MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

19 September 1968

CLINICAL ASPECTS OF FOLIC ACID DEFICIENCY

I Historical Data of Interest:

During studies of a "tropical" macrocytic anemia in Hindu women in Bombay, India in 1930, Lucy Wills identified clinical responses to Marmite (autolyzed yeast) and to crude liver extract but not to a more purified form of extract known to be effective in classical P.A. This so called "Wills factor" was first clinical identification of folic acid:

1. Wills, L.: Tropical Macrocytic Anemia. Proc. Royal Soc. Med. 25:1720, 1932.

2. Wills, L., Clutterbuck, P. W. and Evans, P. D. F.: A New Factor In the Production and Cure of Macrocytic Anaemias and Its Relation to Other Haemopoietic Principles Curative In Pernicious Anaemia. Biochem. J. 31:2136, 1937.

Other studies identified growth factors and were reported as:
- Vitamin M (from dried Brewers yeast)
- Vitamin B_e
- Norite Eluate Factor of Liver (Extract)
- L. casei Factor
- Folic Acid - a term first applied by:

3. Mitchell, H. K., Snell, E. E. and Williams, R. J.: The Concentration of "Folic Acid". J. Am. Chem. Soc. 63:2284, 1941.
   - because it was isolated from a leafy vegetable - spinach.

Subsequently:

4. Hutchings, B. L., Bohonos, N., Hegsted, D. M., Elvehjem, C. A. and Peterson, W. H.: Relation of a Growth Factor Required By Lactobacillus casei to the Nutrition of the Chick. J. Biol. Chem. 140:681, 1941.
   - demonstrated that all of these factors were the same on the basis of their interchangeable ability to support bacterial and avian growth.

II The Sequence of Developing Folate Deficiency:

5. Herbert, V.: Experimental Nutritional Folate Deficiency in Man. Trans. Assoc. Amer. Phys. 75:307, 1962.
   This careful study of the development of folate deficiency proved that intestinal bacteria could not provide adequate folate for man and further delineated the sequence of abnormalities as they occurred:

Duration 5 µgm folate diet (L. casei assay):

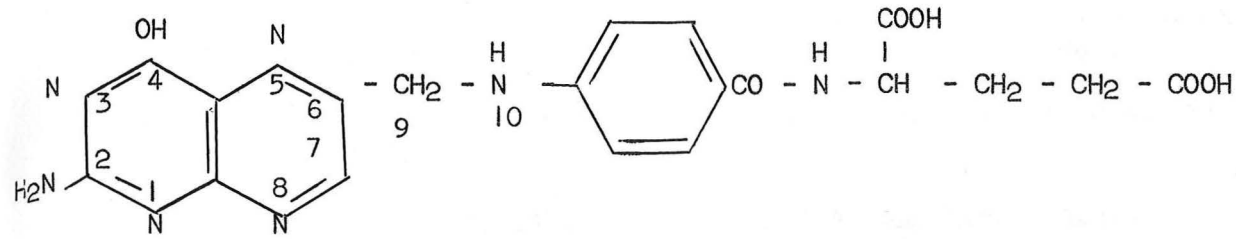
Time	Observation
3 weeks -	Decline in serum folate (less than 3 ng/ml)
7 weeks -	Hypersegmentation of PMN's
14 weeks -	Increased formiminoglutamate (FIGLU) excretion in urine after histidine load
16 weeks -	Decline in red cell folate (less than 20 ng/ml of erythrocytes)
18 weeks -	Peripheral macroovalocytosis
18½ weeks -	Progressive development of sleeplessness and forgetfulness
19 weeks -	Megaloblastic bone marrow
19½ weeks -	Rapid development of anemia
20 weeks -	Irritability and emotional lability Abnormal buccal mucosa

Late Sequelae:

1. Leukopenia
2. Thrombocytopenia
3. Gastric atrophy and achylia gastrica
4. Jejunal mucosal atrophy
5. Malabsorption secondary to mucosal atrophy
6. Hyperferremia, sideroblastosis and tissue hemosiderosis
7. Increased pigmentation of skin

III. The Nature of "Folate":

Ironically the first folate isolated was pteroylglutamic acid (or folic acid), a folate form that probably does not exist in nature:



Pteridine | P-amino-benzoic acid | L-glutamic acid

Pteroic acid

Pteroylglutamic acid
or
folic acid

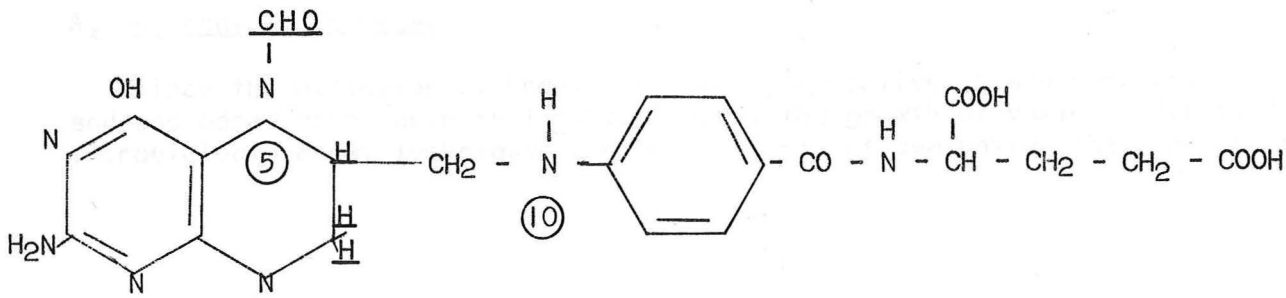
6. Stokstad, E. L. R. and Koch, J.: Folic Acid Metabolism. Physiol. Rev. 47:83, 1967.

The first physiologically active form - so called citrovorum factor:

7. Sauberlich, H. E. and Baumann, C. A.: A Factor Required for the Growth of Leuconostoc Citrovorum. J. Biol. Chem. 176:165, 1948.

8. May, M., Bardos, T. J., Barger, F. L., Lansford, M., Ravel, J. A., Sutherland, G. L. and Shive, W.: Synthetic and Degradative Investigations of the Structure of Folinic Acid. J. Amer. Chem. Soc. 73:3067, 1951.

N^5 Formyl Tetrahydrofolic Acid



9. Rabinowitz, J. C. and Himes, R. H.: Folic Acid Coenzymes. Fed. Proc. 19:963, 1960.

10. Friedkin, M.: Enzymatic Aspects of Folic Acid. Ann. Rev. Biochem. Ed. E. E. Snell. Ann. Rev., Inc., Palo Alto, Calif. 32:185, 1963.

11. Bertino, J. R. and Johns, D. G.: Folate Metabolism In Man. XII Int. Cong. Heme. 12:133, 1968.

Tetrahydrofolate Coenzyme Dependent Enzyme Biosynthetic Reactions

One Carbon Adduct	Coenzyme	System
- CH3	N5-Methyl FH_4	Methionine Biosynthesis
- CHO	N10-Formyl FH_4	Purine Biosynthesis
= CH -	N5,N10-Methenyl FH_4	Purine Biosynthesis
- CH = NH	N5-Formimino FH_4	Histidine Degradation
- CH_2 -	N5-N10-Methylene	Thymidylate Biosynthesis Serine Biosynthesis

In essence, Folic acid, in the form of its tetrahydro-derivative, can function as a carrier of one-carbon fragments.

Two general classes of enzymatic reactions occur:

1. Reactions in which one folate coenzyme form is converted to a different folate coenzyme form.

2. Reactions in which a folate coenzyme donates a one-carbon unit to, or accepts a one-carbon unit from, another compound.

IV Studies of Folate Activity:

A. Microbiologic Assay

Since the evolution of knowledge concerning active folate compounds evolved from and was based upon their ability to support the growth of various microorganisms, these microbiologic assay techniques became the basis of evaluating their presence and potency.

The 3 microorganisms commonly used for assay purposes are:

Lactobacillus casei (ATCC 7469)
Streptococcus faecalis (ATCC 8043)
Pediococcus cerevisiae (ATCC 8081)
(also known as Leuconostoc citrovorum)

Growth Activity of Various Folates for Microorganisms

Folate	P. cerevisiae	S. faecalis	L. casei
Folic Acid	-	+	+
Pteroyl di glutamates	-	+	+
Pteroyl tri glutamates	-	-	+
Reduced pteroyl mono glutamates (except N^5-Methyl)	+	+	+
N^5-Methyl-FH_4	-	-	+
Pteroyl hepta glutamates	-	-	-
Pteroic acid	-	+	-
N5-formyl Pt.	-	+	-
N10-formyl Pt.	-	+	-

12. Baker, H., Herbert, V., Frank, O., Pasher, I., Hutner, S. H., Wasserman, L. R. and Sobotka, H.: A microbiologic method for detecting folic acid deficiency in man. Clin. Chem. 5:275, 1959.

13. Herbert, V., Fisher, R. and Koontz, B. J.: The Assay and Nature of Folic Acid Activity In Human Serum. JCI 40:81, 1961.
Demonstrated that both ascorbic acid and phosphate buffer enhance the growth of L. casei. Thus undoubtedly many of the previous assays of "whole food folate" was really a measurement of a significant contribution of ascorbic acid activity on the organisms. The active serum factor was considered to be a triglutamate.

Subsequent studies:

14. Larrabee, A. R., Rosenthal, S., Cathou, R. E., and Buchanan, J. M.: A Methylated Derivative of Tetrahydrofolate As An Intermediate of Methionine Biosynthesis. J. Amer. Chem. Soc. 83:4094, 1961.
- demonstrated that this monoglutamate was L. casei active material.

15. Keresztesy, J. C. and Donaldson, K. O.: Synthetic Prefolic A. Biochem. Biophys. Res. Comm. 5:286, 1961.
- isolated a similar monoglutamate from horse liver.

These observations were combined and in a cooperative evaluation:

16. Herbert, V., Larrabee, A. R. and Buchanan, J. M. Studies on the Identification of A Folate Compound of Human Serum. JCI 41:1134, 1962.
- demonstrating that all of these as well as the major form of folate found in plasma of man is:

N5-Methyl FH4

17. Herbert, V.: Studies of Folate Deficiency In Man. Proc. Roy. Soc. Med. 57:377, 1964.

18. Woods, D. D.: The Function of Folic Acid In Cellular Metabolism. Proc. Roy. Soc. Med. 57:388, 1964.

These two papers firmly established the L. casei assay validity in man as evidence of folate depletion.

It should be stressed that human tissues may contain a host of folates. Human erythrocytes for instance appear to contain 9 separate folates, three of which (N10-formylpteroic acid, N5-formyl FH4-diglutamate and N10-formylfolic acid) have been identified:

19. Usdin, E.: Blood Folic Acid Studies. VI Chromatographic Resolution of Folic Acid-Active Substances Obtained from Blood. J. Biol. Chem. 234:2373, 1959.

20. Noronha, J. M. and Aboobaker, V. S.: Studies On the Folate Compounds of Human Blood. Arch. Biochem. 101:445, 1963.

From the known biochemical participation of folates other means of evaluating activity and deficiency state developed:

B. Formiminoglutamic Acid Excretion:

The main pathway in the catabolism of histidine is to urocanic acid which is then converted to formiminoglutamic acid (FIGLU) by the opening of the imidazole ring. The step beoynd FIGLU is the transfer of the formimino (CH = NH) group to the folate coenzyme. Since both compounds (urocanic acid and FIGLU) can be easily measured, urinary recovery following a histidine load has been used to evaluate folate depletion:

21. Broquist, H. P. and Lukby, A. L.: Detection and Isolation of Formiminoglutamic Acid From Urine In Folic Acid Deficiency In Humans. Proc. Soc. Exp. Biol. and Med. 100: 349, 1959.

22. Chanarin, I.: Studies on Formiminoglutamic Acid Excretion. Proc. Royal Soc. Med. 57:384, 1964.

23. Merritt, A. D., Rucknagel, D. L., Silverman, M. and Gardner, R. C.: Urinary Urocanic Acid in Man: The Identification of Urocanic Acid and The Comparative Excretions of Urocanic Acid and N-Formiminoglutamic Acid After Oral Histidine In Patients With Liver Disease. JCI 41:1472, 1962.

Although folic depletion in man is indeed associated with increased FIGLU excretion (or its stable precursor urocanic acid) the validity of these techniques as a test of folate depletion is limited because:

1. Even in folate depleted man only 1-2% of the histidine load is recoverable in the urine.

2. The histidine load has been known to precipitate hepatic coma in patients with impaired liver function.

3. Histidine load has yielded cardiovascular collapse in post gastrectomy patients (yielding a profound dumping syndrome).

4. Increased FIGLU excretion (normal output after 15g histidine HCl oral load is 1-17 mgm with mean of 9 mgm) may occur in the absence of megaloblastic changes reflecting its potential sensitivity as a test of subclinical folate deficiency. As such false positive tests (over 20 mgm/24 hrs) have been identified in:

a.) Thyrotoxicosis
b.) Hemolytic Anemia
c.) Hodgkins Disease
d.) Congestive Heart Failure
e.) Psoriasis
f.) Cirrhosis

5. Since the prime value in such tests rest in their ability to differentiate or identify the mechanism for a given megaloblastic anemia, one can look at patients with documented B12 deficiency. Between 30-100% of patients with B12 deficiency have elevated FIGLU's in range of 20-300 mgm/24 hrs.

6. The test is useless in pregnancy since in normal pregnancy the mean output is high (mean 21 mgm/24 hrs) for first 16 weeks of gestation and then very low (mean 7 or less) thereafter yielding many false negatives in spite of folate depletion.

7. In hepatocellular liver damage both urocanic acid and FIGLU may be increased because of reduction in the enzymes urocanase and formiminotransferase.

C. <u>4-amino-imidazole carboxamide (AIC) excretion</u>:

AIC is a purine intermediate originally noted to be increased in the urine of B12 deficient patients. This also occurs in folate depletion:

24. Luhby, L. and Cooperman, J.: AIC Excretion in Vitamin B12 and Folic Acid Deficiencies. Lancet 2:1381, 1962.

25. Herbert, V., Streiff, R., Sullivan, L., McGreer, P.: Accumulation of A Purine Intermediate (Aminoimidazolecarboxamide) In Megaloblastic Anemias Associated With Vitamin B12 Deficiency, Folate Deficiency with Alcoholism and Liver Disease. Fed. Proc. 23:188, 1964.

Although AIC is thus of no differential diagnostic value, it now appears that it results from enhanced purine biosynthesis!! This may suggest that folate deficiency deprives the late phase of DNA synthesis before it affects the earlier pathways of purine synthesis.

D. Fate of Folate Load - Tritiated Folic Acid (H3-FA)

Urinary clearance after a folic acid load as well as plasma clearance and urinary loss of isotopically labeled folic acid have been studied:

1.) Oral Tests:

Balance studies suffer from presence of unmeasurable folate synthetic potential of intestinal bacteria. Isotopic balance studies:

26. Anderson, B., Belcher, E. H., Chanarin, I. and Molen, D. L.: The Urinary and Fecal Excretion of Radioactivity After Oral Doses of H3-Folic Acid. Brit. J. Heme. 6:439, 1960.
- have shown considerable overlap between the values for controls and for those with documented malabsorption of folate and associated folate deficiency.

2.) H3-FA Clearance Studies

27. Johns, D. G., Spekti, S. and Burgen, A.S.V.: Metabolism of Tritiated Folic Acid In Man. JCI 40:1684, 1961.
Clearly the best study revealing:
1.) $T\frac{1}{2}$ clearance after 1 µgm/kg I.V. = 3 minutes
2.) 0.1% remaining = 30 minutes
3.) When f.a. load exceeds 100 ng/ml renal clearance is independent of the plasma level and averages 51 ± 3.1 ml/min.
4.) A fixed upper limit of clearance exists which is lower than the q.f.r. suggesting a considerable fraction of plasma folic acid may be non-filterable due to binding to plasma proteins.

The basis for the rapid tissue uptake of this bulky, polar, lipid insoluble molecule is unknown.

Although clearance studies have been suggested as tests for identification of folate deficiency or abnormal folate handling -

28. Metz, J., Stevens, K., Krawitz, S. and Brandt, V.: The Plasma Clearance of Injected Doses of Folic Acid As an Index of Folic Acid Deficiency. J. Clin. Path. 14:622, 1961.

29. Sheehy, T. W., Santin, R., Guerra, R., Angel, R. and Plough, I. C.: Tritiated Folic Acid As A Diagnostic Aid in Folic Acid Deficiency. J. Lab. and Clin. Med. 61:650, 1963.

30. Klipstein, F. A.: The Urinary Excretion of Orally Administered Tritium-labeled Folic Acid As a Test of Folic Acid Absorption. Blood 21:626, 1963.
- considerable overlap exists between normals and deficient patients, the rapid clearance is not understood and occurs in normals as well as depleted patients and finally pterylglutamic acid (folic acid) is <u>not</u> the physiologic form of folate.

V Diagnostic Criteria of Folic Acid Deficiency:

1. Low serum L. casei folate activity.
2. Elevated Formiminoglutamic acid urinary excretion after histidine loading.
3. Normal serum Vitamin B12 and ascorbic acid levels.
4. Elevated excretion of Urocanic acid.
5. An abnormally rapid clearance of intravenously injected folic acid (suggesting depleted tissue stores).
6. Ultimate response to folic acid therapy with conversion of marrow from megaloblastic to normoblastic and an increase in peripheral erythrocyte values.

Tissue studies (both erythrocyte and hepatic folate) have been done:

31. Chanarin, I., Hutchinson, M., McLean, A. and Moule, M.: Hepatic Folate In Man. Brit. Med. J. 1:396, 1966.

Liver is the site of folate storage in man. Liver folate in man supports only growth of L. casei. Liver folate depletion was usual when serum folate and FIGLU were abnormal <u>except</u> in pregnancy and in patients on anticonvulsant therapy.

VI Clinical Spectrum of Folate Deficiency:

1. Nutritional Deprivation:

Ref. #1 clearly refuted the concept that nutritional deprivation could result in evidence of folic acid deficiency. Probably the first documented case of folate deficiency was:

32. O'Hara, D. and Grewal, J. S.: An Unusual Case of Pernicious Anemia. Boston Med. Surg. J. 197:129, 1927.
- in a patient who lived on bread, milk, potatoes and candy.

In England even today the dietary intake of folic acid generally is considerably less than in the U. S. and dietary folate depletion is not uncommon:

33. Gough, K. R., Read, A. E., McCarthy, C. F. and Waters, A. H.: Megaloblastic Anemia Due to Nutritional Deficiency of Folic Acid. Quart. J. Med. 32:243, 1963.

Folate depletion and megaloblastosis is well known in Kwashiorkor:

34. Velez, H., Ghitis, J., Pradilla, A., Vitale, J. J.: Megaloblastic Anemia in Kwashiorkor. Fed. Proc. 21:386, 1962.
Studies of children in Coli, Columbia. 15-30 µgms of folic acid per day yielded hematologic responses.

35. Adams, E. B., Scragg, J. N., Naidoo, B. T., Liljestrand, S. K. and Cockram, V. I.: Observations on the Aetiology and Treatment of Anaemia In Kwashiorkor. Brit. Med. J. 3:451, 1967.
28% of the children with Kwashiorkor in Durban, South Africa had megaloblastic erythropoiesis with laboratory evidence of folate depletion (ascorbate not evaluated).

Case #1: [redacted] This 64 year old [redacted] man was admitted to [redacted] because of mental depression in 1963. Aside from being very depressed the patient denied all symptoms. Physical examination revealed a pale [redacted] male with normal vital signs. Patchy hyperpigmentation was noted over the trunk and the remainder of the complete physical examination was within normal limits.

His laboratory studies revealed an initial Hgb of 4.9 gms% with a Hct of 15 vols%. The MCV was 120 with evident anisocytosis, poikilocytosis and macroovalocytes. His white blood count was 4,300 with hypersegmentation of the PMN's. The platelet count was 110,000. Gastric analysis demonstrated histamine-fast achlorhydria with the pH rising to 7.2 after histamine stimulation. Bone marrow aspiration demonstrated classic megaloblastic dyspoiesis.

An interesting dietary history was obtained from the patient in that he essentially survived on boiled milk and crackers with the only added staple being that of well cooked beans from a pot that he had continually simmering on his hotplate in his room. The patient had seen a physician in [redacted] Texas who had known of his unusual dietary history and prescribed multivitamins which did not contain either B_{12} or folic acid (they did contain ascorbic acid).

His serum vitamin B_{12} level was 180 µµg/ml (normal 160-660 µµgm/ml) and his serum folate was less than 1 ng/ml (normal 5-18 ng/ml). Unfortunately the patient had been eating sporadically on the psychiatric service and a true physiologic response test could not be carried out. However, on 50 µg of folic acid per day the patient demonstrated an initial increase in reticulocytes from 0.2% to 1.2% on day 2 following the institution of therapy (this was his 12th hospital day). Maximum reticulocyte response occurred five days later and a subsequent return to normal peripheral hematopoietic values resulted.

COMMENT: Our initial diagnostic consideration was that of P.A. and the patient was considered a "Vegans". We did not know the duration of his deprived diet, but felt its folate content was adequate since free folate activity in cow's milk had been reported to be high. The beans (or any well cooked food) has virtually no folate. Recently data has become available to explain the basis of folate deprivation in this case:

36. Ghitis, J.: The Labile Folate of Milk. Amer. J. Clin. Nutr. 18:452, 1966.
- with the demonstration that the free folic acid activity for L. casei in cow's milk averages only 55 µgm/L and more importantly, that over 80% of this activity was lost by boiling for 5 minutes.

The histamine fast achlorhydria always initially raises the question of B12 deficiency, but in spite of:

37. Klipstein, F. A.: Folate Deficiency Secondary to Disease of the Intestinal Tract. Bull. N.Y. Acad. Sci. 42:638, 1966.
- denial that folate depletion leads to mucosal change, we have very commonly seen this in our folate depleted patients with reversibility of the secretory defect associated with folate repletement. Furthermore,

38. Scott, R. B., Kammer, R. B., Burger, W. F. and Middleton, F. G.: Reduced Absorption of Vitamin B12 in Two Patients With Folic Acid Deficiency. Ann. Int. Med. 69:111, 1968.

Folic acid deficiency is a well known cause of abnormal Schilling tests and can alter mucosal function generally.

Another form of nutritional deprivation occurs in association with scurvy. Diets low in Vitamin C are usually low in folate also. In addition, it was long considered that ascorbic acid was considered necessary to convert folic acid to folinic acid (N5-formyl TH4) a reduction step:

39. Nichol, C. A. and Welsh, A. D.: Synthesis of Citrovorum Factor From Folic Acid By Liver Slices: Augmentation by Ascorbic Acid. Proc. Soc. Exp. Biol. and Med. 74:52, 1950.

It is however now clear that the effect of ascorbic acid is not to facilitate this conversion but rather to protect it's reduced end products against oxidative destruction:

40. Doctor, V. M.: Studies in vivo on Conversion of Folic Acid to Citrovorum Factor. J. Biol. Chem. 233:982, 1958.

41. Zalusky, R. and Herbert, V.: Megaloblastic Anemia In Scurvy With Response to 50 Micrograms of Folic Acid Daily. NEJM 265:1033, 1961.

42. Cox, E. V., Meynell, M. J., Northam, B. E. and Cooke, W. T.: The Anaemia of Scurvy. Amer. J. Med. 42:220, 1967.

43. Kahn, S. B. and Brodsky, I.: Metabolic Interrelationship Between Vitamin B12 and Ascorbic Acid In P.A. Blood 31:55, 1968.

Finally the evidence of clinical deficiency has led to extended studies of food folate and studies of the minimal daily adult requirements:

Food Folate:

Folic acid content of diet remains uncertain:

1.) The usual data is based upon microbiologic assay methods that do not protect the labile folates in natural foods.

2.) It is not known how much of the naturally occurring folates are available for absorption.

3.) Cooking of food leads to a variable loss of folate activity.

44. Santini, R., Brewster, C. and Butterworth, C. E.: The distribution of folic Acid Active Compounds in Individual Foods. Amer. J. Clin. Nutr. 14:205, 1964.

45. Blumslag, N. and Metz, J.: Response to Lettuce In A Patient With Megaloblastic Anemia Associated With Pregnancy. S. African Med. J. 38:611, 1964.

One of the very rare studies in which food folate was truly evaluated.

46. Chung, A. S. M., Pearson, William N., Dabry, W. J., Miller, O. N. and Goldsmith, G. A.: Folic Acid, Vitamin B6, Pantothenic Acid and Vitamin B12 In Human Dietaries. Amer. J. Clin. Nutr. 9:573, 1961.

Studied whole diets (using unfortunately Str. faecalis assay) and demonstrated that mean intake in upper income diet was 193 µgm/day, low income diet 157 µgm/day and indigent diet 47 µgm/day. Mean American intake was considered 170 µgm/day. (British mean intake is 110 µgm/day)

The best recent review of the problem of dietary folate and food folate was:

47. Butterworth, C. E.: The Availability of Food Folate. Brit. J. Heme. 14:339, 1968.

Virtually all of the data suggests that 50 µgm/day of folate is the approximate Minimum Daily Adult Folate Requirement:

48. Herbert, V.: Minimal Daily Adult Folate Requirement. Arch. Int. Med. 110:649, 1962.

49. Herbert, V.: Biochemical and Hematologic Lesions In Folic Acid Deficiency. Amer. J. Clin. Nutr. 20:562, 1967.

50. National Academy of Sciences: Recommended Dietary Allowances. Publication #1146 Nat. Acad. Sci. 6:41, 1964.

A daily intake of 50 µgm/day will prevent serum folic acid depletion. An amount of folic acid in excess of 100 µgm/day may prevent the hematologic manifestations of P.A., although the neurologic manifestations may progress. Therefore, sale of vitamin preparations containing 100 µgms of folic acid or greater was prohibited as of 20 July 1963.

2. Excess Folate Loss:

Folate depletion (as well as other water soluble vitamins) are lost in significant enough quantities to yield a deficiency state in chronic renal dialysis:

51. Hampers, C. L., Streiff, R., Nathan, D. G., Snyder, D. and Merrill, J. P.: Megaloblastic Hematopoiesis In Uremia and In Patients On Long Term Hemodialysis. NEJM 276:551, 1967.

3. Anti-Fol Therapy:

Function by interfering with conversion of dihydro to tetra hydrofolate. All of the anti-fols combine firmly with the enzyme dihydrofolic reductase, thus limiting the formation of the active tetrahydrofolate forms.

52. Delmonte, L. and Jukes, T. H.: Folic Acid Antagonists In Cancer Chemotherapy. Pharm. Rev. 14:91, 1962.

53. Condit, P. T. and Grub, D.: Studies on the Folic Acid Vitamins. I. Observations on the Metabolism of Folic Acid in Man and the Effect of Aminopterin. Canc. 11: 525, 1958.

54. Condit, P. T.: Studies on the Folic Acid Vitamins. III. The Duration of Effects of the Folic Acid Antagonists In Man. Canc. 13:229, 1960.

55. Hellman, S., Iannotte, A. T. and Bertino, J. R.: Determinations of Levels of Serum Folate In Patients With Carcinoma of Head and Neck Treated With Methotrexate. Canc. Res. 24:105, 1964.

Demonstrated that the low serum folate following Methotrexate therapy persisted 2-4 weeks and the degree or duration of serum change did not correlate with anti-tumor response.

Although one might presume that folate antagonists should yield a state analogous to pure nutritional folate deficiency this appears not to be true. First the block is highly specific in its site; second, the antagonists produce intracytoplasmic inclusion bodies in mucosa of small bowel that is more common with toxic agents and has not been seen with pure nutritional deprivation:

56. Trier, J. S.: Morphologic Alterations Induced by Methotrexate In the Mucosa of Human Proximal Intestine. Gastro. 42:295, 1962.

57. Bertino, J. R.: The Mechanism of Action of The Folate Antagonists In Man. Canc. Res. 23:1286, 1963.

Finally, it should be noted that oncologists are not the only culprits. Daraprim (pyrimethamine) commonly used to treat malaria and used by ophthalmologists to treat chorioretinitis is an anti-fol.

4. Alcoholic Cirrhosis:

Clinically this represents the largest group at PMH. Reduced serum folate was found in approximately 75% of admissions with megaloblastic erythropoiesis in 18% (1966).

58. Kimber, C., Deller, D. J., Ibbotson, R. N. and Lander, H.: The Mechanism of Anaemia in Chronic Liver Disease. Quart. J. Med. 34:33, 1965.

59. Kimber, C. L., Deller, D. J. and Lander, H.: Megaloblastic and Transitional Megaloblastic Anemia Associated With Chronic Liver Disease. Amer. J. Med. 38: 767, 1965.

60. Herbert, V., Zalusky, R. and Davidson, C. S.: Correlation of Folate Deficiency With Alcoholism and Associated Macrocytosis, Anemia and Liver Disease. Ann. Int. Med. 58:977, 1963.

A critical delineation of the mechanism was the observation that ethanol itself suppressed erythropoiesis and induced increased folate need at the cellular level:

61. Sullivan, L. W. and Herbert, V.: Suppression of Hematopoiesis by Ethanol. JCI 43:2048, 1964.

5. Malabsorption Syndromes:

a.) Site of Folate Absorption:

Although folic acid is capable of being absorbed along the entire small bowel, the primary site of absorption is from the proximal small intestine. In rats, some data suggests that this is an active energy-dependent process at physiologic levels of the Vitamin:

62. Booth, C. C.: The Metabolic Effects of Intestinal Resection in Man. Postgrad. Med. J. 37:725, 1961.

63. Burgen, A. S. V. and Goldberg, N. J.: Absorption of Folic Acid from the Small Intestine of the Rat. Brit. J. Pharmacol. 19:313, 1962.

64. Klipstein, F. A.: The Urinary Excretion of Orally Administered Tritium-Labeled Folic Acid As A Test of Folic Acid Absorption. Blood 21:626, 1963.

65. Whitehead, V. M. and Cooper, B. A.: Absorption of Unaltered Folic Acid From the Gastrointestinal Tract In Man. Brit. J. Heme. 13:679, 1967.

- demonstrated that administered folic acid is absorbed, at least into hepatic portal system, intact, thus methylation probably occurs no earlier than liver.

b.) Problems In Malabsorption Syndromes:

66. Klipstein, Frederick A.: Folate Deficiency Secondary To Disease of The Intestinal Tract. Bull. N. Y. Acad. Med. 42:638, 1966.

67. Klipstein, F. A.: Tropical Sprue. Gastro. 54:275, 1968.
A current review paper.

68. Baker, H., Frank, O. and Sobotka, H.: Mechanisms of Folic Acid Deficiency In Nontropical Sprue. JAMA 187:119, 1964.

The advent of folate depletion in malabsorption syndromes is highly variable. In part this can be explained by intestinal bacterial formation of folate:

69. Hoffbrand, A. V., Toboqchali, S. and Mollin, D. L.: High Serum Folate Levels in Intestinal Blind-Loop Syndrome. Lancet 1:1339, 1966.

c.) Local Mucosal Effects:

Presumably on the basis of close proximity of the nutrient, the jejunum frequently is normal in spite of advanced systemic folate depletion:

70. Winawer, S. J., Sullivan, L. W., Herbert, V. and Zamcheck, N.: The Jejunal Mucosa In Patients With Nutritional Folate Deficiency and Megaloblastic Anemia. NEJM 272:892, 1965.

Finally, induced megaloblastic mucosal changes regardless of etiology do result in decreased absorption (see Ref. #38) and:

71. Carmel, R. and Herbert, V.: Correctable Intestinal Defect of Vitamin B12 Absorption in P.A. Ann. Int. Med. 67:1201, 1967.

6. Folate Depletion Associated With Anticonvulsant Therapy:

Although the first observation of megaloblastic anemia secondary to anticonvulsants was recorded but in 1952:

72. Mannheim, R., Pakesch, F., Reimer, E. F. and Velter, R$_{II}$: Die haematologischen Komplekationen der Epilepsie Behandlung mit Hydantoinkorpern. Med. Klin. 47:1397, 1952.

- it is now well established that although dilantin is the most frequent cause of the megaloblastic changes these can also be caused by barbiturates, mesantoin and primadone. Approximately 50% of all patients on anticonvulsant therapy have low serum folate levels. Although macrocytosis occurs in 78% of those with reduced serum folate, it also occurs in 18% of those with normal serum folate.

73. Klipstein, F. A.: Subnormal Serum Folate and Macrocytosis Associated With Anticonvulsant Therapy. Blood 23:68, 1964.

74. Dahlke, M. B. and Mertens-Roesler, E.: Malabsorption of Folic Acid Due to Diphenylhydantoin. Blood 30:341, 1967.

- contrary to most studies to date, these authors suggest some of the patients have reduced folate absorption.

The mechanism of interference appears to be that of a similar structural configuration with a functional anti-fol effect.

English workers have related the induced folate deficiency to altered neurol function in patients with convulsive disorders on anticonvulsant therapy:

75. Reynolds, E. H., Milner, G., Matthews, D. M. and Chanarin, I.: Anticonvulsant Therapy, Megaloblastic Haemopoiesis and Folic Acid Metabolism. Quart. J. Med. 35: 521, 1966.

76. Reynolds, E. H.: Schizophrenia-like Psychoses of Epilepsy and Disturbances of Folate and Vitamin B12 Metabolism Induced by Anticonvulsant Drugs. Brit. J. Psych. 113:911, 1967.

77. Reynolds, E. H.: Effects of Folic Acid on the Mental State and Fit-Frequency of Drug Induced Epileptic Patients. Lancet 1:1086, 1967.

From these and related studies, virtually all from England, therapy with folic acid has been shown to:

1.) Improve the mental state in 88%.
2.) Increase the fit frequency in 50%.
3.) Clear the schizophrenic-like state in 100%.

In the U. S. the Columbia group have related these same findings to the presence of peripheral neuropathy -

78. Horwitz, S. J., Klipstein, F. A. and Lovelace, R. E.: Relation of Abnormal Folate Metabolism to Neuropathy Developing During Anticonvulsant Therapy. Lancet 1:563, 1968.

These observations have renewed interest in the relationship of folate to the maintenance of neural tissue integrity:

79. Grant, H., Hoffbrand, A. V. and Wells, D. G.: Folate Deficiency and Neurological Disease. Lancet 2:763, 1965.

80. Adams, P., Chalmer, T. M., Foulds, W. S. and Whitley, J. L.: Megaloblastic Anaemia and Vision. Lancet 1:229, 1967.

7. Conditions of Excess Cellular Growth:

a.) Chronic Stimuli to Hematopoietic Cell Production:

This phenomenon is best recognized in chronic hemolytic anemia and was first described:

81. Vaughn, J. M.: The Anaemias: With Notes on Normal and Pathological Erythropoiesis. London. pp. 234-36, 1934.

That this was on the basis of increased folate needs related to high local tissue demands associated with high rate of cellular turnover:

82. Jandl, J. H. and Greenberg, M. J.: Bone-marrow Failure Due to Relative Nutritional Deficiency in Cooley's Hemolytic Anemia: Painful "Erythropoietic Crisis" in Response to Folic Acid. NEJM 260:461, 1959.

When the chronic hemolysis occurs in a population with borderline nutritional status the incidence of megaloblastic anemia can be very high. Thus in Nigeria a 10% incidence is recorded in SS hemoglobinopathy patients:

83. Watson-Williams, E. J.: Folic Acid Deficiency in Sickle Cell Anemia. East Afric. M. J. 39:213, 1962.
- 43 cases in 405 patients studied of newly diagnosed cases of SS disease.

This, of course, can occur in any stimulated drive to erythropoiesis as in any chronic hemolytic anemia:

84. Chanarin, I., Dacie, J. V. and Mollen, D. L.: Folic Acid Deficiency In Hemolytic Anemia. Brit. J. Heme. 5:245, 1959.

The identification of megaloblastosis in such high cellular output states is suggested by:

a.) An increase in the severity of the anemia in any given patient.
b.) Hypersegmented PMN's
c.) Macroovalocytes
d.) A decrease in the previous steady state reticulocyte level
e.) Development of an unexplained leukopenia and/or thrombocytopenia.

The diagnosis, of course, is established by bone marrow aspiration. The etiology can be confirmed by serum folate assay.

b.) Infancy:

85. Matoth, Y., Penkos, A., Zamir, R., Moosllem, F. and Grosswicz, N.: Studies on Folic Acid In Infancy. Ped. 33:507, 1964.

c.) Pregnancy:

As mentioned many of the tests of folate handling are altered in pregnancy and cannot be used to assess depleted states. Indeed the mean PMN lobe average is reduced in pregnancy so that hypersegmentation is also an unreliable index:

86. Lowenstein, L., Lauder, B. and Yang-Shu, H.: Nutritional Anemia and Megaloblastosis In Pregnancy. Can. Med. Assoc. J. 94:636, 1966.

The literature of increased folate needs during pregnancy has almost universally proven increased need during pregnancy. Clearly the best ongoing studies have been from Queen Mother's Hospital in Glasgow where in a series of papers:

87. Willoughby, M. L. N. and Jewell, F. J.: Investigation of Folic Acid Requirements in Pregnancy. B.M.J. 2:1568, 1966.

They have established that the pregnant woman requires 300 µgm of folic acid per day.

More controversial has been the observation that third trimester bleeding and abruptio placenta may be etiologically related to folate depletion:

88. Streiff, R. R. and Little, A. B.: Folic Acid Deficiency In Pregnancy. NEJM 276: 776, 1967.

Virtually all of the subsequent investigations have failed to substantiate this observation:

89. Alpirin, J. B., Haggard, M. E. and McGarrity, W. J.: Incidence of Abruptio Placenta In Pregnant Women With Folic Acid Deficiency. XII Int. Cong. Heme 12:96, 1968.

d.) Malignant Neoplasms:

This was reviewed above.

8. Relationship to Vitamin B12 Deficiency:

90. Ross, J. F., Belding, H. and Paegel, B. L.: The Development and Progression of Subacute Combined Degeneration of the Spinal Cord In Patients With P.A. Treated With Synthetic Pteroylglutamic (Folic) Acid. Blood 3:68, 1948.

91. Conley, C. L., and Krevens, J. R.: Development of Neurologic Manifestations of P.A. During Multivitamin Therapy. NEJM 245:529, 1951.

92. Chanarin, I. and Perry, J.: Metabolism of 5-Methyltetrahydrofolate In Pernicious Anemia. Brit. J. Heme. 14:297, 1968.

9. Homocystinuria:

93. Corey, M.: Folate Metabolism In Homocystinuria. Irish J. Med. Sci. 6:488, 1966.

10. Questionable Relationships:

94. Hines, J. D., Halsted, C. H., Griggs, R. C. and Harris, J. W.: Megaloblastic Anemia Secondary to Folate Deficiency Associated With Hypothyroidism. Ann. Int. Med. 68:792, 1968.

95. Grough, K. R., McCarthy, C., Read, A. E., Mollen, D. L. and Waters, A. H.: Folic Acid Deficiency In Rheumatoid Arthritis. B.M.J. 1:212, 1964.

96. Granville, Norma and Dameshek, William: Hemochromatosis With Megaloblastic Anemia Responding to Folic Acid. NEJM 258:586, 1958.

VII The Sequence of Events With Folate Repletement:

4 hrs - Prompt fall in serum iron
8 hrs - Bone pain - often at sites of previous marrows
Euphoria - often lasting 24-48 hrs
24 hrs - 1st increase in reticulocytes
48 hrs - Bone marrow reverts to normoblastic erythrocytic hyperplasia
4-5 day - Increase in PMN's and platelets
3-9 days - Peak of reticulocytes

The promptness and degree of reticulocyte response as well as the return to normal of peripheral hematologic values can be abtunded by intercurrent infection, antibiotics or severe metabolic derangements.