The Changing Clinical Paradigm of Vitamin B12 (Cobalamin) – Folate Interactions

Neurologic Deficit, Dementia, Vascular Thrombosis and Cancer

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Deficiency of folic acid (folates) or Vitamin B_{12} (cobalamins) are known to be the prime etiologic mechanisms for a macrocytic anemia due to a megaloblastic bone marrow. The past decade has witnessed an important paradigm shift in the clinical features and expression of such deficiencies providing new information on the metabolic sequalae. As a result of these observations, a better understanding of the extent of altered absorption, of folates and cobalamins, recognition of significant neurologic sequalae in the absence of anemia, characterization of the role of these entities in premature occlusive vascular disease, and recently an important correlate with neoplastic transformation has emerged.

I. Classical Patterns of Cobalamin (Vitamin B_{12}) and folate (folic acid) Deficiency

The classical clinical expression of cobalamin or folate deficiency has always been related to the occurrence of bone marrow megaloblastosis and a resultant macrocytic anemia. The recognition of a macrocytic process in the peripheral blood (ie mean corpuscular volume (MCV) > 96 fl/red cell) classically served as the "trigger" to the consideration of an underlying megaloblastic state, particularly when other hallmarks such as macro-ovalocytosis and anisocytosis (with an increased RDW) were found. Since a variety of other clinical states produce macrocytosis (Table I), these need to be considered in the delineation of the red cell changes.

Table I
Causes of Macrocytosis

Artifactual:	Elevated blood sugar Cold Agglutinins
Deficiency or Defect:	Folic Acid (folate) Vitamin B_{12} (Cobalamin)
Induced Defects in DNA:	Chemotherapeutic Drugs Anti-viral Drugs Anti-convulsant Drugs

Metabolic Alterations:
Myelodysplastic Syndromes
Liver Disease
Alcohol
Post-Splenectomy
Hypothyroidism
Defects in Thiamine Metabolism

The presence of hypersegmentation of the granulocytes (six or more nuclear segments) or "twinning" and an associated leukopenici and thrombocytopenia support megaloblastic marrow changes as the cause for the macrocytosis. The

defining feature of the bone marrow is megaloblastosis. Megaloblastic hemotopoiesis is characterized by defective DNA synthesis with continued (permissive) RNA synthesis resulting in large cells of all lineages and evident nuclear dyspoiesis. The anemia that results from the defective erythropoiesis (Table 2) is both hypoproliferative and hemolytic. Thus, there are features of a hypoproliferative defect such as a decrease in red cell production and maturation (with a significant shift to immature erythroblasts) and a relative and absolute reticulocytopenia. The hemolytic component (eg shortened red cell survival) appears to largely be due to ineffective erythropoiesis with significant increased intramedullary cell death (in the marrow) and increased peripheral destruction of red cells due to their defective membrane architecture. These changes result in an increased indirect bilirubin, increased lactic dehydrogenase, and an elevated serum ferritin and serum iron, and hyperuricemia (1,2). The morphologic features can be remarkable. The structural abnormalities in the megaloblastic marrow are not unlike some see in myelodysplasia. In addition, the hypercellularity and remarkable nuclear changes have led to the mis-diagnosis of leukemia.

Table 2
Hematologic Features of Megaloblastic Anemia

Megaloblastic Hematopoiesis:	Defective DNA synthesis: large cells with nuclear dyspoiesis
Macro-ovalocytic anemia:	Ovalocytic large red cells: ↑ MCV with anisocytosis (↑ RDW)
Hypoproliferative:	↓ Reticulocytes
Hemolytic:	↑ Indirect Bilirubin ↑ LDH ↑ Ferritin/Iron ↑ Uric Acid
WBC's:	Hypersegmented PMN's Nuclear Twinning
Platelets:	↓ production

Following recognition of a megaloblastic state, the clinician needs to determine the etiologic mechanisms (Table 3). The clinical features often help focus the laboratory delineation of cause. However, the vast majority of patients have deficiency or defective metabolism of cobalamin or folate as the cause for the megaloblastic state.

Table 3

Etiologic Mechanisms of Megaloblastic States

Deficiency or defects in transport or metabolism of Cobalamin (Vitamin B_{12})

Deficiency or alteration of folate metabolism

Alteration of reductive conversion of ribotide to deoxyribotide
Erythroleukemia (Di Guglielmo syndrome)
Arsenic intoxication
Alterations of orotic aid metabolism
Idiopathic (refractory) megaloblastosis

The clinical features of cobalamin and folate deficiency are shown in Table 4. The megaloblastic anemia is the same regardless of cause and no morphologic features differentiate the two. It is the presence and pattern of the neurologic abnormalities that serves as the best clinical differential diagnostic parameter.

Table 4
Clinical Features of Cobalamin and Folate Deficiency

Cobalamin (B12)	Megaloblastic Anemia	Folate
Postero-lateral spinal column dysmyelinization (paresthesia, loss of vibratory, positional sense, deep tender reflexes, and ataxia)	Neurologic Lesions	Congenital neural tube defects
Peripheral neuropathy		
Cerebral defects (depression, irritability and memory loss)		
Glossitis and papillary atrophy of tongue		
Hyperpigmentation of skin		Increased spontaneous abortions and abruptio placentae
Pseudotumor cerebri (headaches)		
		Decreased weight and body length of infant

Thus, the presence of neurologic deficit essentially defines cobalamin deficiency in the adult. Historically, the focus was on the occurrence of posteo-lateral column dysmyelinization as the governing defect for the neurologic changes. Part of the changing paradigm of cobalamin deficiency is the current common presentation with peripheral neuropathy or with cerebral cognitive defects.

Glossitis, at times a painful "beefy" red tongue with associated papillary atrophy, is seen in cobalamin deficiency and has been related to a defect in DNA synthesis. Although this mechanism has not been proven, symptomatic improvement occurs, but within a few days when proper therapy is instituted. Hyperpigmentation of the skin especially skin creases was shown to be due to the defect in DNA synthesis by the late Dr. James Gilliam (3).

When the diagnosis and etiologic basis for the megaloblastosis is established, the clinician must then pursue the pathophysiologic mechanism that has produced the defect. These mechanisms, shown in Table 5, are in general caused by decreased intake or decreased absorption, although for folate an added basis is increased loss during renal dialysis, increased requirement during pregnancy, significant enterohepatic loss or in the alcoholic. The circumstance of alcoholism merits stress since folate deficiency is common in this setting and although largely due to poor intake, decreased enterohepatic re-absorption occurs. In time, there is also decreased hepatic folate storage due to the alcoholic liver disease and cirrhosis.

Table 5

Pathophysiologic Mechanisms of Cobalamin and Folate Deficiency

Cobalamin	Mechanism	Laboratory Evaluation
Deficient Intake:	Dietary Deficiency (Vegans: Absence of all animal protein)	History
Deficient Absorption	Intrinsic Factor (IF) Deficiency (ie P.A., gastric atrophy, long use of H2 blockers)	Serum IF Antibodies Gastric IF, post pentagastrin Schilling I, II
	Defective Food Proteolysis and B12 liberation (ie the "aged" stomach)	Schilling with Protein Bound B_{12}
	Cleavage of B12 – IF complex (ie stasis states: small bowel diverticulae fish tape work)	Schilling III
	Defect ileal Absorption Site (ie resection, enteritis)	Schilling I, II
Defective Transport:	Transcobalamin (TC) Deficiency	TC II assay
Enzymatic Defects:	Congenital defects of Cbl enzymes	Assays of Ado Cbl and methyl Cbl enzymes
	Acquired defects (ie nitrous oxide)	History
Folate:		
Deficient Intake:	Dietary Deficiency	History
Deficient Absorption	Malabsorption States	Small bowel biopsy
Increased Folate Loss	Drug Interference	History
Increased Folate Requirement	Renal Dialysis	History
	Pregnancy	History
	Alcoholism	History

It is very important to stress that in spite of the decades of study of the laboratory, in manifestations and pathophysiologic features of the megaloblastic state we still do not understand the specific mechanisms whereby the deficiencies of folate or cobalamin actually impair DNA synthesis, nor do we have an understanding of the molecular changes that are present (2,6).

II Cobalamin Absorption: Expanding Clinical Pattern of Mechanisms of Altered Absorption or Interference

The classical concepts of the facilitated absorption of cobalamin by gastric intrinsic factor (IF) (2,7,8) was a brilliant story in human physiology. We now know that this is a more complex multistep process. Thus, a variety of physiologic binders of cobalamins other than IF exist and actually are present in almost every body fluid. Current evidence is that cobalamins from food are liberated in the stomach and at the low pH of gastric juice bind to a family of B_{12} binding proteins largely available from salivary secretions; this binding serves to protect the B_{12}. As the gastric contents enter the small intestinal high pH site, the cobalamins are liberated from these (so called "rapid") binders, at least in part due to the function of pancreatic secretions (9). Gastric intrinsic factor has favorable binding characteristics to cobalamin at the high pH of the intestine, and a tightly bound IF- B_{12} complex forms and traverses the gastrointestinal tract to the terminal ileum where receptor activated endocytic absorption occurs. Interference with this mechanism can occur if there is untoward cleavage of the complex by selected parasites (eg Diphyllobothrium latum) or inappropriate bacterial utilization (ie as in duodenal diverticulae). It is of interest that B_{12} is synthesized by normal intestinal flora; and, in addition significant conservation of cobalamin occurs because of the enterohepatic circulation (2, 10, 11, 12).

A. <u>The "Aging" Stomach</u>

Over two decades ago, Doscherholmen and colleagues (13, 14, 15) demonstrated that the absorption of food B_{12} was significantly different than crystalline B_{12} (as is used in the Schilling test). This was subsequently confirmed and the complex relationships of the liberation of food B_{12} for appropriate intrinsic factor mediated absorption began to be recognized and understood (16, 17, 18). Carmel has carefully examined the issue and has helped define the concept of "aging stomach" (19, 20, 21). His data to implies that as many as 5 million Americans over the age of 60 have subnormal cobalamin concentrations (22)! In essence, the release of cobalamins from food proteins requires pepsin activity at a low pH. Decreased gastric acidity with aging may be the most important aspect of such a decreased "extraction of food B_{12}", but clearly it is not the only factor (23). Whether this mechanism should be acknowledged as a natural consequence of aging is presently argued. Other separate events also occur with aging that include a decrease in cobalamins bound to the normal physiologic B_{12} transport protein, transcobalamin II; and decreased hepatic coenzyme formation (24). Nevertheless, it is now evident that decreased oral absorption of cobalamins occurs with aging and this is separate from the issues of intrinsic factor secretion. The clinical issue of note is that patients with the aging stomach have normal

absorption of crystalline B_{12} while their absorption of fond B_{12} is reduced. As expected, their Schilling tests are normal. In addition, they have an excellent physiologic response to oral vitamin B_{12}.

B. Other Factors affecting Cobalamin Absorption

Prolonged therapy with inhibitors of histamine – 2 receptors or the gastric proton pump result in cobalamin deficiency which is reversible with cessation of therapy (25). Although the development of deficiency depends on the duration of treatment and the existent tissue stores, two weeks of therapy with a proton pump inhibitor does result in an almost complete malabsorption of B_{12} (from 3.4% to <0.4%). Although Heliobacter pylori infection has serially been proposed as the new "era" mechanism for B_{12} malabsorption, most studies have failed to implicate this sequence (26).

C. Factors affecting Cobalamin Integrity Nitrous Oxide Exposure

In 1956 Lassen et al described an unusual event; that is, when prolonged exposure to the anesthetic nitrous oxide (N_20) was given as a form of treatment for patients with tetanus their bone marrows became pancytopenic and megaloblastic (27). This has subsequently been confirmed in patients whose exposure was merely that of nitrous oxide during open heart surgery (28). Almost immediately a rapid onset of neuropathy with such exposure was described (29). Perry et al (30, 31, 32) showed that even brief exposure of N_20 resulted in the abrupt inactivation of coenzyme methylacobalamin with the resultant immediate interference with DNA synthesis and resultant megaloblastosis. Precedence for such a prompt effect had been previously reported by our laboratory (6, 33). Parenthetically, since sudden neurologic deficit was also seen in these patients (29), the implication drawn was that the methylcobalamin methyltransferase biochemical pathway was the basis for the neurologic deficit in B_{12} deficiency. Subsequently, we identified a similar functional inactivation and interference with the methylmalonyl coenzyme – mutase reaction (34). It is of interest, that severe neurologic deficit (Brown-Sequard like syndrome) has been seen with N_20 used for only 2 – 3 hours for oral surgery in an otherwise normal woman (35).

AIDS

Reduced Vitamin B_{12} and folate levels have been seen in approximately 15% of patients with AIDS. Although this has generally been attributed to malabsorption and the inter-relationships with multiple drugs utilized in their therapy, some evidence suggests that a decrease and functional alteration in transport (ie such as transcobalamins) proteins also occurs (36).

III. Physiologic Issues Relative to cobalamins and Folates Vitamin B_{12}

The average American diet contains 5 – 30 μg of cobalamin. The average absorption has been considered to be 2 – 4 μg/day and the projected daily requirement is 0.5 to 1.0 μg /day (37). Issues relative to the efficiency of the

enterohepatic circulation make these numbers far from precise, since it has been projected that 1 to 10 mg of cobalamins are "re-cycled" daily (2).

Folate

The folate content of the mean American diet is said to be 280 μg for men and 210 μg for women. Although previous estimates of "daily requirements" had been 400 μg /day, recent daily recommendations have been 200 μg /day for men and 180 μg /day for women (2, 38, 39).

It is noteworthy that folate levels begin to decline within 2 weeks of deprivation. Indeed, acute clinical deprivation is a recognized event in intensive care units. Studies of this rapid deprivation suggests that our daily needs are closer to 400 μg per day and that the mean storage content is approximately 5000 μg (38, 39).

IV The Diagnosis of Cobalamin and Folate Deficiency

Laboratory tests to define the etiologic basis for megaloblastic anemia have largely been in transition as a clearer recognition of deficiency of B_{12} or folate have been understood.

Table 6

Laboratory Diagnosis of Megaloblastic Anemia

Deficiency:

Cobalamin (B_{12})		Folate
	Macro - Ovalocytic Anemia Hypersegmentation of PMN's Ineffective Erythropoiesis Ineffective Hematopoiesis "Megaloblastic Bone Marrow"	
Decreased Serum B_{12}		Decreased RBC Folate Serum Folate
Elevated Serum Methyl - Malonic Acid (MMA)		Normal MMA
Elevated Serum Total Homocysteine (HCYS)		Elevated HCYS
Serum Intrinsic Factor Antibody		
Elevated Serum Gastrin		
Abnormal Schilling Test		
Abnormal Absorption of Food Bound Cobalamin		

Recent evidence has confirmed the value of a lower serum B_{12} value in patients with megaloblastic anemia. However, evaluation of patients with little or no hematologic abnormalities, but with a variety of neurologic and/or psychiatric changes particularly in the older age group, has defined a significantly decreased sensitivity and specificity for the classical serum B_{12} assay (50-53).

Characterization of the only two metabolic pathways for cobalamin metabolism had long ago provided assays of intermediates of the two functional coenzymes, methylcobalamin (MeCbl) active in the homocysteine to methonine pathway, and adenosylcobalamine (Ad Cbl). In quite a clear derivative, cobalamin deficiency results in an increase in the serum methylmalonic acid and serum (or plasma) total homocysteine (HGCYS) (54, 55). Normally, serum methylmalonic acid (MMA) is undetectable (or as usually defined less than 0.4 m mol/L). An increase in serum and urine MMA has been shown to be highly sensitive and specific for the diagnosis of cobalamin deficiency. It is now clearly the gold standard in the clinical evaluation of suspected B12 deficiency. As would be expected, HCYS is also increased when tissue cobalamin deficiency exists.

The normal levels and ranges for homocysteine (HCYS) are shown in Table 7; and the causes of increased levels are shown in Table 8.

Table 7

Plasma Total Homocysteine

Normal:	**Range**	**Mean**
Men	6.5 - 15.8 µmoL/L	12
Women	5.7 - 16.5 µmoL/L	10
Hyperhomocysteinemia		
Moderate:	15 - 30 µmoL/L	
Intermediate:	31 - 100 µmoL/L	
Severe:	>100 µmoL/L	

Table 8

Causes of Elevated Plasma Total Homocysteine

Genetic Defects:	Cystathionine B-Synthase
	Methylene - Tetrahydrofolate reductase
Deficiency:	Vitamin B_{12} (Cobalamin)
	Folic Acid
	Pyridoxine
Impaired Renal Function	
Aging	
Smoking	
Heavy coffee consumption	

The laboratory diagnosis of folate deficiency has been even more difficult that that of cobalamin deficiency (54, 55). The serum folate assay is the commonly mode of measurement. Unfortunately, it is remarkably affected by a short period of dietary deprivation and/or recent alcohol ingestion. Red cell folate levels are a log greater than those in serum. It is invalid in pregnancy. In addition, even a slight degree of hemolysis will artificially increase the serum level in the assay because measurement of red cell folate provides a parameter of tissue folate status. Unfortunately, it is more difficult to perform and it is insensitive to issues relating to alcohol ingestion and pregnancy. Since the red cell life span is 120 days, the red cell folate measurement is a mean of the events over that prolonged period of time.

By contrast, measurement of HCYS levels are very sensitive and are an excellent assessment of folate deficiency when the serum MMA is normal. The assay of MMA and HCYS provide approximately a 99% sensitivity and specificity for the diagnosis of cobalamin or folate deficient states (56 – 62).

V The Current Paradigm of Patterns of Neurologic Deficit

One of the most significant shifts in the clinical paradigm of cobalamin deficiency has been related to the patterns of neurologic deficit. Classically, the neurologic lesion of cobalamin deficiency was a myelopathy expressed with the clinical neurologic features of combined system injury (63). The late John Lindenbaum (50, 51, 53) with other correlate observations (64 – 68) helped refine our understanding of the current clinical expression of neurologic abnormalities in cobalamin deficiency. There has been clear recognition that an early clinical manifestation of neurologic involvement is the presence of paresthesias often transient and recurrent, particularly of the hands (and then later the feet). These may occur with a description of transient ataxia, but on examination no neurologic findings are identified. Peripheral neuropathy is the most common neurologic deficit and these occur with measurable neurophysiologic changes (68) have been documented. Peroneal nerve abnormalities by physiologic evaluation are the most common and consistent finding (68); an issue of personal note, since our nerve studies in man were done on the peroneal nerve (69).

A second important change in the pattern of expression is the evidence that neuropsychiatric disturbances are very common, although difficult to measure (50, 51, 52, 53). Of particular note relative to these symptoms and signs is the general recognition that these neurologic and neuropsychiatric abnormalities occur in 10 – 15% of patients who have normal or borderline serum B_{12} values. However, they do have clear evidence of increased MMA and HCYS; and, a response to B_{12} therapy. In addition, approximately 25% of the patients do not have anemia or other hematologic stigmata of the B_{12} deficiency (50-67). It is of interest that the neurologic deficit seen in these patients who do not have anemia appear to be more severe then in the classical patient with pernicious anemia; and predict for residual neurologic findings after otherwise successful replacement therapy.

The mechanism(s) of the neurologic lesions in cobalamin deficiency have not been completely resolved. Because the neuropsychiatric abnormalities (such as paranoia, irrational behavior, and even clear dementia) clear rapidly, usually in 3 – 5 days following institution of therapy, not unlike the glassitis, it has been assumed these are on a metabolic basis, rather than a defendable neurologic abnormality. By contrast, all of the other neurologic findings clear very slowly; or, for advanced spinal column lesions, not at all. It is this broad constellation of neural deficits that have been the major focus of the pursuit of mechanisms.

Our own studies were based on the observations that when patients with pernicious anemia were treated purposely or inadvertently with folic acid, the anemia corrected, but fulminant and severe neurologic deficits ensued (69 – 71). On the basis of 60 such cases at New York Hospital, in which, when plantar responses were present, they saw no complete resolution of the neurologic lesion a "rule" was passed to begin "proper" treatment of megaloblastic anemias only with B_{12} (71). Since only two metabolic pathways of cobalamin metabolish exist in man and since the methylcobalamin pathway could be "bypassed" by folate therapy, we felt the likely mechanism for the neurologic lesion related to the adenosylcobalamin metabolic pathway.

Our studies in nerve biopsies from normal volunteers and patients with pernicious anemia demonstrated the presence of increased (abnormal) odd chain fatty acids. The degree of neurologic dysfunction and identified dysmyelinization of the nerves directly correlated with the measured amount of abnormal odd chain fatty acids (72). Subsequently, we were able to show that B_{12} deprivation had an unusual alteration in the enzymes of fatty acid synthesis since the deficiency results in a marked compensatory increase. (73, 74) In addition, the measured in vivo coenzyme A intermediates of the propionate pathway were approximately increased (75). Finally, an effect on the citrate synthase pathway carefully delineated by Paul Srere was also affected (76, 77). Thus, propionyl CoA which was increased in the B_{12} deficient state replaced acetyl CoA in fatty acid biosynthesis, resulting in the increased odd chain fatty acid production.

Furthermore, these observations provided correlative evidence that these fatty acid changes resulted in defective myelin synthesis and turnover and an ultrastructural pattern of the axon changes were secondary the classical pattern of neural injury associated with B_{12} deficiency. In addition, neurologic deficit resolution was consistent with the measurable myelin sheet turnover rates.

Studies of some congenital defects in cobalamin metabolism (78), and the attractive consideration that defective methyl group availability when the homocysteine – methionene shuttle was adversely affected by deficiency of methylcobalain led to extended focus on this metabolic site as the basis of the neural injury. Studies in our laboratory as well as in many others who espoused the methylcobalamin lesion however, failed to identify methyl deprivation as the basis of the neural lesion. There is now a return to and acceptance of the adenosylcobalamin defect as the biochemical site and basis for the neurological lesion in cobalamin deficiency (79).

VI Pregnancy and Cobalamin and Folate

An extensive review of the issues of Vitamin B_{12} and folate deficiency in pregnancy and obstetrics is in press and will not be re-reviewed here (80).

The classical concept of megaloblastic anemia in pregnancy was that it was incredibly rare because lack of B_{12} or folate resulted in infertility. As serum Vitamin B_{12} levels begin to be measured, it became evident that approximately 20% of women had low serum levels during the third trimester of pregnancy reaching nadir values at term (40, 41). The patients did not have other stigmata of cobalamin deficiency and, in general, it was not clear what the true body economy of cobalamin was during pregnancy. Clearly, an important aspect of these low values was that they were an antifact of measurement related to changes in B_{12} binding proteins during pregnancy (41), since measured values increased by 3 to 4 weeks post-partum with no specific therapy. Chanarin (82) demonstrated active cobalamin transfer to the fetus with measurable cord levels two fold that of the mother. The placenta does have active receptors for transcobalamin II, so an active transport mechanism could be defined.

Folate deficiency was similarly recognized during pregnancy; again, the problems in measurement of serum folate were even more complex than those related to B_{12}. Pritchard and his colleagues extensively described the Dallas experience and began an important focus on fetal wastage and fetal malformations (83).

It was, however, the spectacular observation of Smithhells and coworkers (84) who in 1976 found significantly lower red cell folate levels during the first trimester of pregnancy in 6 women who subsequently gave birth to infants with neural tube defects, when compared to controls. Their subsequent studies defined by 1981 that neural tube defects could be prevented by periconceptual vitamin supplementation (84).

VII Hyperhomocysteinemia and Vascular Occlusive Lesions

The link of hyperhomocysteinemia to vascular disease was importantly focused by the observations that patients with the rare inborn error of metabolism cystathionine B-synthase deficiency was associated with an increase in vascular occlusive lesions. The causes and clinical correlates are shown in Table 9. The clinical relationships of hyperhomocysteinemia as well as the characteristics of the vascular bed have been well reviewed (85, 86). The observations from the congenital defects have been related to the patterns seen in the acquired lesions.

Table 9

Causes of Hyperhomocysteinemia

A. Inherited Defects
1. Enzyme deficiencies
   a. Cystathionine β-synthase
   b. Methylenetetrahydrofolate reductase
   c. Methionine synthase (Cbl E, Cbl G)
   d. Cobalamin coenzyme synthesis (Cbl C and Cbl D)
2. Transport defects
   a. Transcobalamin II deficiency
   b. Cobalamin lysosomal transporter (Cbl F)

B. Acquired Defects
1. Nutritional
   a. Cobalamin deficiency
   b. Folic acid deficiency
   c. Pyridoxine deficiency
2. Metabolic
   a. Chronic renal disease
   b. Hypothyroidism
3. Drug-induced
   a. Methotrexate and other folate antagonists
   b. Nitrous oxide and other cobalamin antagonists
   c. Azaribine and other pyridoxine antagonists

It is important to stress that in spite of extensive study, we do not know the specific pathophysiologic steps whereby hyperhomocysteinemia causes vascular injury. At least seven different effects of elevated homocysteine levels have been described. Harjai (88) has just reviewed these; they include: 1) Endothelial dysfunction; 2) Endothelial cell injury; 3) Smooth muscle cell proliferation; 4) Enhanced thromboxane A_2 formation; 5) Enhanced platelet aggregation; 6) Increased binding of lipoprotein (a) to fibrin; 6) Enhanced procoagulant action; and 7) Decreased (protective effect) of endothelial-derived relaxing factor.

Clearly, our focused interest relates to the critical role of methylcobalamin and folate intermediates in acquired hyperhomocysteinemenia, and the relationship to vascular occlusive disease. It merits note that in the data base on nutrient deficiencies of folate and/or B_{12} a significant association with thromboembolic disease has not been recognized. This is troubling to some who question the global equation of hyperhomocytsteinemia and vascular disease and thrombosis. However, the B_{12}/folate data relates primarily to patients with clear clinical megaloblastic states; in that event anemia and frequently thrombocytopenia may have had a protective effect.

The interest in cobalamin and folate has had a specific focus on a particularly important subset of patients. These patients have an enzymatic defect that further predisposes to hyperhomocysteinemia : 5,10 methylene tetrahydrofolate

reductase (MTHFR) defect. This enzyme is responsible for the methyl donor re-methylation of homocysteine to methionine. Genetic polymorphism of the enzyme has been identified in approximately 11% of the American population. A well defined gene mutation at C677T where a valine for alanine substitution produces a thermolabile form of the enzyme which results in decreased enzyme activity and elevated plasma homocysteine levels. The truly exciting aspect of this congenital defect is that folate and cobalamin supplements (ie environmental changes) can overcome the genetic predisposition and abnormality (89).

Although exciting, and folate and cobalamin supplements have already a commonly exploited therapeutic approach in some areas, definitive clinical correlative relationships have been difficult to define. In just the recent weeks, the Farmingham Study has shown non fasting homocysteine levels as an independent risk factor for strokes in the elderly (91) and data from Israel are in agreement (92). Careful epidemiologic reviews from Europe (93) and this hemisphere (94) express serious caution primarily focused on the still unresolved question as to whether nutrient supplements will alter the cardiovascular lesions or their natural history. An interesting confounding aspect of these studies is the recognition that prothrombotic polymorphisms, such as the founder lesions Factor V Leiden (nt 1691 G→A) or Factor II (prothrombin 20210 G→A) have clinically synergistic effects on thrombotic risk (94, 95).

Until randomized clinical trials of the risks of vascular disease and the role of folate-B_{12} supplements on these risks are completed, some caution continues to be appropriate.

VIII Folate and Cancer

It has long been known that B_{12} or folate deficiency produces the same megaloblast- like (ie increased RNA and defective DNA synthesis) changes in most non-hematopoietic tissues as those seen in the bone marrow. Indeed changes when viewed in non-marrow sites (particularly uterine cervix, gastric and oral mucosa) have at times been called cancer. The histologic characteristics are consistent with changes termed dysplasia in many tissues. Such dysplasia and subsequent neoplastic transformation has been considered to be the basis for the increased incidence of gastric adenocarcinoma in patients with pernicious anemia. This concept grew out of the observations of Massey and Rubin in 1954 (96) that the cytologic examination of gastric mucosal cells in patients with pernicious anemia who had had complete B_{12} repletement persistently were abnormal. Similar changes were seen in the uterine cervix, but those had complete reversal with therapy (97). The focus on dysplasia and its relationship to folate status was emphasized by a case-control study in patients with chronic ulcerative colitis, a lesion with a known 10% rate of malignant transformation. Lashner et al (98) showed that folate supplementation resulted in a greater than 60% reduction in the incidence of dysplasia and neoplastic transformation; and, moreover those on sulfasalazine therapy (known to inhibit intestinal absorption of folate) had the greatest risk of initial recognition of dysplasia.

The interest in the molecular characterization during the change of normal cells to dysplastic cells and then to a frank neoplasm has led to broadened interest in the role of folate deficiency at the cellular level, even when measurable levels in serum or red cells are normal. This is further highlighted by the evidence that increasing dietary folate to four times the identified basal requirement resulted in a progressive reduction in the evolution of macroscopic cancers from microscopi foci (99). Studies by Ames group (100) have demonstrated that folate deficiency caused massive misincorporation of uracil into human DNA and there were associated chromosome breaks, all markers of neoplastic transformation. Data from an adenoma prevention trial demonstrated that dietary folate had a significant protective association with the risk of recurrence of large-bowel adenomas; although excess supplementation did not further reduce the risk (101). Population epidemiologic studies are always difficult to evaluate. Nonetheless, the Harvard Nurses Health Study of over 88,000 women who were deemed free of cancer in 1980, when followed with dietary logs, were shown to reduce their relative risk for cancer to 0.25 for those on at least 400 μg of folate per day.

These gastrointestinal studies highlight the interest in focal site folate (or cobalamin) deficiency as a factor in the dysplastic changes. These are difficult studies to perform and all of the data to date must be considered interesting, but inconclusive.

IX Current Therapeutic Approaches (103)

General Principles of Therapy

The first and most critical therapeutic concern is the clinical stability of the patient who presents with severe anemia or rapidly progressive neurologic deficit, especially where an impending or "pseudo" spinal cord transection appears to be developing. Since megaloblastic anemias develop slowly, compensatory cardiopulmonary responses are often associated with only modest symptoms, even when patients present with hemoglobin levels below 3 or 4 grams per dL. Often the sense is to "quickly treat with multiple hematemics" while awaiting diagnostic data from the laboratory. The appropriate approach is to recognize that even with specific diagnosis and therapy, the red cell values will not improve for at least 7 to 14 days. The red cell needs must be judged solely on the cardiopulmonary and cerebral functional status of the patient; and, if required, cautious transfusion is the urgent treatment of choice. Commonly, only a single unit of packed red cells is needed. Transfusion(s) should be given slowly (over 3 to 4 hours), because rapid volume shifts may precipitate functional problems related to the precarious hemodynamic status of these patients.

Less commonly, the neurologic deterioration of the patient poses urgency of therapy. Such rapid progression virtually defines the etiology to be due to cobalamin. Fulminate neurologic progression can be seen with nitrous oxide anesthesia (which produces inhibition of cobalamin dependent enzymes) or with folate therapy inappropriately given when cobalamin was the cause of the megaloblastic anemia. Although rare, if progression appears rapid, serum should be collected and treatment with cobalamin instituted immediately.

The second important principle is the requirement for a specific etiologic diagnosis. This is important, since the pathophysiologic mechanism that produced the defect must be defined. Such delineation is critical in order to determine reversibility of the cause and the duration of therapy (i.e. short term, lifetime, etc.). Thus, (genetic) pernicious anemia will demand a lifetime of cobalamin replacement therapy whereas cobalamin deficiency due to jejunal diverticula can be approached with short term B12 therapy, antibiotics and the consideration of surgical repair. Similarly, the "aged stomach syndrome" can easily be managed with oral B_{12} supplements.

The third issue relates to an understanding of the rate and pattern of repair of the clinical abnormalities. When the anemia fails to respond in the expected time frame, the question of an incorrect diagnosis or an unrecognized associated lesion must be considered. Thus, iron deficiency goes unrecognized when associated with megaloblastosis; it will, however, result in suboptimial therapeutic response to the identified cause. This can be particularly noteworthy in cobalamin deficiency where patients with pernicious anemia. have an increased risk of gastric cancer; and, the finding of iron deficiency may provide the clue to its diagnostic pursuit.
Fourth, the serial follow-up of patients after therapeutic restitution requires an understanding of the natural history of the underlying disease status. Patient education to the need for therapy and follow-up can only be done when the physician understands the cause and mechanism. Such education is important, since the ease with which repair can be achieved, sometimes belies the significance of the problem.

For instance, pernicious anemia patients have an increased incidence of gastric cancer and have the potential to develop endocrinopathies (especially hypothyroidism and hypodrenolism) secondary to organ related auto-antibodies. Thus, they will need a lifetime of therapy and serial clinical evaluation.

Finally, the concept of a "therapeutic trial" in patients with megaloblastic states has evolved from the era of "shot gun" multihematemic therapy to our present view of the need to define the physiologic significance of a possible deficiency state. Subtle or atypical presentation of cobalamin deficiency, particularly in the elderly, where anemia may be absent and neurologic change prominent, as well as the absence of true tissue deficiency in some patients with low serum B12 values has expanded the need for a clear diagnosis. Metabolate assays are of particular value in such suspected circumstances. An elevated MMA and/or HCYS can be used as a parameter for a trial of therapy; correction of the defect should similarly correct the metabolic abnormality in 10 - 14 days. This allows affirmation of the diagnosis and confirms the presence of tissue deficiency. I do not favor therapeutic trials with <u>both</u> B12 and folate, since such approaches still leave the clinical dilemma of specificity. A sequential trial (first B12 and then, if needed, folate) allows reasonable characterization under these circumstances.

<u>Treatment of Cobalamin (B12) Deficiency</u>
Normal tissue cobalamin stores (primarily in liver bone marrow) range between 7 - 15 mg. Clinically significant deficiency is expressed when tissue stores are reduced to 30 - 50% of normal. The goal of therapy is to replete tissue stores. However, with each dose of cobalamin, the percentage of the given dose retained by tissues declines. Therefore, significantly greater amounts of cobalamin must be administered then one would

calculate from that known to exist in total body tissues. In essence, fractional urinary excretion of an administered dose increases as the stores are progressively repleted. The fractional retention is better when temporal gaps (ie daily or every few days) exist between doses. These physiologic issues help explain the variable repletement schedules found in the literature, and further allow the clinician to adapt a sequence most appropriate to the patient and the related clinical issues.

Since most of the mechanisms of cobalamin deficiency relate to decreased absorption the initial therapy should began with cyano (or hydroxy) cobalamin 1 milligram (1000 micrograms) given subcutaneously (SC) or intramuscularly (IM). This is rapidly absorbed from either site, with peak serum levels in approximately one hour after injection; and, following this initial dose approximately 65% will be retained. Intravenous injection produces a much greater urinary loss and should not be used. A simple repletement schedule from that point is 1 milligram given daily or every other day during the first two weeks and then weekly for the next month, by which time normal peripheral hematologic values are expected.

Thereafter, the pathophysiologic mechanism of the deficiency will determine the approach to future therapy. For most, where gastric intrinsic factor secretion is defective (ie pernicious anemia), cobalamin must be given for life. Monthly or bimonthly injections of 1 milligram provide simple, inexpensive, and effective therapy (subcutaneous or intramuscular) that requires no special monitoring. In patients with neurologic deficit, more frequent administration of cobalamin has been used in the first 6 months, a time when neurologic repair is at the maximum. It must be emphasized that such an increased frequency is empirical, with no supportive data. Similarly, shortening the interval between injections, often requested by elderly patients who express having an "improved sense of well being" with the treatment has no special support.

The elderly often have cobalamin deficiency due to ineffective liberation of cobalamin bound to protein in food. In these patients the absorption of crystalline B12 is normal. In such circumstances, as well as in the strict vegans (ie no animal product ingestion) patient, oral cobalamin can be utilized after tissue stores have been repleted with parenteral therapy. Oral 1 milligram (1000 micrograms) tablets are available for such use and should be given daily. We have successfully treated patients with the "aged stomach" with oral B_{12} alone (ie without initial parenteral repair) and, in general, 100 μg per day orally has been very successful.

Increased daily cobalamin requirements occur in pregnancy and lactation, thyrotoxicosis, and in liver or renal disease (especially where protein loss is extensive). Since tissue concentrations of cobalamin are in the milligram range and daily requirements in the microgram range, the normal stores are adequate for 1 - 3 years in the absence of supplementation. Deficiency is therefore uncommonly associated with such an increased need, except is pregnancy in the vigans patient, where cobalamin deficiency can occur in infants from a clinically asymptomatic mother.
Side effects from cobalamin therapy are incredibly rare. Patients with the very rare early Leber's disease (hereditary optic nerve atrophy) have been reported in the past to have increased atrophy with institution of high dose therapy. Rarely, pruritus and skin rash have occurred. Anaphylatic shock has been reported.

Short term sequalae of repletement therapy in megaloblastic states occur regardless of the etiology of the deficiency. These include hypokalemia and hyperuricemia, especially in the first 48 - 72 hours of institution of therapy therefore, potassium supplementation is wise when therapy is started.

Treatment of Folate Deficiency

Normal tissue stores of folate are approximately 5000 micrograms (5 milligrams) with a projected daily requirement of 100 - 200 micrograms. These limited stores result in folate deficiency more quickly with dietary deprivation, than in cobalamin deficiency. Since most clinical circumstances of folate deficiency are due to inadequate intake or drug interference, oral repletement is the usual mode, giving 1 milligram (or 5 milligrams) folic acid pills (the commonly available form) per day. In general, 1 milligram per day provides a significant excess, and allows repletement of tissue stores. In known malabsorption syndromes the 5 milligram daily folic acid oral dose is preferable. An intravenous formulation is available in 15 milligram dose form. Tissue stores can be repleted easily in a few weeks with daily oral therapy; and, therefore the duration of therapy is determined on the continued presence of cause.

Folate prophylaxis is recommended through pregnancy where at least 600 micrograms of folic acid per day is desirable, because of its potential to eliminate neural tube defects. If a previous pregnancy has been associated with a neural tube defect, it is recommended that 4 milligrams per day be used, beginning 4 weeks before the pregnancy and continuing at least through the first 3 months. High doses of folate (greater than 500 micrograms per day) have allegedly reduced zinc absorption; thus, mineral supplementation during pregnancy is appropriate. Another circumstance that merits folate prophylaxis is in patients on long standing anticonvalesent therapy. Folate deficiency has been associated with an increased fit frequency. This can be circumvented by giving one milligram per day of folic acid.

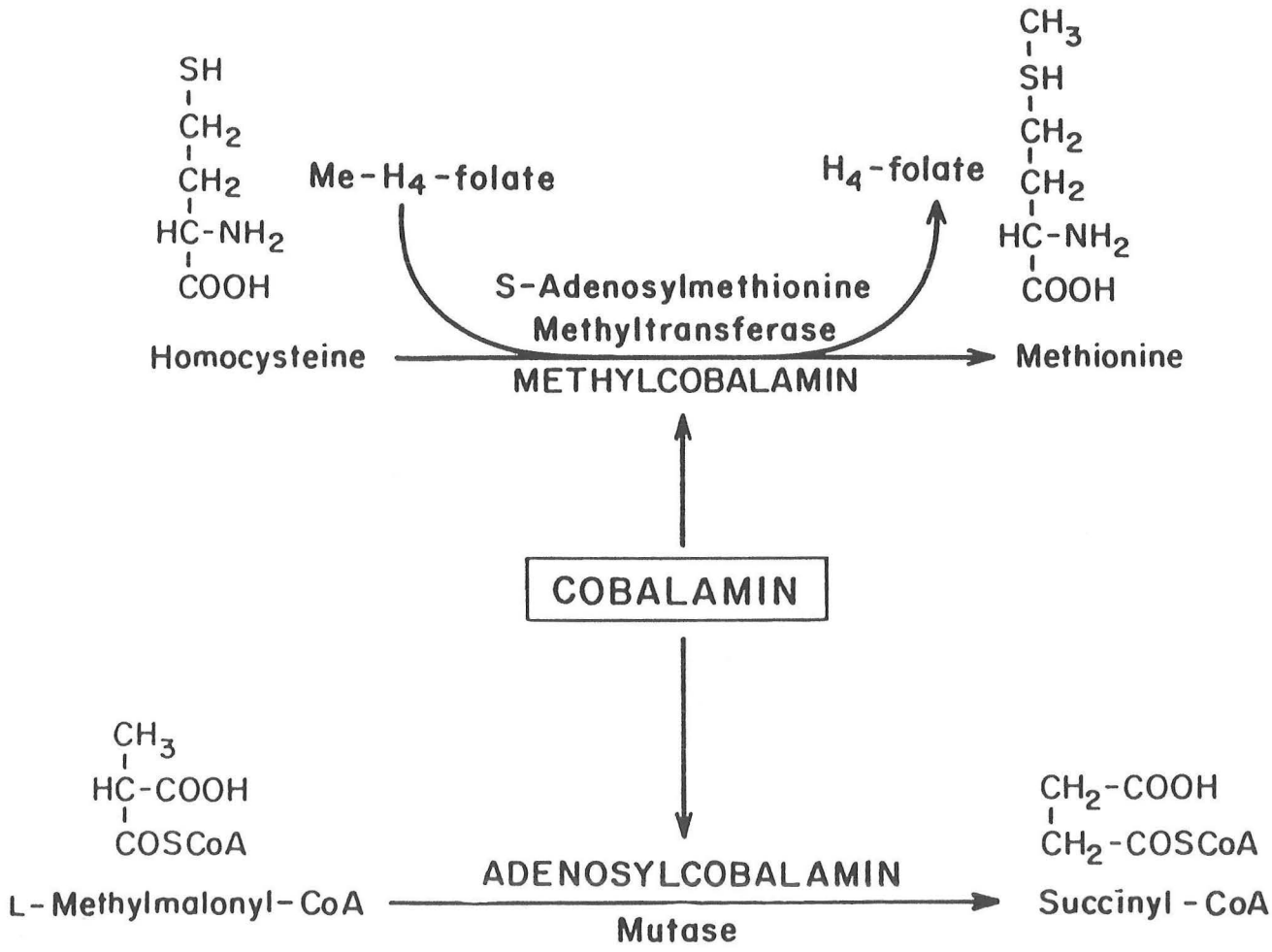
It again merits emphasis, that empiric folate therapy in megaloblastic anemia will repair the anemia, but if the correct diagnosis is cobalamin deficiency, a fulminant neurologic deficit may ensue.

Sequence of Repair After Therapy and Followup Care

The sequence of repair immediately following institution of therapy in megaloblastic anemia is shown in Table 10. Since an increased incidence of gastric polyps (up to 5%) and gastric cancer (2 to 3%) occur in patients with pernicious anemia, long term surveillance is needed. Less common is the development of endocrinopathy (especially thyroid or adrenal) secondary to the organ related autoantibodies. Such surveillance provides for continued patient education, treatment compliance, and confirmation that a reversible pathophysiologic mechanism has been corrected.

Table 10
Pattern of Repair of Megaloblastic Anemia with Therapy

Time	Response
8 - 12 hours	Decrease in serum iron and ferritin
12 - 36 hours	Hypokalemia; Hyperuricemia
24 - 48 hours	Normalization of bone marrow Normalization of deoxyuridine suppression
Day 2 - 3	Increased sense of well being and appetite
	Increased reticulocytes
	Decrease in indirect hyperbilirubenemia
Day 5 - 9	Reticulocyte Peak
Days 7 - 10	Decrease in serum lactic dehydrogenase
Day 7 - 14	Decrease in serum MMA and HCSY Increasing RBC Values
Weeks 2 - 4	Normalization of MCV Disappearance of hypersegmented polymorphonuclear leukocytes



Reactions catalyzed by Cbl coenzymes in mammalian tissues. Note the specificity of AdoCbl for the isomerization of methylmalonyl CoA and of MeCbl for the methylation of homocysteine. Me-H_4folate = N^5-methyltetrahydrofolate; H_4folate = tetrahydrofolate.

Historical Events in Pernicious Anemia

1855: Thomas Addison: Describes an unusual anemia in preface to monographon adrenal insufficiency.

1880: Paul Ehrlich: Described megaloblasts in the blood.

1883: Otto Leichtenstern: Described postero-lateral spinal column degeneration.

1887: George S. Hayem: Described macrocytes in blood.

1897: William Hunter: Emphasized presence of sore tongue.

1907: Joseph Arneth: Described multilobed PMN's

1908: Richard Cabot: Defined entire clinical picture in 1200 patients he studied.

1926: George Minot, William Murphy: Anemia abolished by diet (1/2 lb lightly cooked beef liver per day). (Presented at AAP on May 4, 1926. Nobel Prize: 1934)

1928: Edwin J. Cohn & G.H.A. Clowes: Cohn's liver fraction G contained the "active" material.

1929: William B. Castle: Described an "intrinsic factor" from stomach needed to correct PA.

1938: Lucy Wills & Barbara Evans: Nutritional anemia in pregnant women failed to respond to liver extract: thus, a second form of megaloblastic anemia.

1943: JJ Pfiffner & ELR Stokstad: Identified growth factor in yeast and green plants: Named it folic acid.

1947: Thomas Wood and Edward Rickes: Precipitated some "red junk" from liver.

1948: Karl Folkers: Identified and crystallized Vitamin B_{12}.

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