

Homocysteine and Cobalamin metabolism



- Homocysteine metabolism and related pathways
- Inherited disorders
- Clinical presentation
- Diagnostic approaches
- Treatment
- Homocysteine in common diseases

**THE FORMATION OF A HOMOLOGUE OF CYSTINE BY
THE DECOMPOSITION OF METHIONINE WITH
SULFURIC ACID***

BY LEWIS W. BUTZ AND VINCENT DU VIGNEAUD

(From the Laboratory of Physiological Chemistry, University of Illinois,
J. Biol. Chem. 1932 99: 135-142. *Urbana*)

(Received for publication, October 15, 1932)

Methionine upon being heated with strong sulfuric acid was

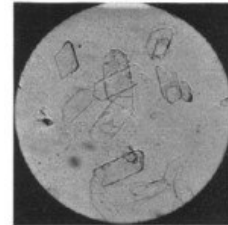
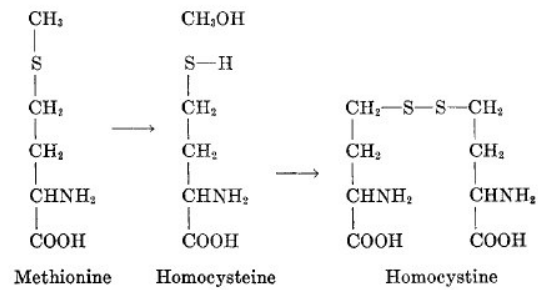
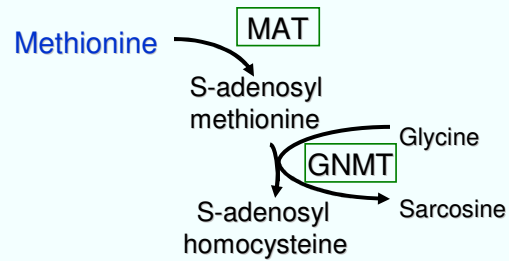


FIG. 1. Homocystine crystals. $\times 215$

Homocysteine originates from food



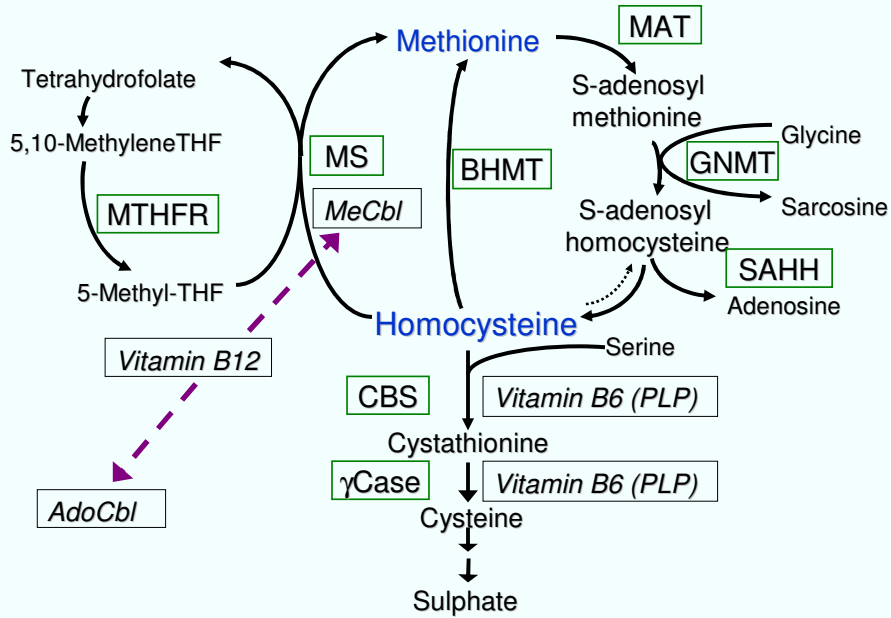
Outline of Homocysteine Metabolism



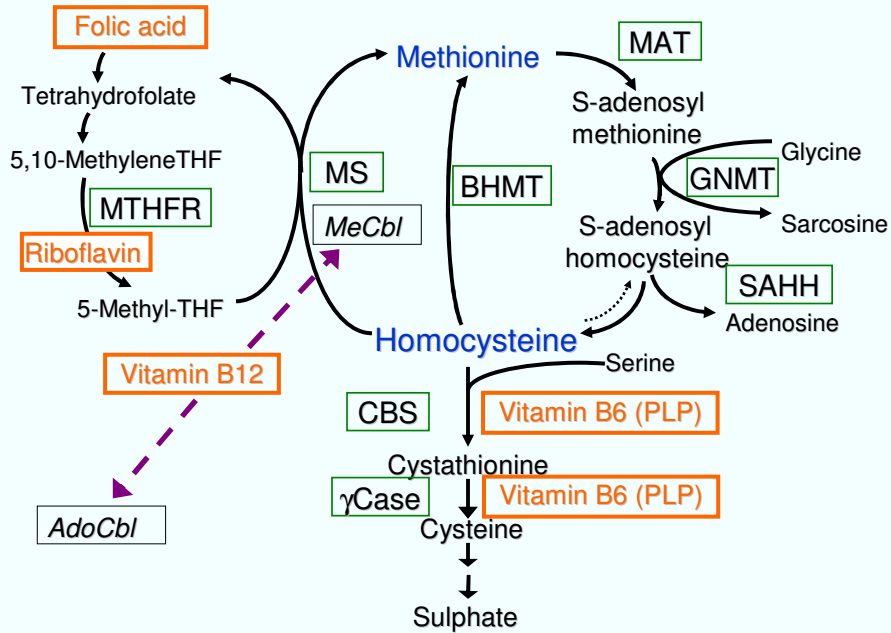
Examples of transmethylation reactions

Group	Methyl group acceptor / function
DNA	DNA-cytosine / gene expression regulation, inactivation, imprinting
RNA	mRNA-guanine / capping of mRNA tRNA-cytosine, -guanine, -adenine / alteration of tRNA flexibility
Proteins carboxyl groups	L-isoaspartate (D-aspartate) in protein repair
Proteins amino acid residues	lysine in histones arginine, e.g. in basic myelin protein
Lipids	Phosphatidylethanolamine / synthesis of phosphatidylcholine
N-methyltransferases	Guanidinoacetic acid / creatine synthesis
O-methyltransferases	Catechols e.g. norepinephrine, epinephrine, dopamine / inactivation

Outline of Homocysteine Metabolism



Vitamins Regulate Homocysteine Metabolism




Foods rich in Vitamin B12 (Cobalamin)

Bedarfsempfehlung der Deutschen Gesellschaft für Ernährung (DGE): **Daily requirement 3µg**

weil die Speicher den Menschen den meisten Mangelen

Metabolism most complicated

Vitamin B12 requirement lowest of all vitamins



4 g	Kalbs- oder Rindsleber
40 g	Miesmuscheln
70 g	Forelle
100 g	Schweinefleisch
150 g	Kalb- oder Rindfleisch
2	Hühnereier
150 g	Emmentaler
7,5 dl	Vollmilch

Foods that are rich in Folic acid

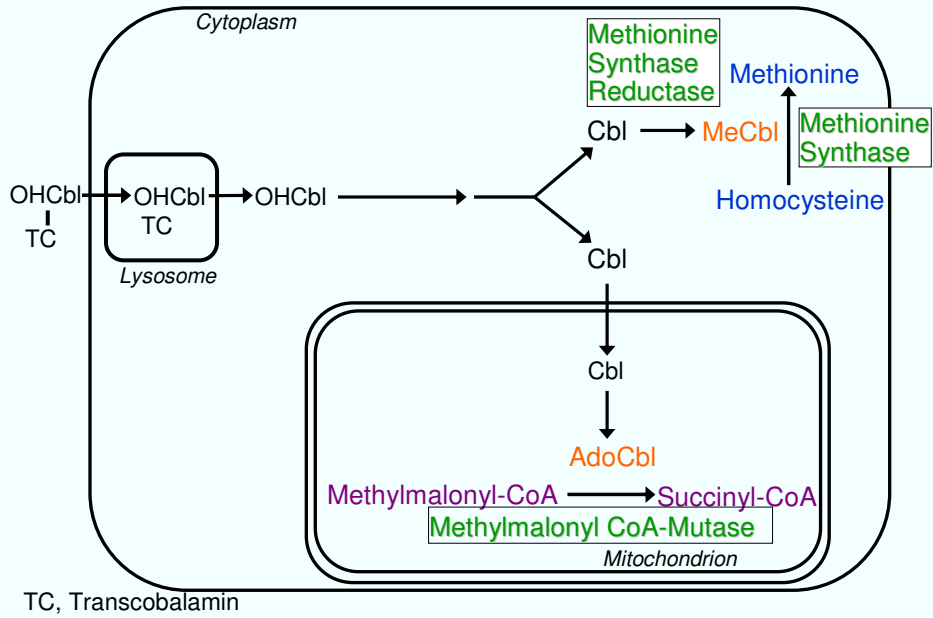
Bedarfsempfehlungen der Deutschen Gesellschaft für Ernährung (DGE): **Tagesdosis enthalten in:**
(roh, ohne Berücksichtigung der Bioverfügbarkeit)

Um das beim fähigen 4.1 Folsäure sich ben

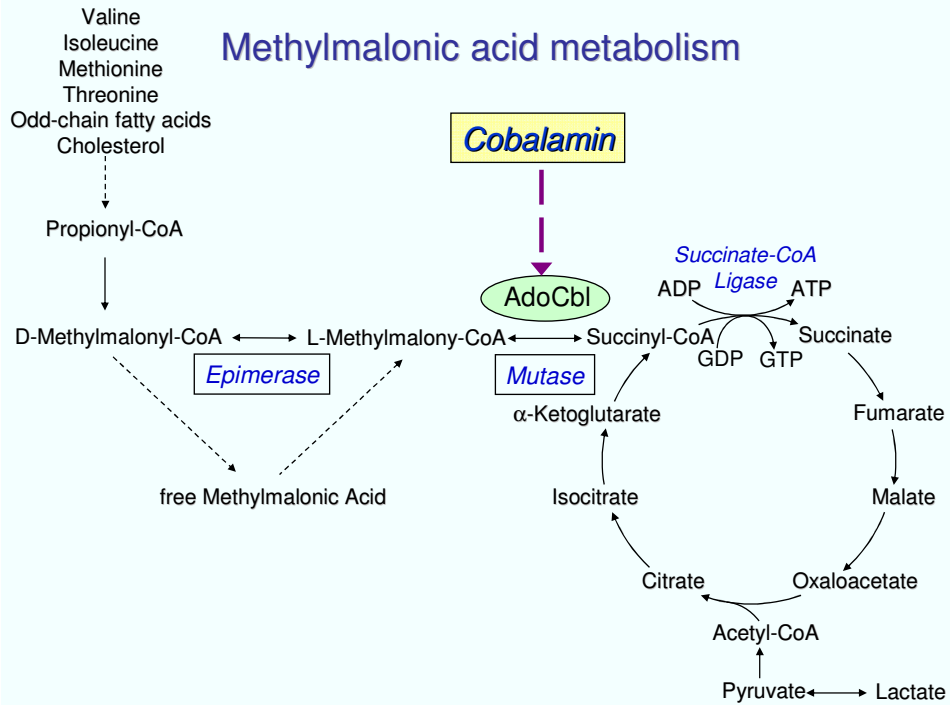


30 g	Vollkornknäckebrötchen
50 g	Rindsleber
90 g	Weisse Bohnen
140 g	Grünkohl
190 g	Grüne Erbsen
220 g	Vollkornbrot
240 g	Blumenkohl
270 g	Broccoli
400 g	Kopfsalat
450 g	Erdbeeren
1,5 kg	Kartoffeln

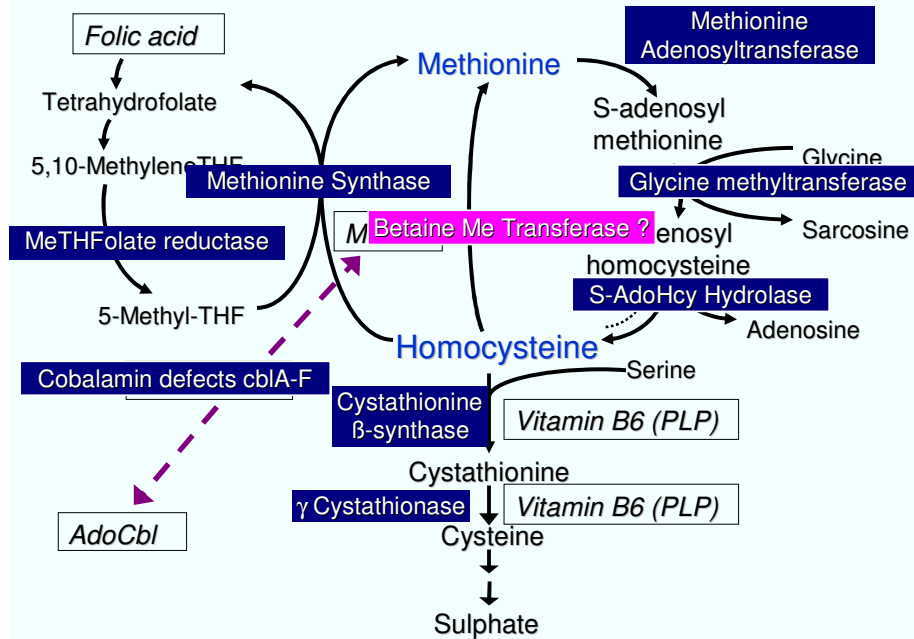
Intracellular Cobalamin Metabolism



Methylmalonic acid metabolism



Disorders of homocysteine metabolism



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Gene Identification for the *cb1D* Defect of Vitamin B₁₂ Metabolism

David Coelho, Ph.D., Terttu Suormala, Ph.D., Martin Stucki, M.Sc.,
Jordan P. Lerner-Ellis, Ph.D., David S. Rosenblatt, M.D.,
Robert F. Newbold, Ph.D., Matthias R. Baumgartner, M.D.,
and Brian Fowler, Ph.D.

N ENGL J MED 358;14 WWW.NEJM.ORG APRIL 3, 2008

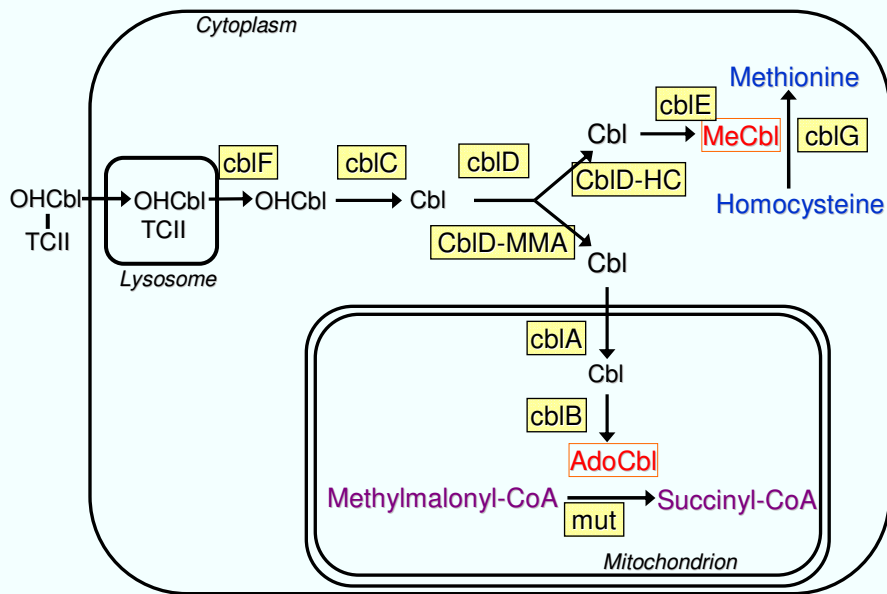
Identification of a putative lysosomal cobalamin exporter altered in the *cbIF* defect of vitamin B₁₂ metabolism

Frank Rutsch¹, Susann Gailus¹, Isabelle R Miousse², Terttu Suormala³, Corinne Sagné⁴, Mohammad Reza Toliati⁵, Gudrun Nürnberg⁵, Tanja Wittkamp¹, Insa Buers⁶, Azita Sharifi⁴, Martin Stucki^{7,8}, Christian Becker⁵, Matthias Baumgartner⁷, Horst Robenek⁶, Thorsten Marquardt¹, Wolfgang Höhne⁹, Bruno Gasnier⁴, David S Rosenblatt², Brian Fowler³ & Peter Nürnberg^{5,10}

- Mutations in the *LMBRD1* gene encoding LMBD1, a lysosomal membrane protein found in *cbIF* patients
- Transfection of *cbIF* fibroblasts wild-type *LMBD1* rescued cobalamin coenzyme synthesis
- *LMBRD1* identified as *cbIF* gene and suggests that LMBD1 is a lysosomal membrane exporter for cobalamin

Homozygosity mapping

Intracellular Cobalamin Metabolism



Disorders of Cobalamin metabolism

- **non-genetic** **Nutritional deficiency**
 - (strict vegetarian diet / Vegans)
 - reduced intestinal absorption (elderly persons)
- **genetic** **Disorders of Absorption and Transport**
 - Hereditary Intrinsic Factor Deficiency
 - Defective Transport of Cbl by Enterocytes (Imerslund-Gräsbeck Syndrome)
 - Haptocorrin (R Binder) Deficiency
 - Transcobalamin (TC) Deficiency

Disorders of Intracellular Utilization of Cbl

 - Combined Deficiencies of AdoCbl and MeCbl
 - AdoCbl Deficiency
 - MeCbl Deficiency

How to detect patients with homocysteine disorders

Selection by clinical suspicion

Simple and sophisticated methodology available

Wide range of clinical and biochemical variation

Mass Screening (Newborns)

Methionine measurement unreliable for:

some cases of CBS deficiency

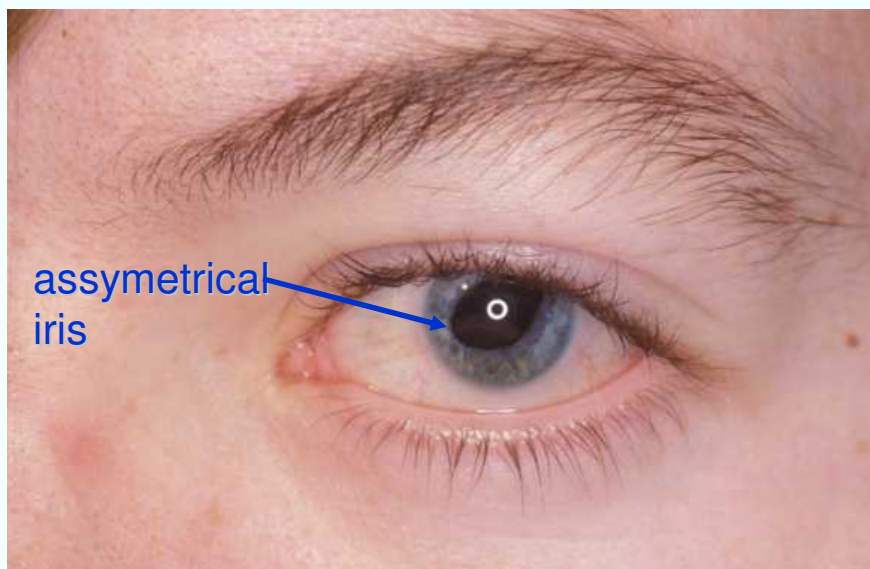
remethylation defects

New approaches needed

Clinical Features of Homocystinuria

System	Feature	Cause
Ocular	Lens dislocation Iridodonesis Glaucoma	↓ Cystine
Central Nervous System	Mental retardation Seizures Psychiatric disorders	Toxicity of ↑ metabolites
Skeleton	Osteoporosis Scoliosis Genua Valgum Arachnodactyly	Interference with Collagen "crosslinking" ↓ SO ₄
Vascular	Malar Flush Thromboembolism of Arteries and Veins	Homocysteine Toxicity on Endothelial cells

Homocystinuria Eye Damage

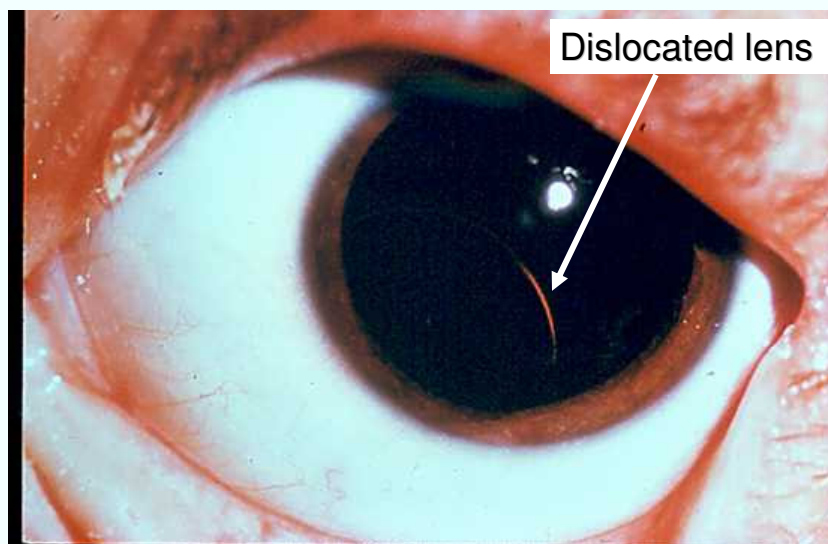


Homocystinuria

Eye Damage
Short
sightedness



Homocystinuria Eye Damage



Homocystinuria Eye Damage - Glaucoma



Homocystinuria Bone Abnormalities



Osteoporosis

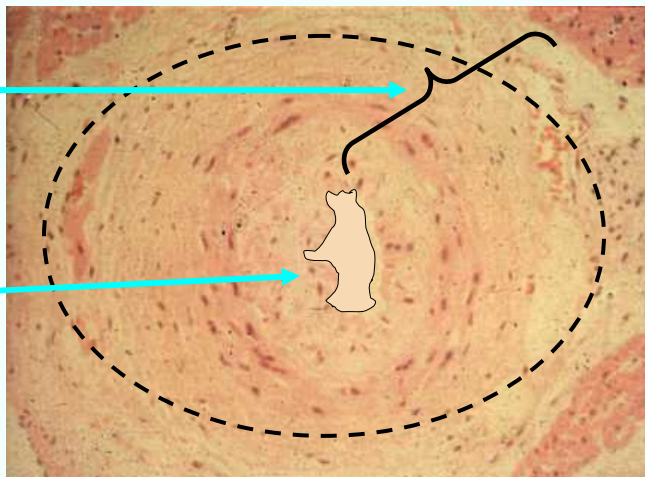
Homocystinuria (CBS Deficiency) A progressive multisystem disorder



Homocystinuria Arterial damage

“Arteriosclerosis”
thickened vessel
wall

Narrowing of
the lumen



Homocysteine Remethylation defects: Clinical Presentation

- Age of onset few weeks → adulthood
- Predominantly neurological abnormalities, encephalopathy
Psychomotor retardation, ataxia, lens dislocation not seen
- Skeletal changes usually not present
- Vascular changes sometimes seen
- Disordered folate metabolism
- Methionine, AdoMet ↓
- MethyleneTHF reductase def. - ↓ MeTHF
Methionine Synthase def. - reduced folates ↓
- megaloblastic anaemia

cbIF defect

13 patients

infections	↓ white blood cells
neurological abnormalities	megaloblastic anaemia
poor development	

Plasma Hcy ↑↑↑	Methionine ↓
cobalamin ↓ / Normal	
Urine Methylmalonic acid ↑↑↑	Schilling test abnormal

CblC defect: early clinical presentation

3.5 w. Feeding problems
temperature dysregulation
Pale, irritable, unconscious, dystrophic
poor growth
neurological abnormalities, tachycardia
anaemia

Plasma Hcy ↑↑↑ Methionine ↓
Urine Methylmalonic acid ↑↑↑
Treated OH-Cbl i.m 1mg/d. betaine, carnitine

4 months re-admitted to hospital
died one day later with multi-organ failure / hyperthermia

Prof. Sengers, Nijmegen

CblC defect, late Clinical presentation

Clinical

12y- 21y.

Unsteady gait, urinary incontinence
Spinal cord involvement, neuropathy
inability to walk
respiratory insufficiency (respirator)
Thought to have multiple sclerosis: Steroid treatment

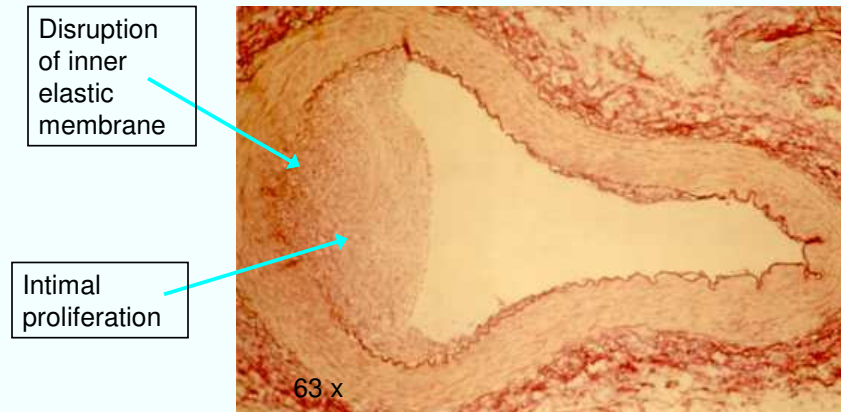
Laboratory

Urine MMA ↑↑↑
Plasma total homocysteine ↑↑
Treated i.m. OH-Cbl 500µg / d. - 10mg /week

Gold et al. 1995

Vascular changes in remethylation defects

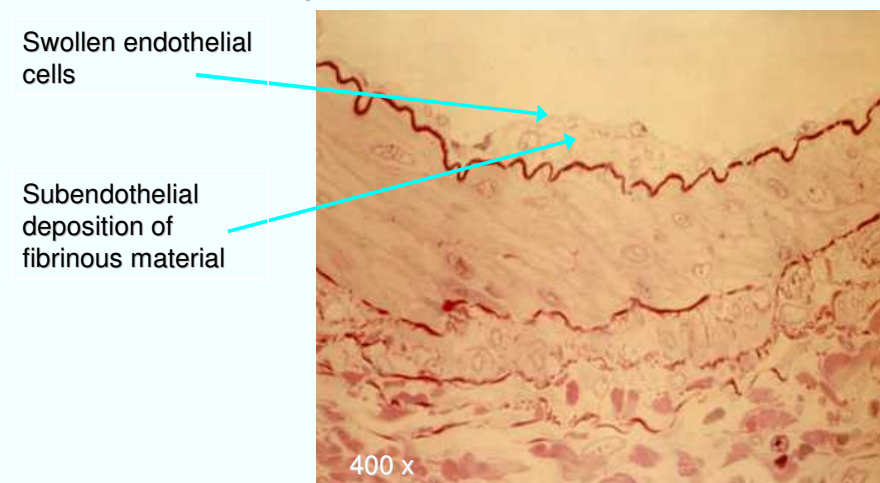
Section of the narrowed coronary artery of a 4 month old patient with the cb1C defect



Baumgartner et al. 1979 *Helv Pediat Acta* 34:465

Vascular changes in remethylation defects

Section of the small renal cortical artery of a 4 month patient with the cb1C defect



Baumgartner et al. 1979 *Helv Pediat Acta* 34:465

Intracellular Cobalamin Defects Patient with CblC



Diagnosed at 2 months

Microcephaly

Hypotonia

Feeding difficulties

Cerebral atrophy

Treatment

OH-Cbl (1mg./day)

Folate 10 mg./day

Betaine

Prof. G. Hüner (Istanbul)

8 months old

Methods for the Post- and Prenatal Diagnosis of the Homocystinurias

Routine laboratory parameters

Haematological, folate, cobalamin

Metabolite measurement

Sulphur amino acids in plasma and urine

Enzyme activities

Direct enzyme assays
Whole cell pathway assays,
Complementation analysis

Mutation Analysis

e.g. CBS, MR, MS, MSR deficiencies

European
Reference
Center

Methods for Diagnosis of the Homocystinurias Metabolite measurements

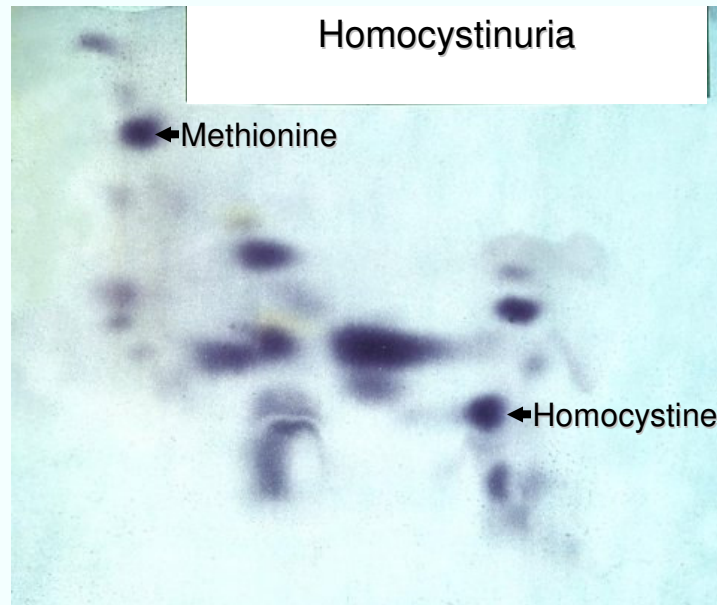
Urine

- Homocystine, methionine
Thin layer chromatography, electrophoresis
- Methylmalonic acid
Gas chromatography-mass spectrometry

Cyanide-Nitroprusside
Spot test for
Homocystine in Urine



2-dimensional thin-layer chromatography: Urine



Methods for Diagnosis of the Homocystinurias Metabolite measurements

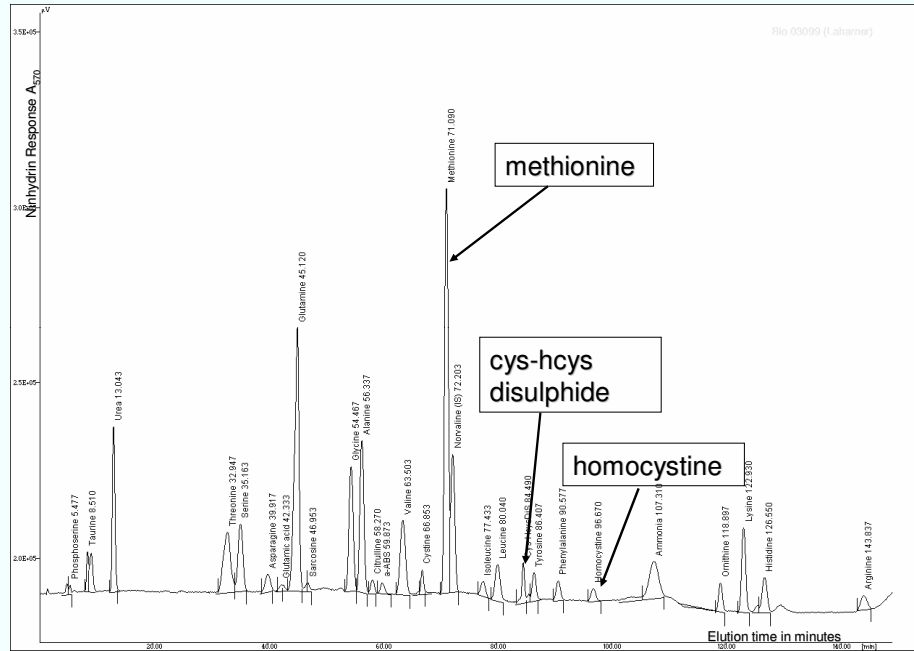
Urine

- Homocystine, methionine
Thin layer chromatography, electrophoresis
- Methylmalonic acid
Gas chromatography-mass spectrometry

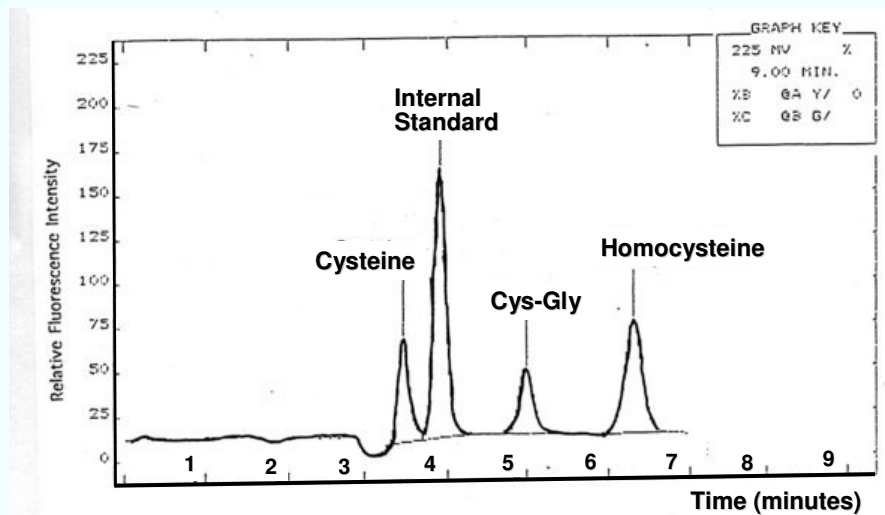
Plasma

- Free homocystine, methionine, cystine, Cys-Hcys disulphide
Ion exchange chromatography
- Total homocysteine
HPLC (mild hyperhomocysteinaemia)

Ion-exchange chromatogram Plasma1 Homocystinuria



HPLC Analysis of thiols in plasma derivatised with Fluoro-Benzo-oxa-Diazole-Sulphonic acid (SBDF)



Methods for Diagnosis of the Homocystinurias Specific Enzyme Assays

Cystathionine β synthase (Fibroblasts)	\pm Pyrdoxal 5' phosphate \pm S adenosylmethionine
Methylene THF reductase (Fibros., Leukocytes)	\pm Flavin-adenine dinucleotide \pm Heating at 46° (Thermolabile Mutation)
Methionine synthase (Fibros., Leukocytes)	\pm varying reducing agent for cblE

Good discrimination between control and homozygous affected individuals in most cases
Exceptions known, variant mild forms

Methods for Diagnosis of the Homocystinurias Indirect Pathway Assays in Intact Cultured Fibroblasts

Formation of methionine and serine from [^{14}C] formate

Remethylation defects (MR, MS, cblCD)

Complementation analysis

Cobalamin uptake and coenzyme formation

cblC/D, cblF, cblE, cblG (MTHFR def.)

[^{14}C] propionate incorporation into cell proteins

\pm Hydroxo-Cbl

cblC/D, Methylmalonic acidurias

Treatment of CBS deficiency Homocystinuria



Treatment Strategies in the Homocystinurias

Cystathionine synthase deficiency

Pyridoxine 600 mg/d oral

Folic acid 5mg / day

Methionine intake restriction -intensive as practicable

(compliance difficult) 160 - 900 (median 230) mg /d

Betaine Maximum effect 150 mg/kg/ day, in 2 doses

(Bonham et al. pharmaco-kinetic model)

Vitamin B12

Cystine supplementation (debatable value but not harmful)

Treatment Strategies in the homocystinurias

Methionine synthase deficiencies

hydroxocobalamin (intramuscular) 1 mg / d - week
biochemical response in virtually all reported patients

betaine should be given additionally if sufficiently high methionine levels not reached

folate in high oral doses (30 mg/day), can be helpful in isolated defect

methionine supplementation (50 mg/kg/day)

Treatment Strategies in the Homocystinurias

Methylenetetrahydrofolate reductase deficiency

Betaine (as for cystathionine β -synthase deficiency),

Methionine supplementation when its levels remain low on betaine

Pyridoxine beneficial in lowering homocystine levels in isolated cases

Folate (30 mg/d oral) effective in exceptional "folate-responsive" late-onset patients

Treatment Strategies in the Homocystinurias

Monitoring

Plasma total homocysteine (most sensitive and reliable)

Aim of treatment (CS, MR, MS deficiencies)

reduce total homocysteine levels to normal ($<15\mu\text{mol/L}$).

In practice levels below 40 - 50 $\mu\text{mol/L}$ are achieved rarely, only in mildly effected cases

Dilemma

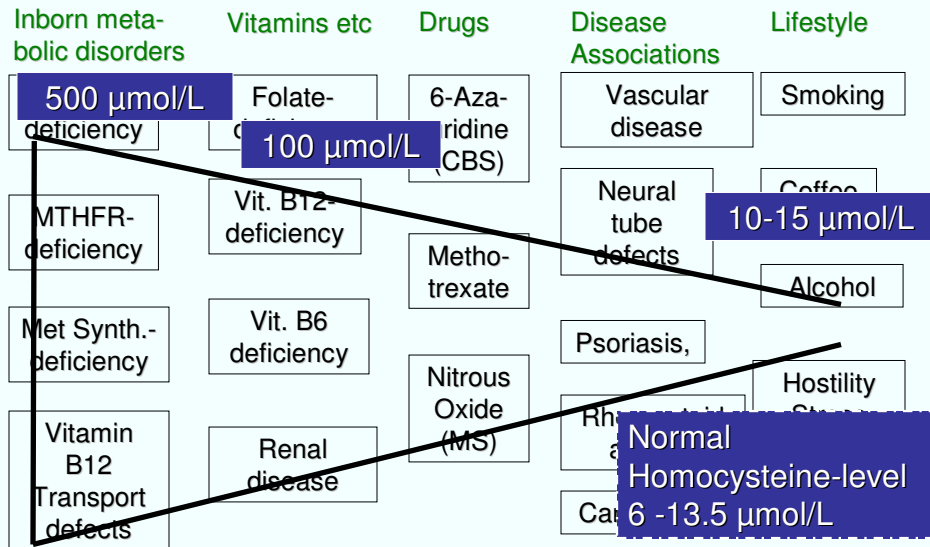
Moderately increased homocysteine (15-30 $\mu\text{mol/L}$) as risk factor for vascular disease.

Consequence for treatment of the homocystinurias ?

Conditions associated with increased homocysteine levels

Inborn meta-bolic disorders	Vitamins etc	Drugs	Disease Associations	Lifestyle
CBS-deficiency	Folate-deficiency	6-Aza-uridine (CBS)	Vascular disease	Smoking
MTHFR-deficiency	Vit. B12-deficiency	Metho-trexate	Neural tube defects	Coffee
Met Synth.-deficiency	Vit. B6 deficiency	Nitrous Oxide (MS)	Psoriasis,	Alcohol
Vitamin B12 Transport defects	Renal disease		Rheumatoid arthritis	Hostility Stress
			Cancer	

Conditions associated with increased homocysteine levels



Homocysteine: vascular disease risk factor ?

Ongoing studies – clinical effect of reduction of homocysteine by B-vitamin treatment

Schnyder, G., M. Roffi, et al. (2001). "Decreased rate of coronary restenosis after lowering of plasma homocysteine levels."

Lange et al. (2004) "vitamin-administration led to an increased risk of restenosis"

negative results

VISP Studie - JAMA 2004;291:565–575.

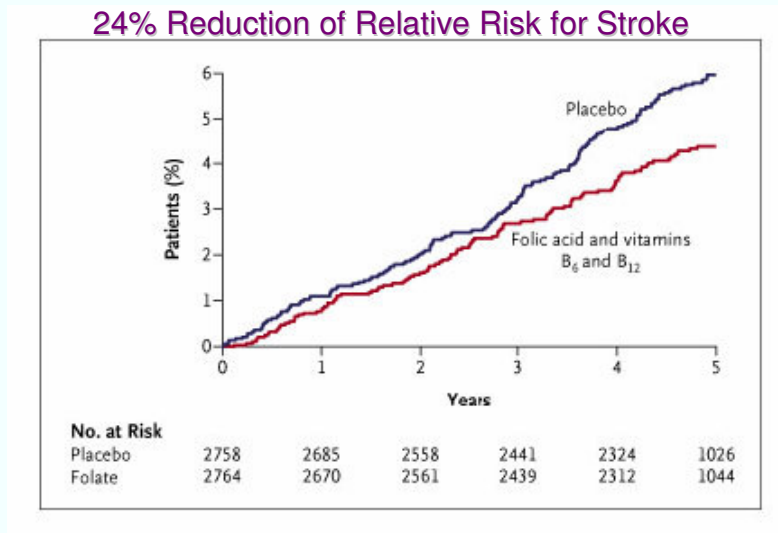
NORVIT Studie - N Engl J Med 2006; 354:1578-1588.

HOPE-2 Studie - N Engl J Med 2006; 354:1567–1577.

positive results

Improvement in Stroke mortality in Canada and US, 1990-2002 (Circulation 2006;113:1335 → 9.3% reduction of stroke following fortification flour with folic acid (1998)

Homocysteine: risk factor for vascular disease ?



Probability of cerebral ischaemia within 5 years
Placebo versus vitamin treatment. HOPE-2

The English Breakfast



The Swiss Breakfast



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