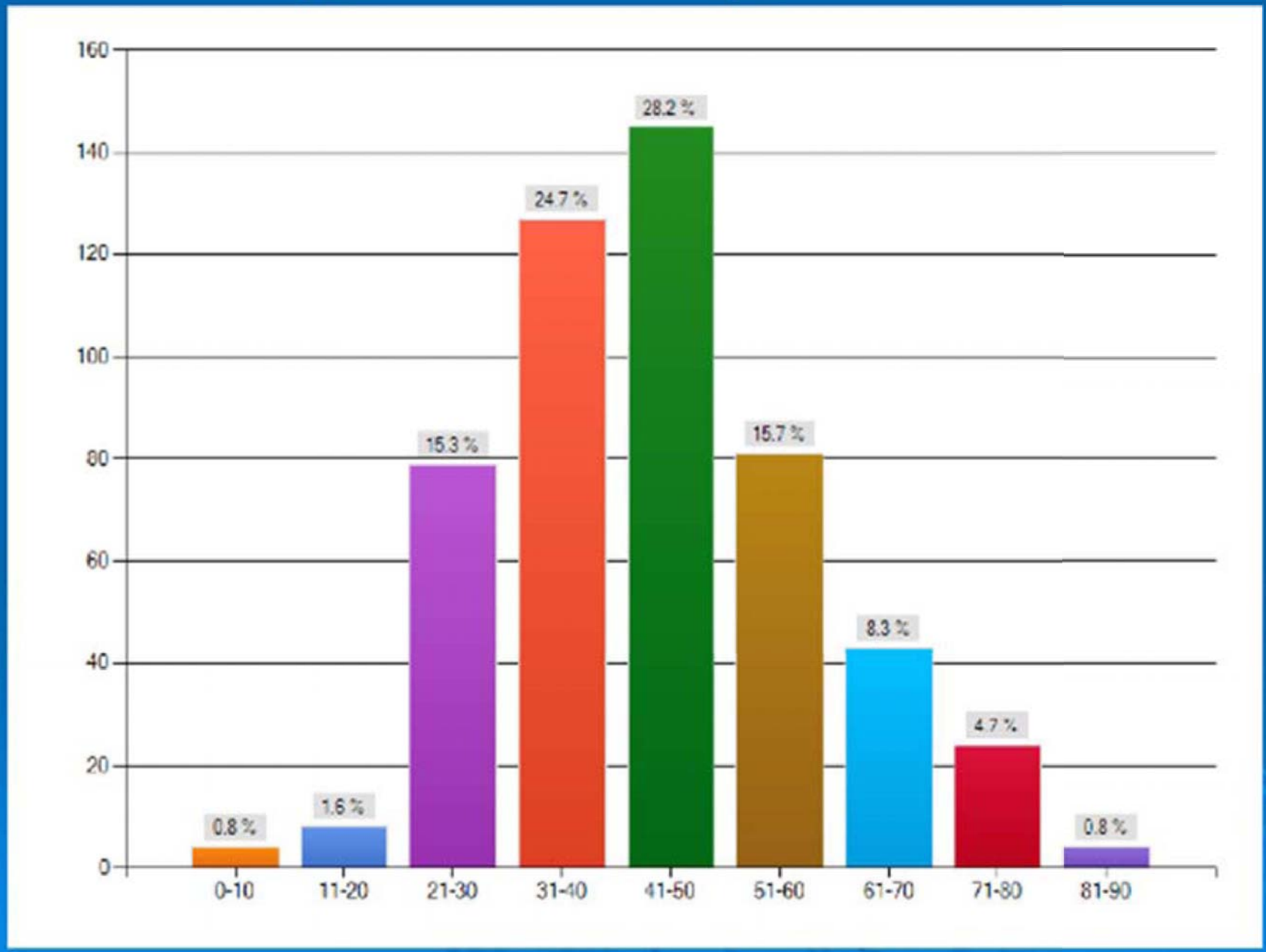
www.pernicious-anaemia-society.org


Ado
Cbi
Nucleotide Loop
DMB
Co
OH OH
H2C
O
N
NH2
H2NOC
CONH2
CH3
H3C
H
NH
HO
P
O⁻

Time Taken To Diagnose



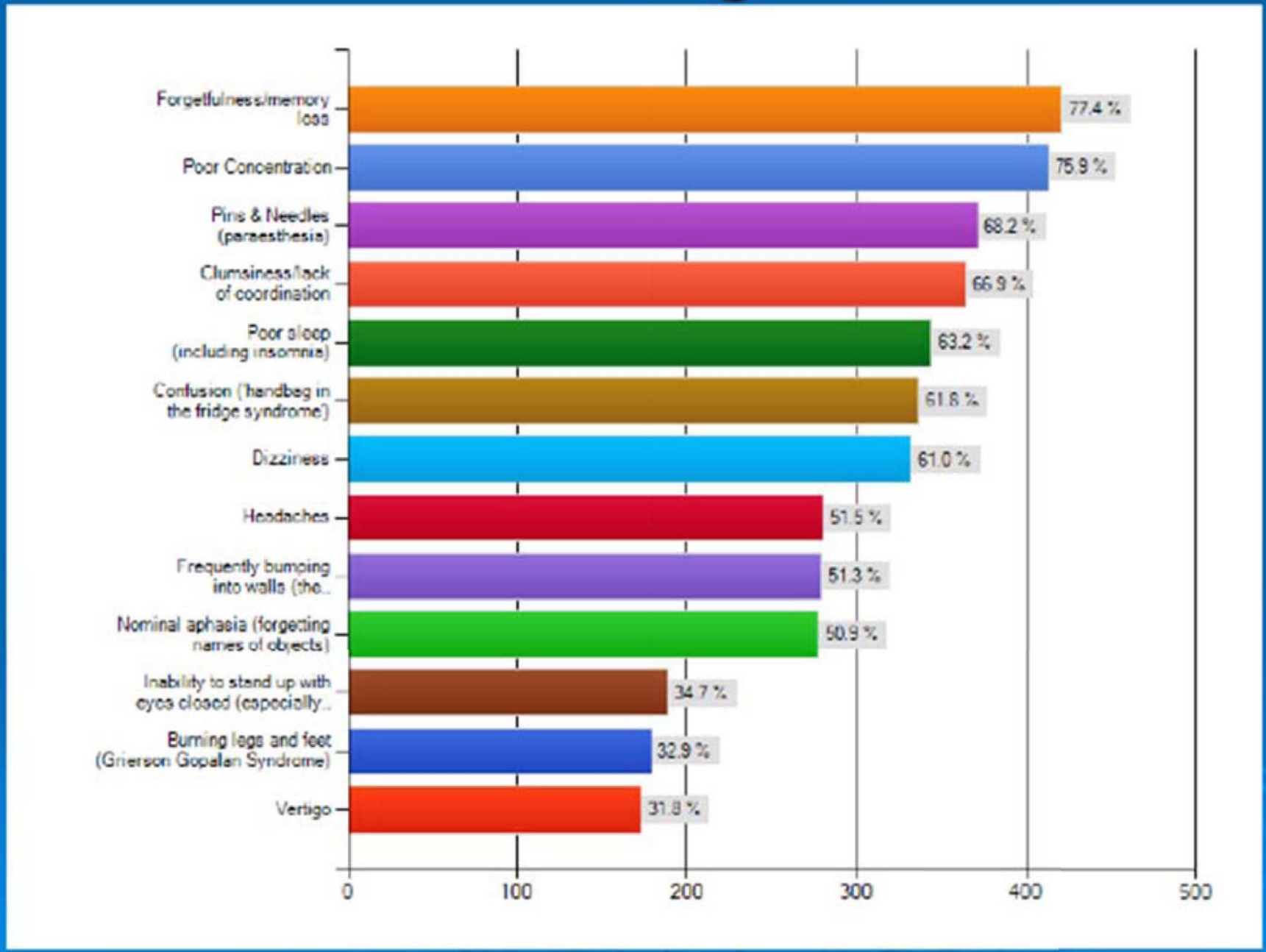
Age at Diagnosis



Emotional Symptoms

- Irritability 77%
- Isolation 65%
- Mood Swings 57%
- Suicidal Thoughts 20%

Neurological



Frequency Of Injection

More than once a day	1.2%
Daily	2.0%
2-3 times per week	2.2%
Weekly	6.3%
Every 2-3 weeks	2.9%
Every 4-6 weeks	20.5%
Every 8-10 weeks	20.7%
Every 12 weeks	48.1%
None	1.6%

www.pernicious-anaemia-society.org

Could you be B12 Deficient?

Tick the boxes which correspond to your symptoms.

Strange Tiredness	☐	5 points
The Fogs – lack of clarity/difficulty in concentrating	☐	5 points
Breathlessness - 'The Sighs' or 'The Gulps'	☐	5 points
Brittle nails	☐	5 points
Brittle nails ***with ridges***	☐	+ extra 5 points
Pins and needles - usually in your hands and feet	☐	5 points
Swollen and/or sore Tongue	☐	5 points
Sudden unaccountable bouts of diarrhoea	☐	5 points
Balance problems	☐	5 points
General unsteadiness	☐	5 points
Vertigo	☐	5 points
Burning legs or feet	☐	5 points
Tinnitus	☐	2 points
Irritability/anger/lacking patience	☐	2 points

Active B12- The transport of this key vitamin

B_{12} is attached to protein when ingested

peptic digestion

B_{12} complexed to HC to form HoloHC and transported to the small intestine

Pancreatic proteases

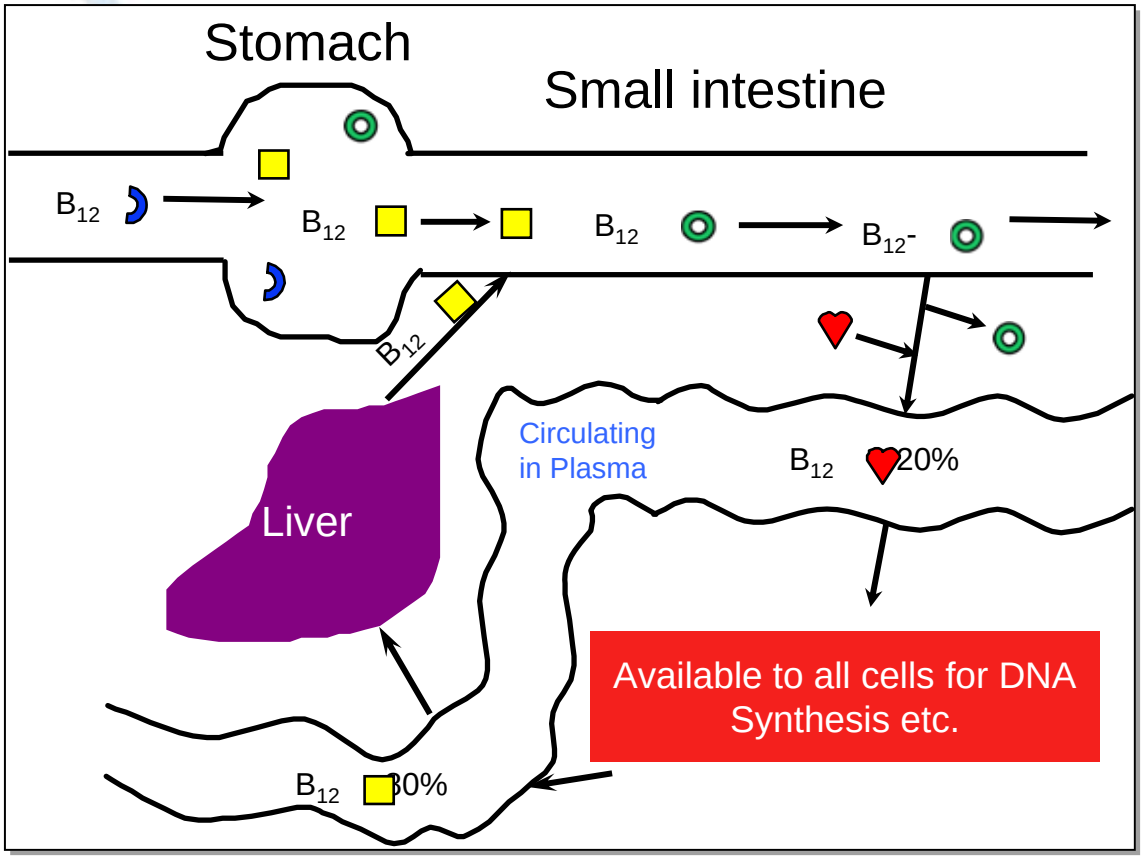
B_{12} complexed to IF and absorbed to receptor in the intestine

B12 release in enterocyte

B_{12} complexed to TC, to form Active-B12 (holotranscobalamin)

Released in Blood

HoloTC taken up by specific cell receptors

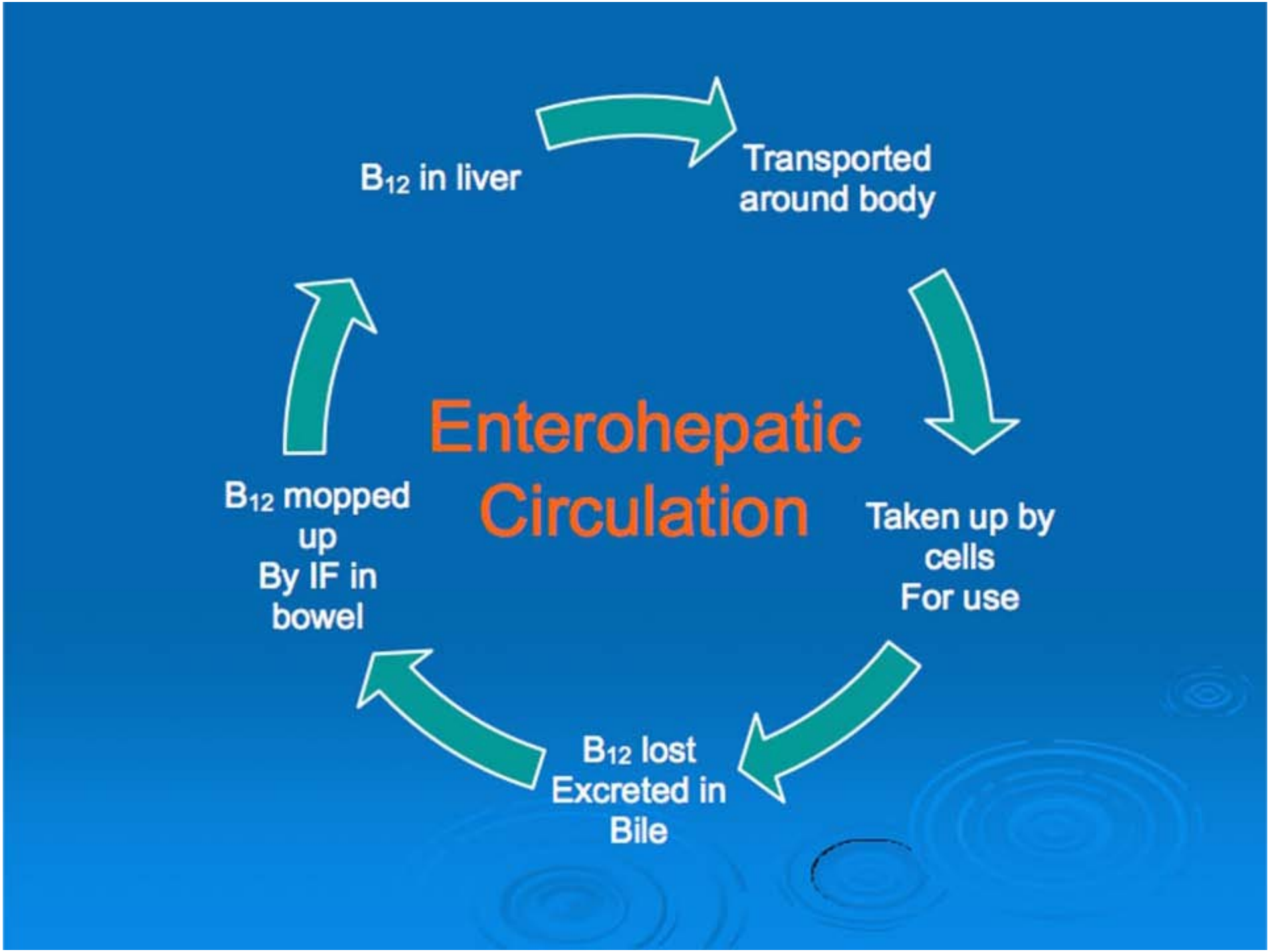


Food protein

Haptocorrin (HC)

Intrinsic Factor (IF)
(B12 uptake to intestine)

Transcobalamin (TC)
(Transport B12 to all cells)


Enterohepatic Circulation
B_{12} in liver
Transported around body
Taken up by cells For use
B_{12} lost Excreted in Bile
B_{12} mopped up By IF in bowel

Vitamin B12 deficiency

- Vegan diet
  - No dietary B12
  - Recycling of biliary B12 by Intrinsic Factor
  - Onset after 20 years
- Pernicious anaemia
  - Dietary B12
  - No recycling of biliary B12
  - Onset after 2-3 years

We have known about this for 100 years - e.g.

Nervous and mental manifestations of pre-pernicious anaemia

FW Langdon MD
JAMA 1905

Dr. Donoghue has never punctured an ovarian cyst. Malignant disease of the peritoneum is not uncommon following the puncture of a cyst which may appear benign. The danger of the large incision is much less than the danger of dissemination papilloma of the ovary by puncture.

NERVOUS AND MENTAL MANIFESTATIONS OF PRE-PERNICIOUS ANEMIA.*

F. W. LANGDON, M.D.
CINCINNATI, OHIO.

Most practitioners of wide experience have probably been puzzled at times by a class of patients presenting an anomalous grouping of general and nervous symptoms, which have passed from one physician to another; perhaps labelled as "general debility" by one, "neurasthenia" by another, "crankiness" by a third, "hysteria" by others; cases which steadily progress toward a fatal termination in spite of supporting treatment, "rest cures" and optimistic prognoses.

Occasionally a residence for a more or less prolonged period in a well-equipped hospital, or an accidental falling into the hands of some young "up-to-date" village doctor, leads to a routine blood examination and the surprising discovery is made that the patient has "pernicious anemia."

by control, patience, good temper, etc., f
shorter intervals.

Two of my cases, both women, presente
bility to a remarkable extent, the mental p
ing abruptly for better or worse within a fe

During the "better" periods some patien
"angelic" in disposition and conduct.

One patient had a distinct history of au
cinations.

3. Sensory disturbances: (a) Subjecti
of intramuscular and articular pains. Th
never, in my experience, of "lightning" ch
tabes, but rather of the nature of "aches"

The articular pains are seldom accompa
sions and never by true inflammatory acti
ness, etc.

Other subjective sensory complaints wh
haps more frequent than the above are
numbness, tingling and weight in the extre
ly more marked in the feet and legs. The
with the ataxia noted later, have occasiona
erroneous diagnosis of tabes, as in Case 1.

(b) Objective sensory disturbances: Th
mon and consist chiefly of losses of tactile
sibility about the feet and ankles; "patchy
tion, i. e., not segmental. Sometimes i
stages only delay in transmission of sens

Most practitioners of wide experience have probably been puzzled at times by a class of patients presenting an anomalous grouping of general and nervous symptoms, which have passed from one physician to another; perhaps labelled as "general debility" by one, "neurasthenia" by another, "crankiness" by a third, "hysteria" by others; cases which steadily progress toward a fatal termination in spite of supporting treatment, "rest cures" and optimistic prognoses.

George Whipple (1920)

- bled dogs
- fed them various foods
- liver had most benefit

Journal of Nutritional Medicine (1991) *2*, 89–90

CLASSIC PAPER REVIEWED

Cerebral Manifestations of Vitamin B12 Deficiency

J. MACDONALD HOLMES

British Medical Journal 1956; 2: 1394–1398

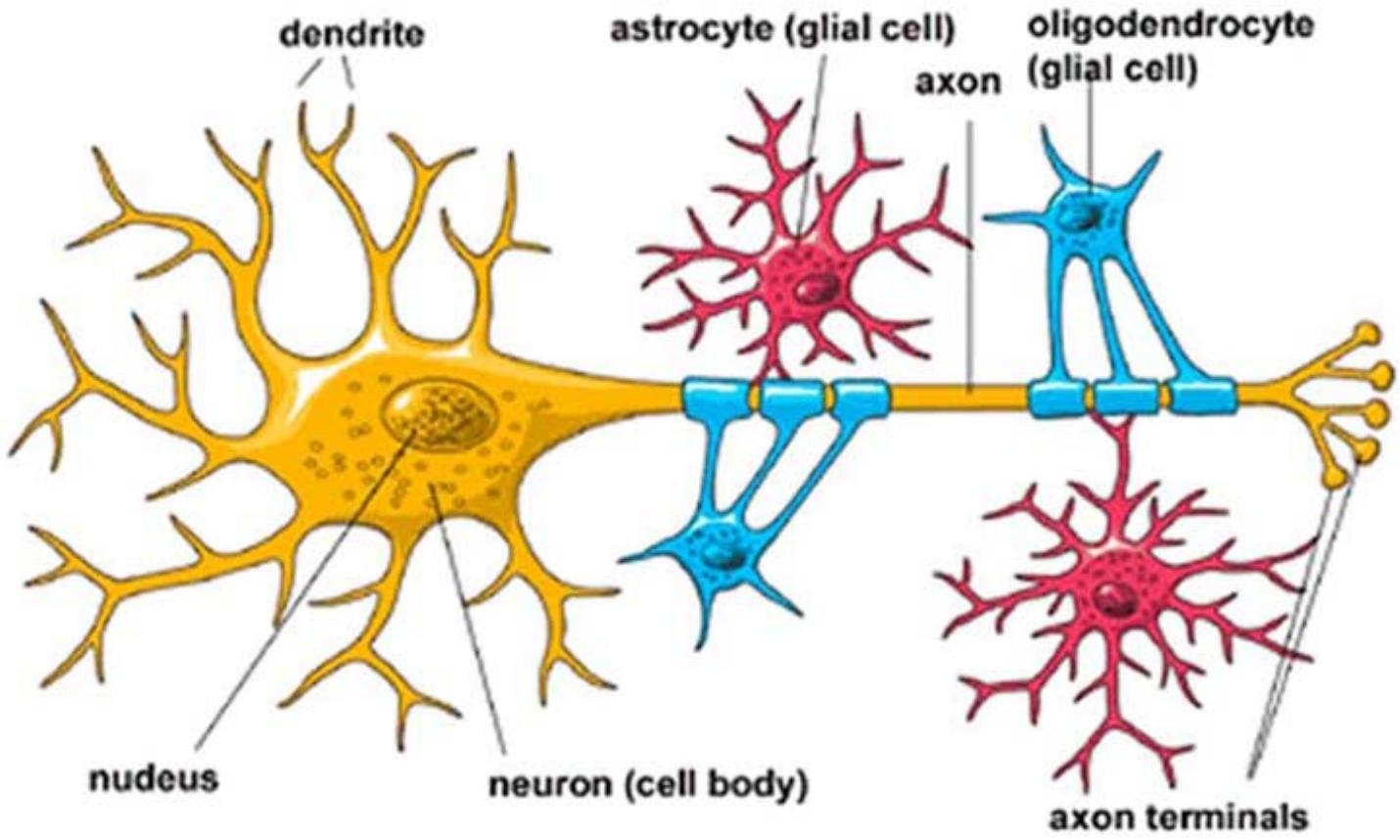
Reviewed by Damien Downing MBBS

The pattern of psychological symptoms was as follows:

Pronounced slowing of mental processes	100%
Confusion and memory defect	100%
Depression	50%
Delusions	35%
Hallucinations	21%
Agitation	7%
Mania	7%

Glial cells

- Feed neurones
- Smaller & more numerous
- Run out of B12 fast



Journal of Nutritional Medicine (1991) *2*, 91–92

KEY PAPER REVIEWED

Neuropsychiatric Disorders Caused by Cobalamin Deficiency in the Absence of Anaemia or Macrocytosis

J. LINDENBAUM, E. B. HEALTON, D. G. SAVAGE *ET AL.*

New Engl J Med 1988; 318: 1720–1728

Reviewed by Stephen Davies MA BM BCh

141 consecutive patients with neuro-psychiatric abnormalities due to cobalamin deficiency; 40 (28 percent) had no anemia or macrocytosis.

Characteristic features;

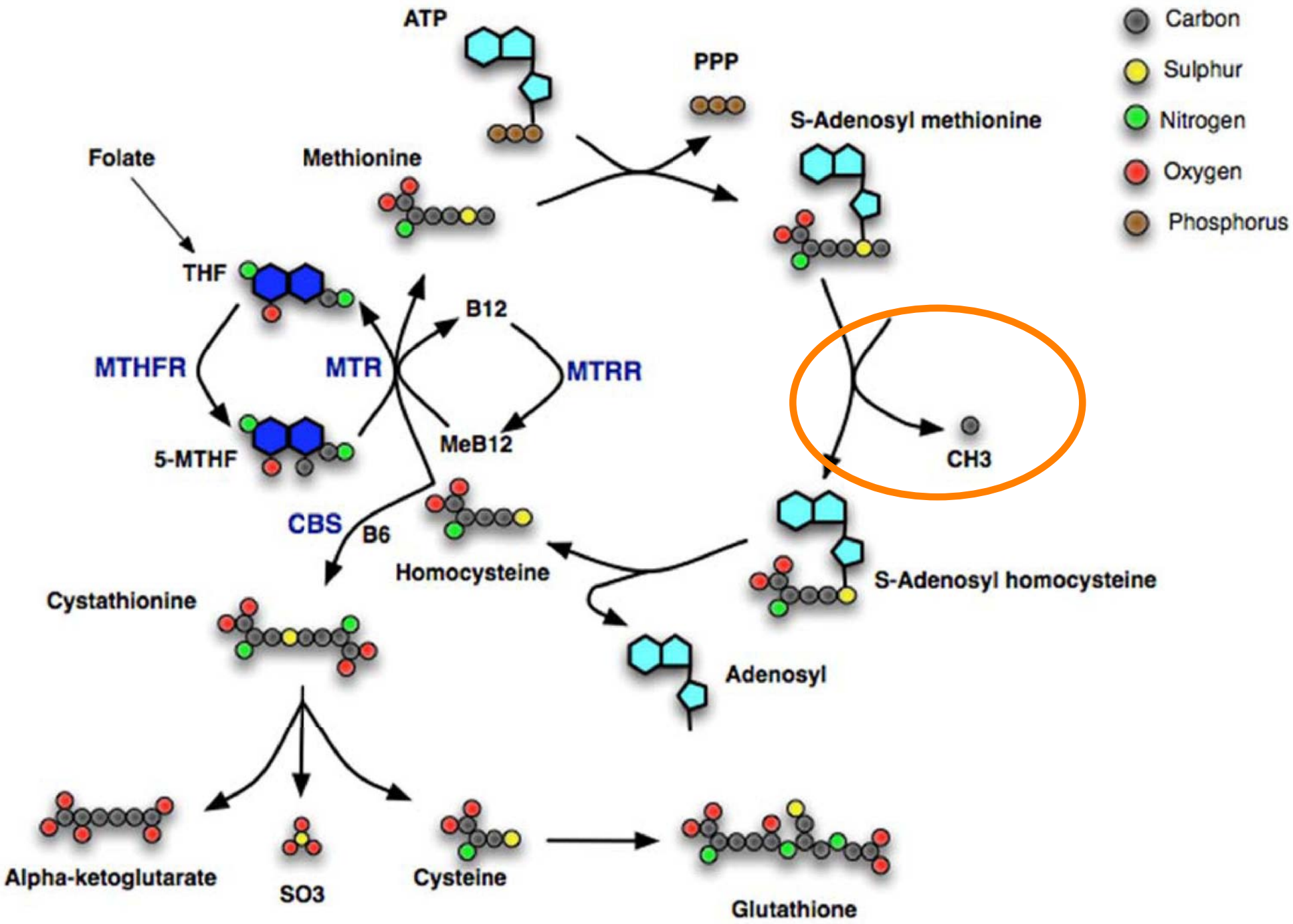
- paresthesia, sensory loss, ataxia,
- dementia, and psychiatric disorders
- longstanding neurologic symptoms without anemia
- normal white-cell, platelet counts, serum bilirubin and lactate dehydrogenase levels
- markedly elevated methylmalonic acid and total homocysteine

Methylation cycle

- donates single carbons
- major metabolic “crossroads”

The Methyl Deficiency Hypothesis
-first proposed in;

Miller, J. A. and Miller, E. C. 1953. The Carcinogenic Aminoazo Dyes. *Adv. Cancer Res*. 1: 339-396.


ATP
PPP
S-Adenosyl methionine
Folate
Methionine
THF
B12
MTHFR
MTR
MTRR
5-MTHF
MeB12
CH3
CBS
B6
Homocysteine
S-Adenosyl homocysteine
Cystathionine
Adenosyl
Alpha-ketoglutarate
SO3
Cysteine
Glutathione
Carbon
Sulphur
Nitrogen
Oxygen
Phosphorus

Disorders linked to impaired methylation

- Atherosclerosis, Coronary Artery Disease, Deep Vein Thrombosis, Stroke
- Neural Tube Defects, Spontaneous Abortion, Placental Abruption, Pre-eclampsia
- Cervical Dysplasia
- Cancers
- Autism, Depression, Schizophrenia
- Cognitive Impairment, Senility, Late-Onset Alzheimer's Disease
- Migraine
- Fatigue, ME/CFS
- Osteoporosis, Rheumatoid Arthritis, Diabetes

Main uses of methyl groups

Methylation of:	Role
DNA	Regulates (silences) gene expression
Phospholipids	Increases membrane fluidity and transmembrane signalling
Catecholamines	Breaks down adrenaline, histamine, dopamine
Carnitine precursors	Improves cellular energy provision
Oestrogens	Reduces cancer risks

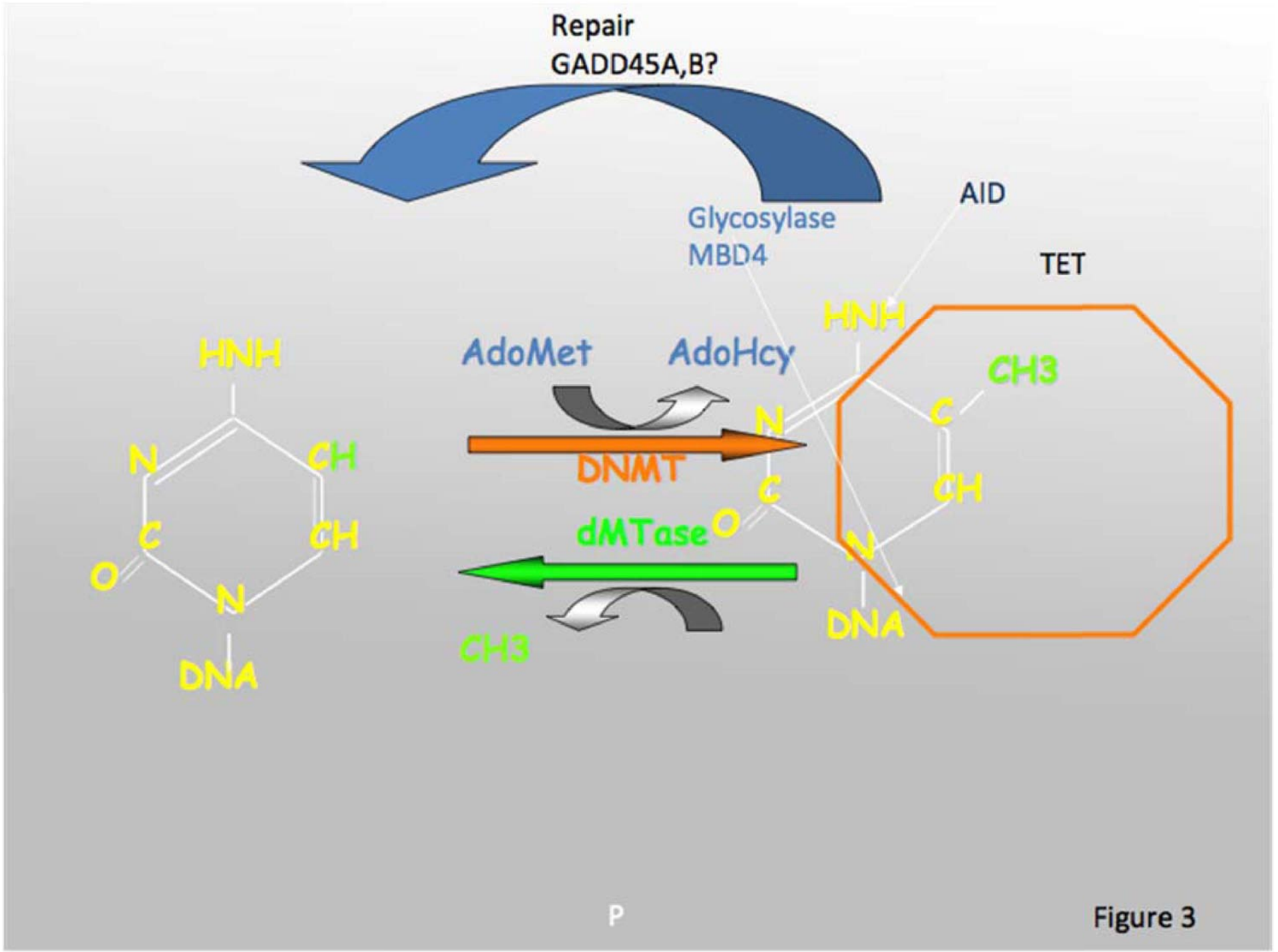
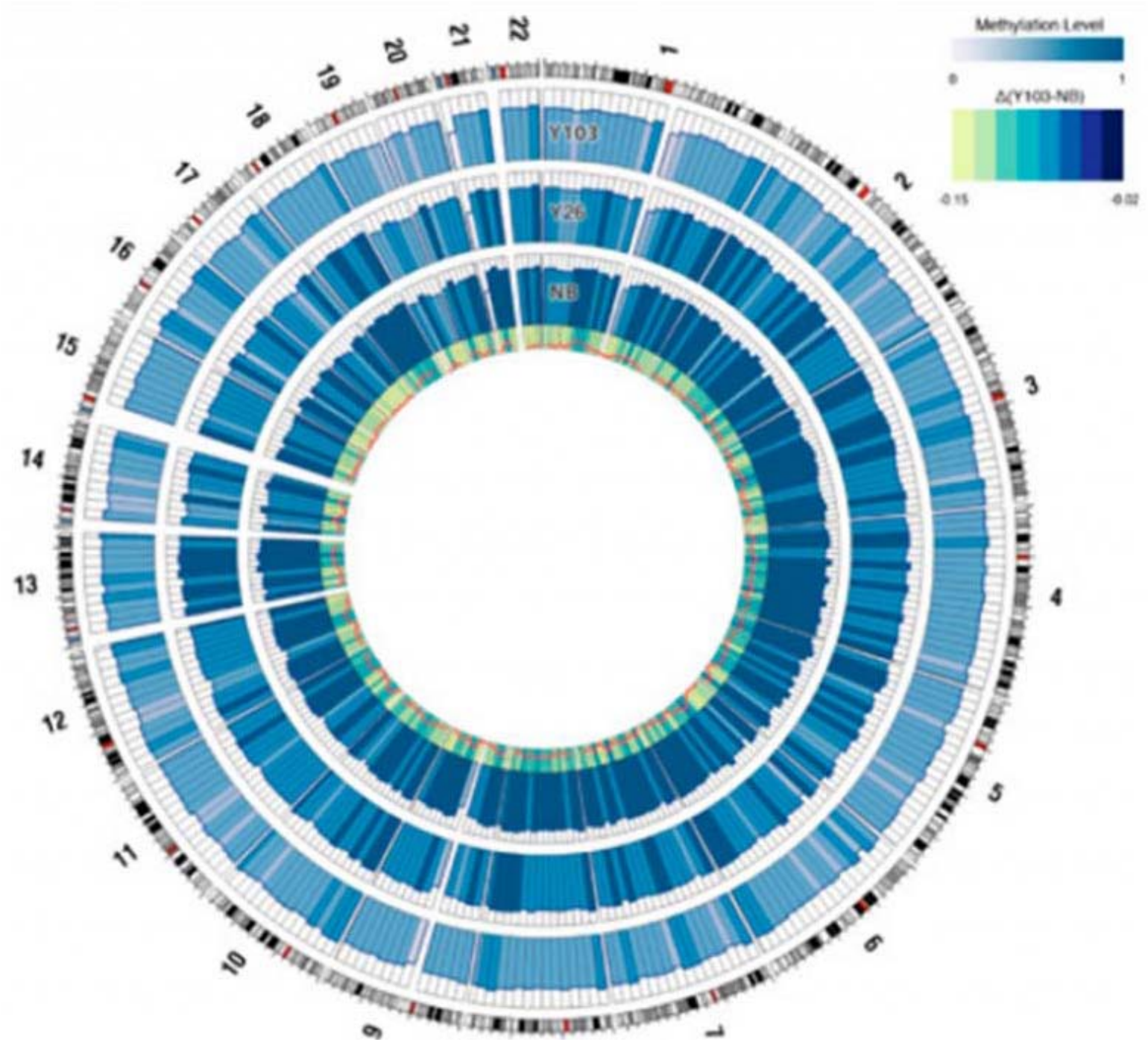
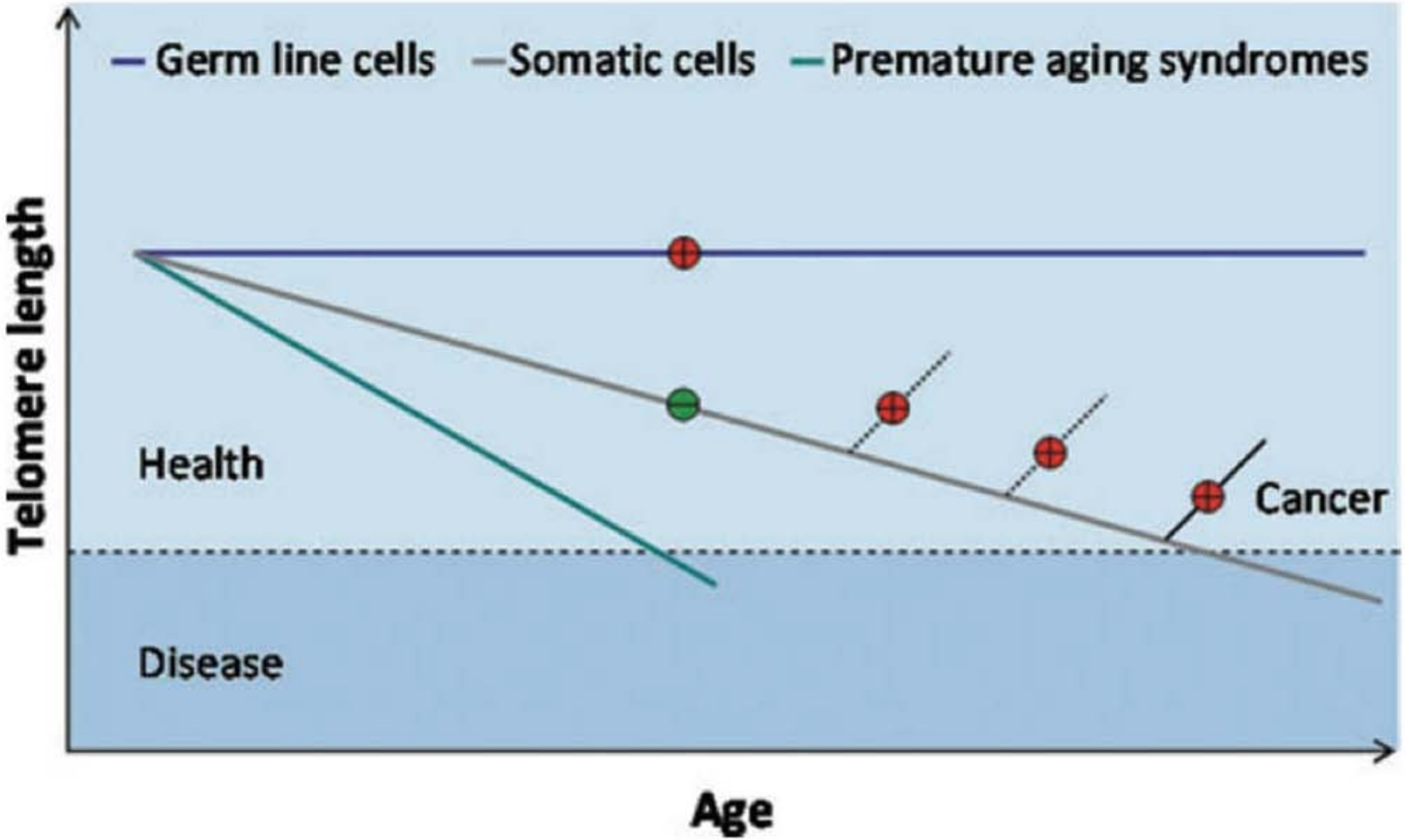

Repair
GADD45A,B?
Glycosylase
MBD4
AID
TET
HNH
AdoMet
AdoHcy
CH3
N
CH
C
CH
O
N
DNA
DNMT
dMTase
HNH
CH3
C
N
CH
C
O
N
DNA
CH3
P

Figure 3


Methylation Level
0
1
Δ(Y103-NB)
-0.15
-0.02
Y103
Y26
NB
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

DNA methylation decreases with age

- Neonate: 80.5%
- 26-yo: 78.0%
- 100-yo: 73.0%


Germ line cells
Somatic cells
Premature aging syndromes
Telomere length
Health
Cancer
Disease
Age

1,319 healthy subjects were recruited from a population-based cohort. Leukocyte Telomere Length was negatively correlated with plasma homocysteine levels, after adjustment for smoking, obesity, physical activity, menopause, hormone replacement therapy use and creatinine clearance.

The difference in multiply-adjusted LTL between the highest and lowest tertile of homocysteine levels was 111 base pairs ($p=0.004$), corresponding to 6.0 years of telomeric aging. This relationship was further accentuated by decreased concentrations of serum folate and increased levels of C-reactive protein.

Main uses of methyl groups

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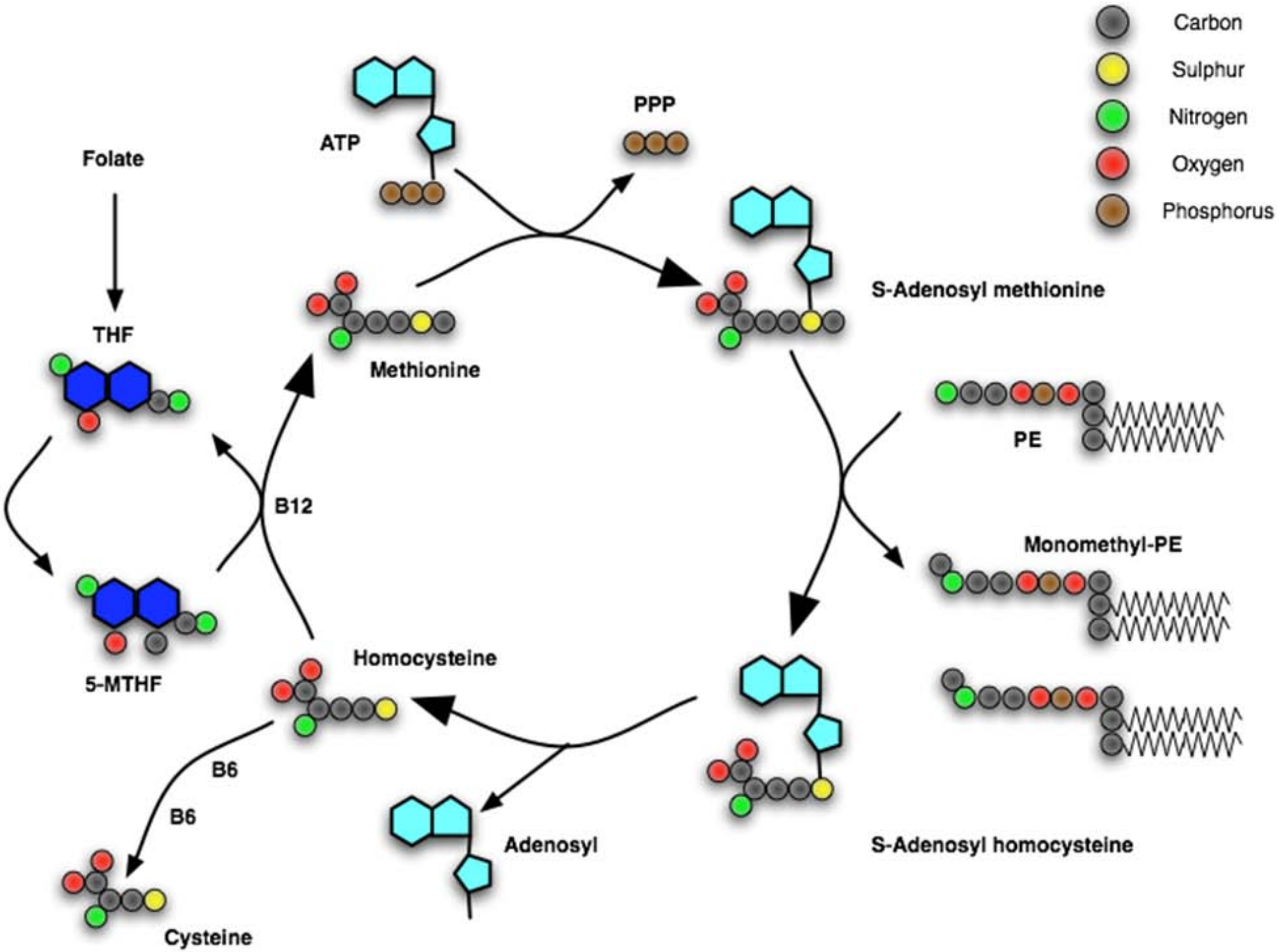
Carbon
Sulphur
Nitrogen
Oxygen
Phosphorus
ATP
PPP
Folate
THF
Methionine
S-Adenosyl methionine
PE
Monomethyl-PE
B12
5-MTHF
Homocysteine
B6
B6
Adenosyl
S-Adenosyl homocysteine
Cysteine

Composition of membrane lipids

- Cholesterol 50%
- Ph-choline 25%
- Ph-ethanolamine 12.5%
- Ph-serine 5%
- Ph-inositol 2.5%
- Sphyngomyelin 1.5%
- Other lipids 3.5%

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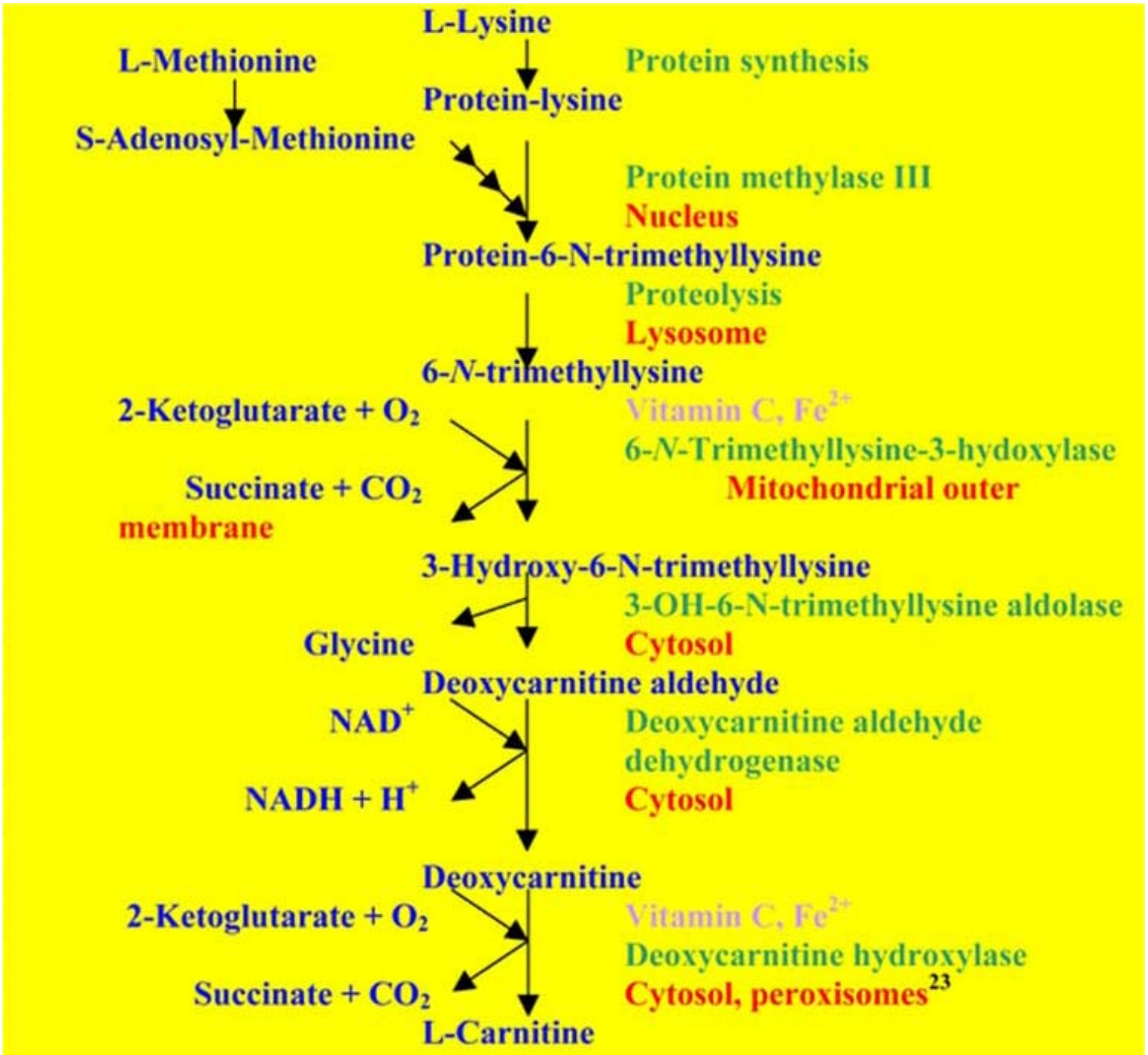
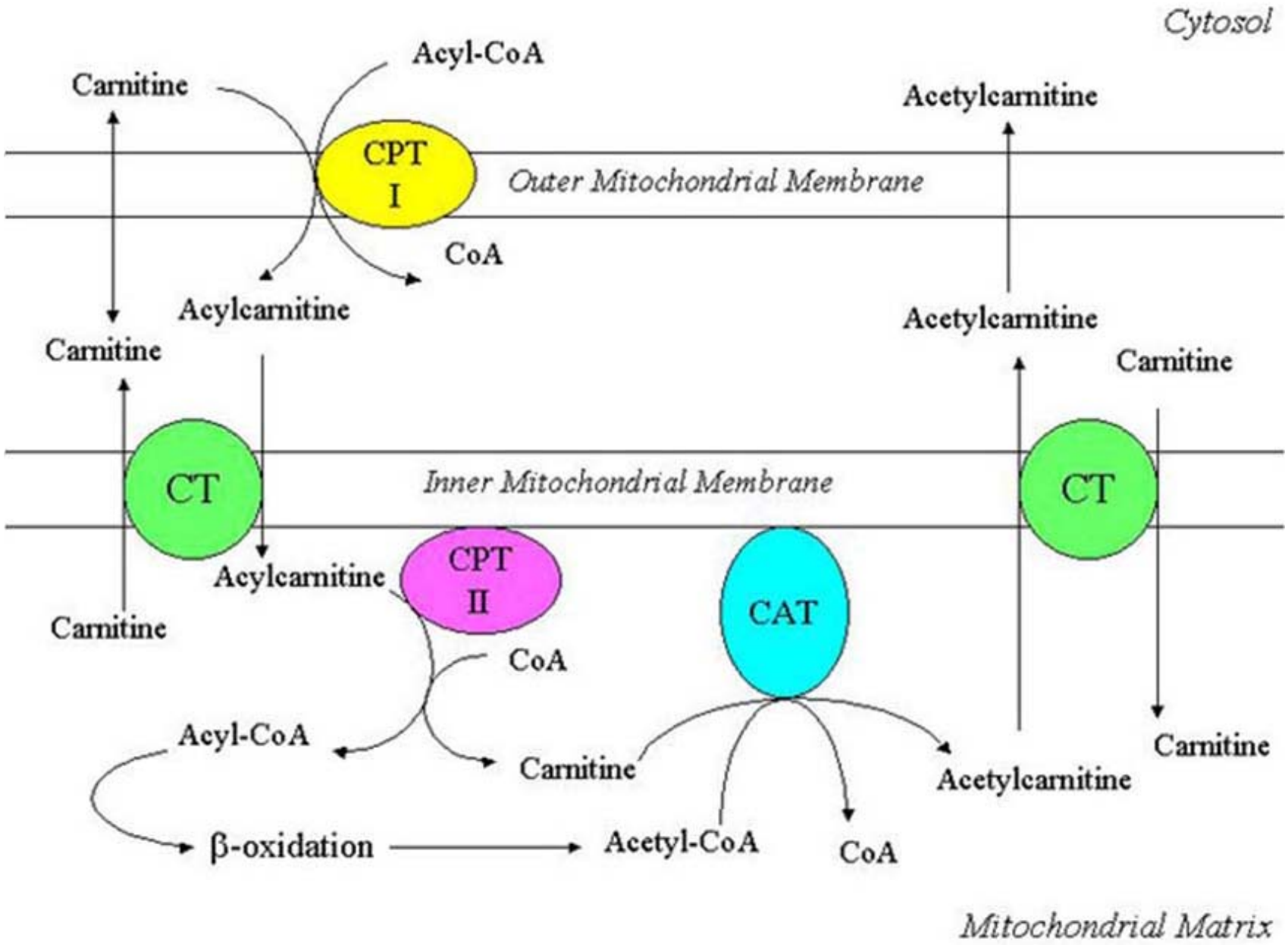


Carnitine biosynthesis

Substrates, products, cofactors, enzyme names, localization. [2, 23-25]


Cytosol
Acyl-CoA
Carnitine
Acetylcarnitine
CPT I
Outer Mitochondrial Membrane
CoA
Acylcarnitine
Acetylcarnitine
Carnitine
Carnitine
CT
Inner Mitochondrial Membrane
CT
Carnitine
CPT II
Acylcarnitine
CAT
Carnitine
CoA
Acyl-CoA
Carnitine
Acetylcarnitine
Carnitine
β-oxidation
Acetyl-CoA
CoA
Mitochondrial Matrix

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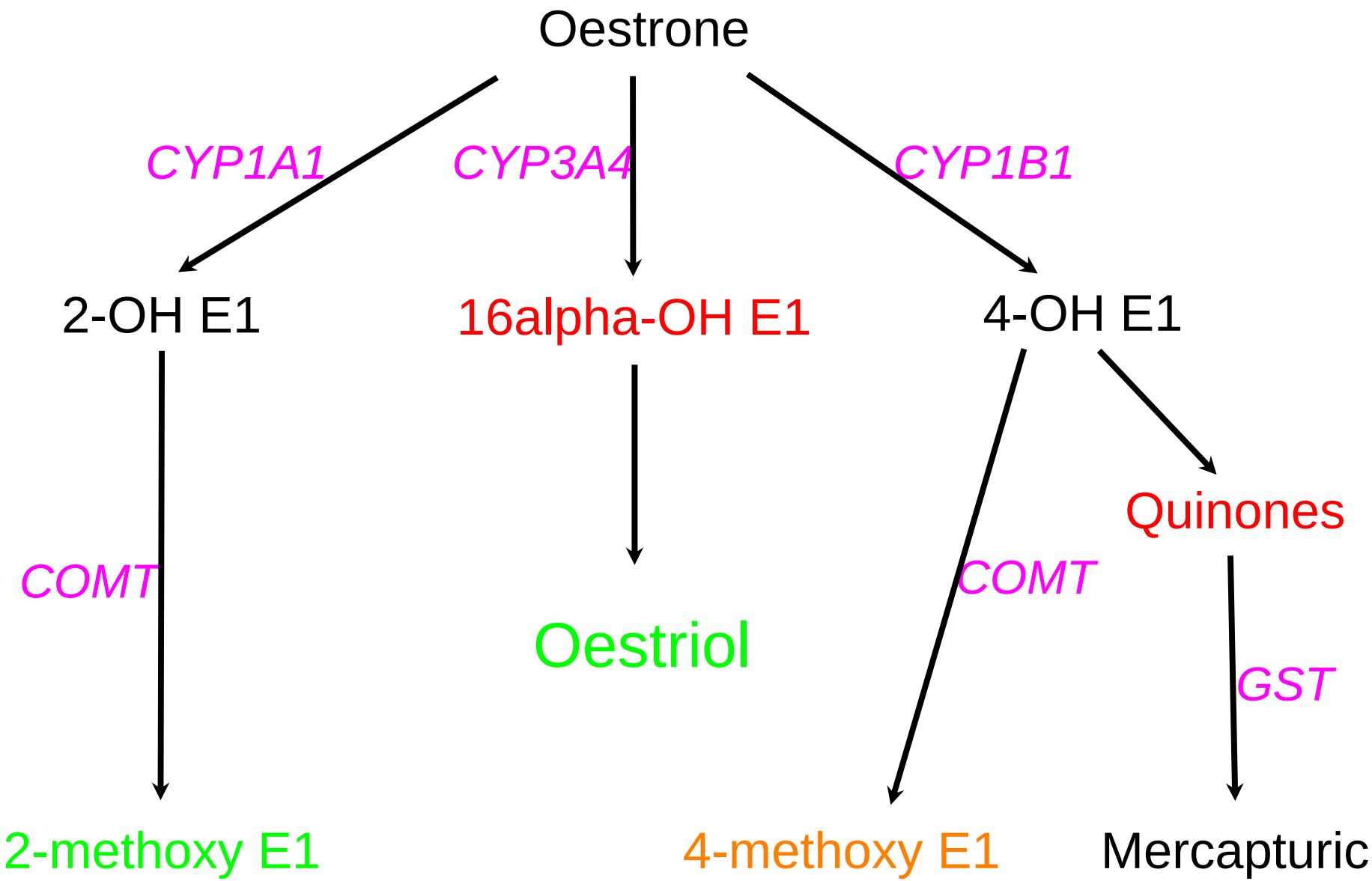
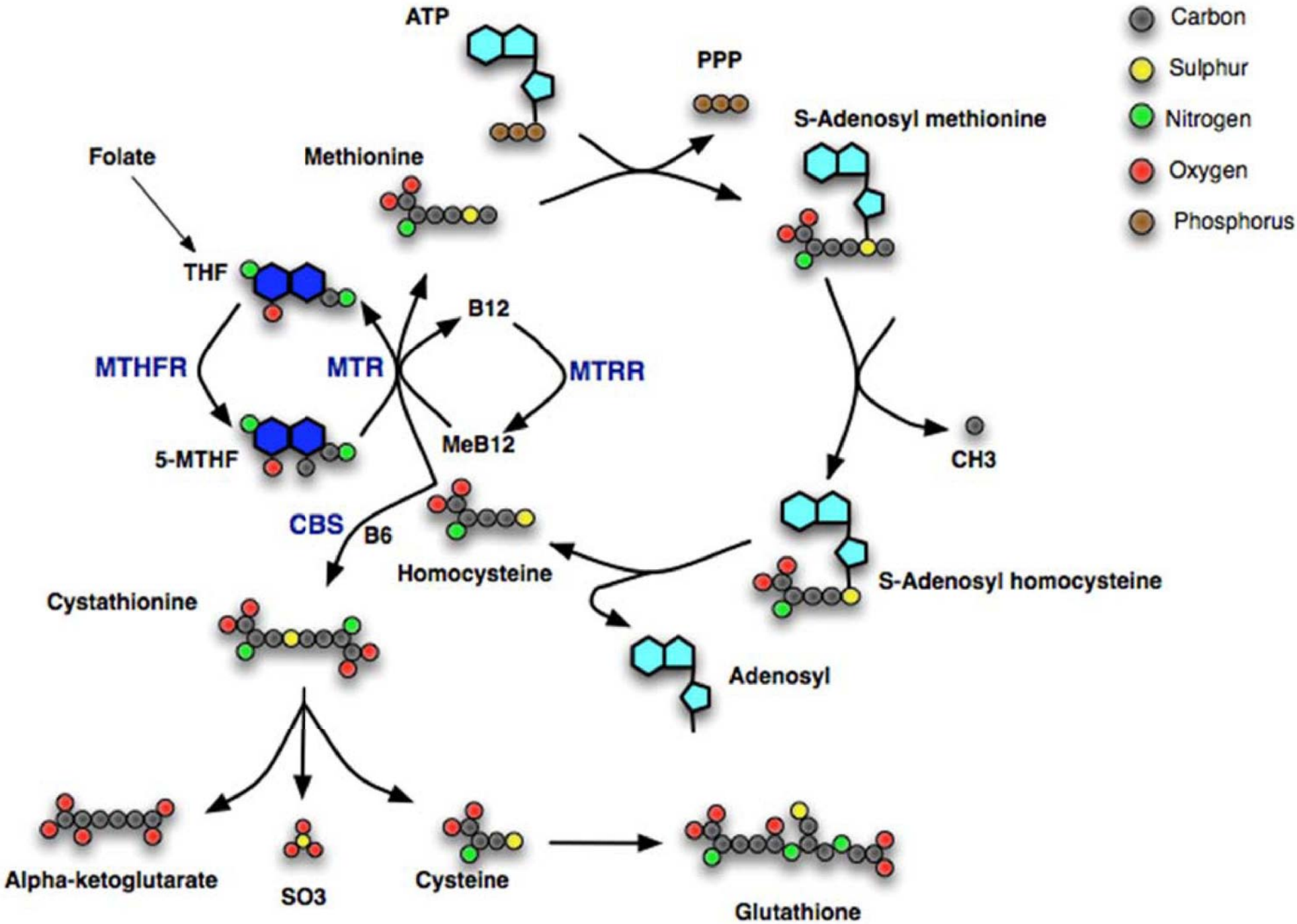

Oestrone
CYP1A1
CYP3A4
CYP1B1
2-OH E1
16alpha-OH E1
4-OH E1
COMT
Oestriol
COMT
Quinones
GST
2-methoxy E1
4-methoxy E1
Mercapturic

Factors that impair methylation

- **Genetic polymorphisms**
- Nutritional deficiencies
- Toxic exposures


ATP
PPP
S-Adenosyl methionine
Folate
Methionine
THF
B12
MTHFR
MTR
MTRR
5-MTHF
MeB12
CH3
CBS
B6
Homocysteine
S-Adenosyl homocysteine
Cystathionine
Adenosyl
Alpha-ketoglutarate
SO3
Cysteine
Glutathione
Carbon
Sulphur
Nitrogen
Oxygen
Phosphorus

Genetic polymorphisms

Genes/Alleles	wtwt	wtmt	mtmt
CYP1A2*C	wtwt		
CYP1A2*F			mtmt
CYP1B1*3			mtmt
GSTM1	wtwt		
GSTP1		wtmt	
GSTT1	wtwt		
MTHFR (A1298C)	AA		
MTHFR (C677T)		CT	
MTR (A2756G)		AG	
MTRR (A66G)		AG	

MTHFR C677T

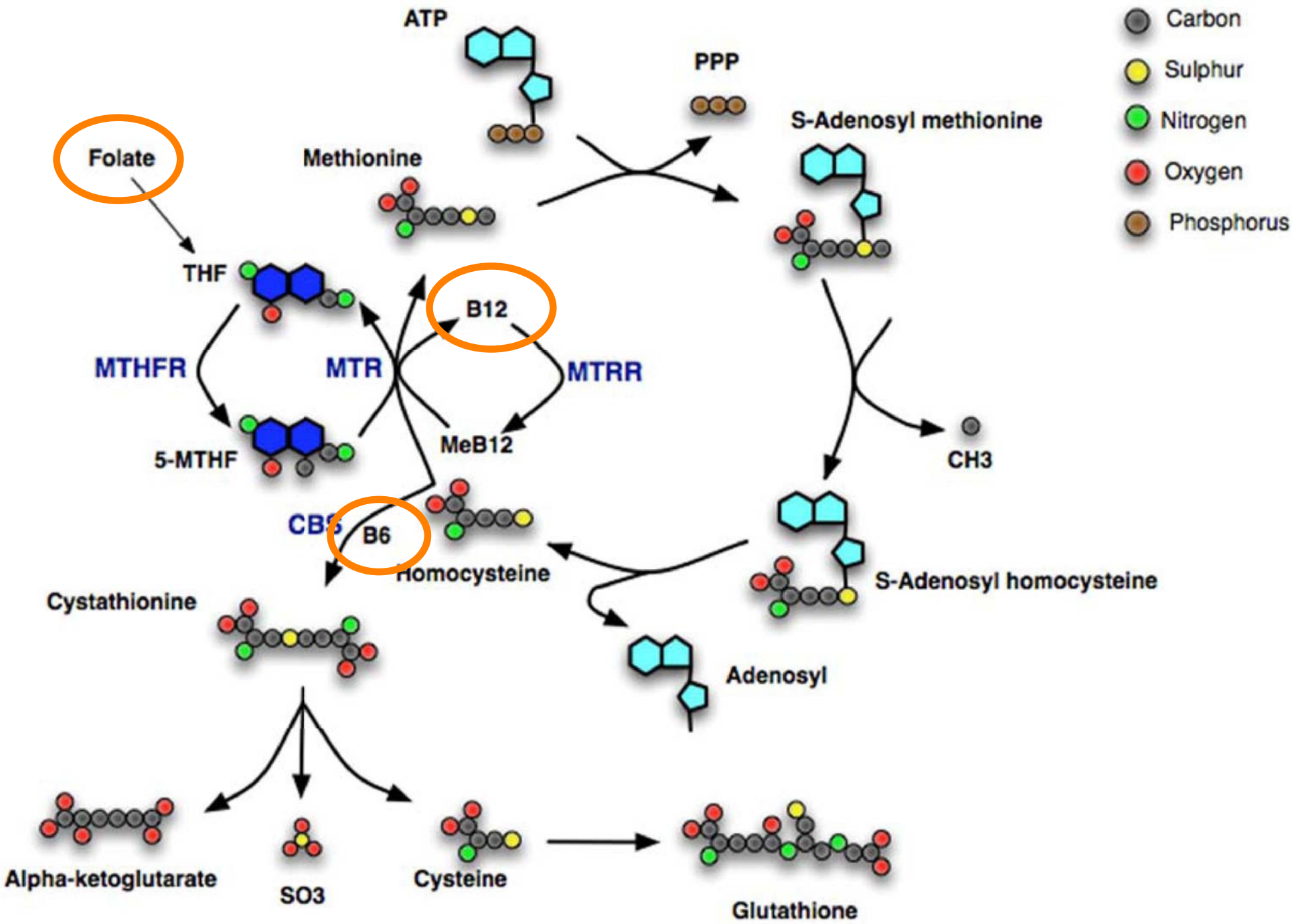
Genotype	Function	Frequency (Europe)
CC	100%	45%
CT	65%	40%
TT	30%	15%

Factors that impair methylation

- Genetic
- **Nutritional deficiencies**
- Toxic

Homocysteine

- Responds to;
  - B12
  - Folate
  - B6


ATP
PPP
S-Adenosyl methionine
Folate
Methionine
THF
B12
MTHFR
MTR
MTRR
5-MTHF
MeB12
CH3
CBS
B6
Homocysteine
S-Adenosyl homocysteine
Cystathionine
Adenosyl
Alpha-ketoglutarate
SO3
Cysteine
Glutathione
Carbon
Sulphur
Nitrogen
Oxygen
Phosphorus

Am J Clin Nutr 2002;75:616–58.

High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K_m): relevance to genetic disease and polymorphisms[1–3]

Bruce N Ames, Ilan Elson-Schwab, and Eli A Silver

ABSTRACT As many as one-third of mutations in a gene result in the corresponding enzyme having an increased Michaelis constant, or K_m, (decreased binding affinity) for a coenzyme, resulting in a lower rate of reaction. About 50 human genetic diseases due to defective enzymes can be remedied or ameliorated by the administration of high doses of the vitamin component of the corresponding coenzyme, which at least partially restores enzymatic activity. Several single-nucleotide polymorphisms, in which the variant amino acid reduces coenzyme binding and thus enzymatic activity, are likely to be remediable by raising cellular concentrations of the cofactor through high-dose vitamin therapy. Some examples include the alanine-to-valine substitution at codon 222 (Ala222→Val) [DNA: C-to-T substitution at nucleotide 677 (677C→T)] in methylenetetrahydrofolate reductase (NADPH) and the cofactor FAD (in relation to cardiovascular disease, migraines, and rages), the Pro187→Ser (DNA: 609C→T) mutation in NAD(P):quinone oxidoreductase 1 [NAD(P)H dehydrogenase (quinone)] and FAD (in relation to cancer), the Ala44→Gly (DNA: 131C→G) mutation in glucose-6-phosphate 1-dehydrogenase and NADP (in relation to favism and hemolytic anemia), and the Glu487→Lys mutation (present in one-half of Asians) in aldehyde dehydrogenase (NAD^+) and NAD (in relation to alcohol intolerance, Alzheimer disease, and cancer). *Am J Clin Nutr* 2002;75:616–58.

the primary defect and remediates the disease. We show in this review that ≈50 human genetic diseases involving defective enzymes can be remedied by high concentrations of the vitamin component of the coenzyme, and that this therapeutic technique can be applied in several other cases, including polymorphisms associated with disease risks, for which molecular evidence suggests that a mutation affects a coenzyme binding site.

The nutrients discussed in this review are pyridoxine (page 618); thiamine (page 625); riboflavin (page 627); niacin (page 632); biotin (page 637); cobalamin (page 638); folic acid (page 641); vitamin K (page 643); calciferol (page 645); tocopherol (page 645); tetrahydrobiopterin (page 646); *S*-adenosylmethionine (page 646); pantothenic acid (page 646); lipoic acid (page 647); carnitine (page 647); hormones, amino acids, and metals (page 648); and maxi B vitamins (page 649).

The proportion of mutations in a disease gene that is responsive to high concentrations of a vitamin or substrate may be one-third or greater (1–3). Determining the true percentage from the literature is difficult because exact response rates in patients are not always reported and much of the literature deals only with individual case reports. The true percentages depend on several factors, such as the nature of the enzyme, the degree of enzyme loss that results in a particular phenotype, how much a small conformational change disrupts the binding site of the particular enzyme, whether the binding site is a hot spot for mutations, and whether dietary admin-

MTHFR

- converts 5,10 MTHF to 5 MTHF
- co-factors are;
  - NADPH (from B3)
  - FAD (from B2)
- C677T SNiP affects FAD binding, not folate
  - in CT and TT B2 lowers Hcy

MTHFR

- >50% of migraineurs respond to B2
  - only 677 CT and TT

Factors that impair methylation

- Genetic
- Nutritional
- **Toxic exposures**

Volume 3, Issue 1

January 2003

REGULATORY RESEARCH PERSPECTIVES

Impact on Public Health

On the Chemical Causation of Methyl Deficiency and its Attendant Pathologies

Lionel A. Poirier [1], Luis A. Herrera [2], and Carolyn K. Wise [1]

[1] FDA's National Center for Toxicological Research (NCTR), Jefferson, Arkansas 72079,
[2] Investigacion Biomedica en Cancer, IB-INCan, Box 70-228, Mexico City 04510, Mexico (LAH)

Trans-HHS Workshop: Diet, DNA Methylation Processes and Health [*Journal of Nutrition,* 132 (8S):2329S-2484S, 2002].

- Genetic
- Nutritional
- **Toxic exposures**

- Antimetabolites
- Anticonvulsants
- Halogen compounds
- Heavy metals

Table I
Associations between disease and methyl insufficiency caused by diet or enzyme defects

PARAMETER	AGENTS	
	DIET	ENZYME DEFECT
Disease		
Cancer	+ (2-5,16,25,53,54)	+ (4,18,63,68-70)
Pancreatic toxicity	+ (6,29)	+ (66,67)
Atherosclerosis	+ (8,26-28)	+ (40,41,79)
Birth defects	+ (11,12,31,32,55,56)	+ (42,64,65)
Neurotoxicity	+ (14,15,33,34)	+ (73)
Biochemical alterations		
SAM/SAH	+ (3,18,19,33,34)	+ (19,20,30,73,74)
Homocysteine	+ (13,18,19,44-46,48,51,52,80,81)	+ (19,79)
DNA methylation	+ (3,11,16,17,19)	+ (19,20)
MTase	+ (82)	

TABLE III THE ANTICONVULSANTS				
PARAMETER	**AGENTS**			
	PHENOBARBITOL	**PHENYTOIN**	**DIAZEPAM**	**OTHER**
Disease				
Cancer	+ (157-160)	+ (161,162)	+ (163,164)	+ (163,165)
Pancreatic toxicity	+ (177,214,215)	+ (167)		+ (168-174)
Atherosclerosis	-/+ (178,179,181,182,184)	-/+ (182,184)	- (180)	-/+ (181,182,184)
Birth defects	+ (185-188)	+ (185-187,189-191)	+ (186,187,192)	+ (161,185,186,188-190,193,194)
Neurotoxicity		+ (196-198)		+ (199)
Biochemical alterations				
SAM/SAH	+ (200-202)	+ (202,203)	+ (204)	+ (193,205,206)
Homocysteine	+ (155,209)	+ (155,208,209)		+ (208-210)
DNA methylation	+ (201,211-213)			+ (205)
MTase				

TABLE IV POLYHALOGENATED COMPOUNDS				
PARAMETER	**AGENTS**			
	CCl_4	**DDT**	**DIOXIN**	**OTHER**
Disease				
Cancer	+ (216,217)	+ (158,231-236)	+ (250-254)	+ (278,296)
Pancreatic toxicity	+ (218,219)	+ (241-243)	+ (252,255,256,258)	+ (279-281)
Atherosclerosis		+ (244)	+ (262-264)	+ (244,282,283)
Birth defects	+ (221,222)	+ (246,247)	+ (251,272-274)	+ (284-287)
Neurotoxicity	+ (223-226)	+ (232,248,249)	+ (224,225,274-277)	+ (285,288)
Biochemical alterations				
SAM/SAH	+ (227)	+ (200)		
Homocysteine	+ (227)			
DNA methylation	+ (227)			+ (289-292)
MTase				+ (293)

- Organochlorines
- PCBs, PBBs
- DCA, TCA

TABLE V THE METALS				
PARAMETER	**AGENTS**			
	As	**Ni**	**Cd**	**Zn Deficiency**
Disease				
Cancer	+ (311-313,316,319,452,453)	+ (340,341)	+(353)	+ (385-392,454)
Pancreatic toxicity	+ (242,301-303,309,312)	+ (341,342,455)	+ (354-360,456)	+ (394-397,401,403,404)
Atherosclerosis	+ (264,298,299,307,308,312,322,453,457)	+ (343,344)	+ (361-368)	+ (398,399,406-411)
Birth defects	+ (337,453,458-460)	+ (345-347)	+(353,373,374,421,461)	+ (412-419,421)
Neurotoxicity	+ (312,336,453)		+ (375-380)	+/- (422-427)
Biochemical alterations				
SAM/SAH	+ (326,327,335,462,463)	- (349)		+ (428,429)
Homocysteine		- (352)		+/- (429,430)
DNA methylation	+ (313,315,316,323,326)	+ (350,351)		+ (429)
MTase	+ (328)	+ (350,351)	+ (384)	+ (384)

- Pb
- Hg

Nutrient-toxin interface

BRITISH SOCIETY FOR ECOLOGICAL MEDICINE
allergy environment nutrition

Journal of Nutritional Medicine (1990) *1*, 231–232

CLASSIC PAPER

Teratogenic Effects of Thalidomide in Rabbits, Rats, Hamsters and Mice

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Toxicology and Applied Pharmacology 1965; 7: 268–86

Reviewed by Stephen Davies MA BM BCH

In rats (Long-Evans and Dunning-Fischer strains) and hamsters, 150 mgkg of thalidomide from days 3 to 12 of pregnancy did not produce significant fetal changes.

In Long-Evans rats, rendered hypovitaminotic by diets deficient in riboflavine, pantothenic acid or a-tocopherol, the embryotoxic effect was enhanced by the daily peroral administration of 150 mgkg of thalidomide during pregnancy, as evidenced by the increased number of fetal resorptions or malformations.

Whilst as a profession we have learned, at least to some degree, the lesson of extreme caution in prescribing drugs during pregnancy, we have still not learned the other lesson which this paper, some 25 years after its publication, can teach us: that the way an individual reacts to an environmental challenge is, in part, dependent on his or her nutritional status.

Tests of drug and chemical mutagenicity are carried out on laboratory animals fed a nutrient-rich diet, whose nutritional status is presumably, therefore, adequate. Humans are then exposed to drugs and chemicals which have been passed as 'safe' on the basis of such laboratory experiments. However, this screening process evidently dangerously and unjustifiably assumes nutritional adequacy of humans exposed to such substances.

Nutritional deficiency *per se* has been demonstrated to be mutagenic. Nutrient deficiencies in conjunction with a chemical challenge may result in an increased rate of mutagenesis, and thus carcinogenesis and teratogenesis.