METABOLIC INTER-RELATIONSHIPS BETWEEN FOLIC ACID AND VITAMIN B_{12}

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SEVERAL recent reports indicate that folic acid and vitamin B_{12} have more or less defined biochemical functions other than their established involvement in hæmopoiesis. However, there has been no direct experimental proof hitherto of specific enzyme systems associated with either vitamin and regulating cellular metabolism. The fact that folic acid and vitamin B_{12}, despite their dissimilarity from a chemical standpoint, should function so interrelatedly in diverse processes would only make such studies more intriguing.

Among systems known to be influenced by the two hæmatinics may be mentioned those involved in (*i*) the oxidations of purines, choline, glycine and D-amino acids, (*ii*) the metabolism of tyrosine, serine and glycine, (*iii*) nucleic acid biogenesis, and (*iv*) transmethylations. Relevant literature has been cited in recent publications from this laboratory.[1-5] An explanation of the observed metabolic interrelationships between the two vitamins has been sought for on the basis of the following well-known synthetic step in nucleic acid metabolism:

$$\text{Precursors} \xrightarrow{(1)} \text{Thymine} \xrightarrow{(2)} \text{Thymidine}$$

in which steps (1) and (2) are catalysed respectively by folic acid and vitamin B_{12}. Indeed, clinically and biochemically, such a step could be taken as having been established unequivocally.[6] Nevertheless, this inter-relationship would seem obviously insufficient to account for the other apparently unrelated enzyme systems enumerated above.

Unpublished data (referred to in[1]) have indicated that plasma levels of folic acid as well as stored folic acid in livers of experimental chicks are influenced by dietary vitamin B_{12} fed as condensed fish solubles especially at low levels of folic acid ingestion. Typical results are summarised in Table I.

This potentiating effect of vitamin B_{12} on the mobilisation of folic acid has since been confirmed with crystalline vitamin B_{12}.[8]

It is pertinent to refer here to certain significant observations on the metabolic economy of folic acid. Micro-organisms which synthesize their own requirement of folic acid from precursors elaborate this vitamin largely as folinic acid or the *citrovorum factor*[4] which is undoubtedly the physiologically more active form of folic acid;[2] folinic acid is the form present both in the cells and in culture filtrate. On the other hand, organisms requiring exogenous folic acid for growth do not synthesize appreciable folinic acid activity in culture filtrates whereas the folic acid taken in the cells is present as folinic acid (unpublished data). Likewise, stored folic acid in rat liver which is nearly equally distributed between the particulate and supernatant fractions exists almost entirely as folinic acid.[9] A similar intracellular distribution pattern for vitamin B_{12} would show that it is concentrated in the mitochondria. These findings suggest that folinic acid is dynamic and functions in varied enzyme systems while the role of vitamin B_{12} may be more specific and confined only to certain enzymes of the mitochondria. It is tempting to speculate whether the effect of vitamin B_{12} on the mobilisation of folic acid is in part at least attributable to a function for it in the conversion of folic acid to folinic acid. A recent report observes increased urinary folinic acid excretion in immature rats following administration of vitamin B_{12} or whole liver powder.[10]

TABLE I

Influence of Vitamin B_{12} on the Utilization of Dietary Folic Acid

Dietary folic acid (*gamma* per cent.)	Without iodinated casein		With 0·03% iodinated casein	
	Basal diet	Basal diet +3·0% fish solubles	Basal diet	Basal diet +3·0% fish solubles
	Blood folic acid (*gamma* per 100 c.c.)			
0	0·30	0·38	0·30	0·43
10	0·36	0·46	0·35	0·49
50	0·46	0·55	0·36	0·49
200	0·84	1·10	0·60	0·95
	Liver folic acid (*gamma* per g.)			
0	1·31	1·77	1·34	2·15
10	1·70	1·93	1·48	1·96
50	1·93	2·23	1·75	1·83
200	3·42	3·63	2·80	2·78

Day-old chicks, ten to a group, were employed; the basal diet was a purified dextrin-casein ration as given in[7] with the substitution of pure corn dextrin for sucrose. Data above relate to samples at end of four weeks.

We may examine further the inter-relation between folic acid and vitamin B_{12} with special reference to transmethylations. Of all biochemical functions attributed to the two vitamins, that relating to choline and methionine metabolism has perhaps been the most significant. An inter-dependence between these two methylating compounds on the one hand and folic acid and vitamin B_{12} on the other has been observed in the nutrition of several species, mice, rats, dogs, chicks, etc.[11] also.[1,3,4] More specifically it has been demonstrated that methionine,[12] creatine[3] and nicotinamide (unpublished observations) metabolism are influenced by one or both of the vitamins. Choline oxidase activity is also related to vitamin B_{12}[13,14] and to folic acid.[15]

For a proper attempt at interpreting these observations, one should take into account established facts concerning the biosynthesis of methyl groups which could occur in the tissues of the rat[16] from the *alpha* carbon of glycine and the *beta* carbon of serine as well as from formate and methanol[17,18]; the ethanolamine moiety of choline could be derived from serine by decarboxylation.[19,20] The reverse step, namely, the degradation of labile methyl to formate could also take place.[16–20] There are indications that the neogenesis of methyl groups is mediated by folic acid and vitamin B_{12}[21–23] but their exact significance in methyl economy has as yet remained obscure.

Since folic acid is concerned directly with formate production from glycine[24] and with its utilization for serine synthesis[3,24] and possibly in other single carbon addition reactions such as nucleic acid formation,[5] its sparing action on labile methyl requirement would seem explicable on the basis that the latter functions in transmethylations[3] as well as in the transfer of glycine to serine and in other one carbon fragment fixation processes mediated by formate. In folic acid deficiency methyl drain to formate could occur excessively. Some proof of this possibility has been forthcoming from our observations on the impairment of normal creatine and nicotinamide metabolism in folic acid deficiency and on its partial restoration by administered methanol or formate (Table II).

The possibility is not excluded that folic acid in the above experiments might also act by accelerating methyl biogenesis from administered precursors. However, this latter argument is not in harmony with our later findings that in folic acid deficient mice there occurs no more synthesis of choline or methionine from exogenously administered precursors, methanol and serine, as a result of folic acid supplementation than from metabolically derived precursors (Table III).

TABLE II

Effect of Formate or Methanol on Creatine and Nicotinamide Metabolism in Folic Acid deficient and Replete Mice

Group	Urinary creatine[3]	Urinary N^1-methyl-nicotinamide *[25]
	(mg./100 g body wt./24 hrs.)	
Basal diet ..	1·57	71·0
Basal diet + formate ..	1·68	88·0
Basal diet + methanol ..	1·73	102·2
Basal diet + folic acid ..	1·80	119·0
Basal diet + folic acid + formate	1·86	117·6
Basal diet + folic acid + methanol	1·88	122·9

Adult mice, inbred Swiss strain, were used, not less than four to a group in each case. Basal diet was the folic acid-free purified ration[3] with 15 gamma per cent. of vitamin B_{12}. Folic acid was administered orally at 10 *gamma*/mouse/day while methanol or formate was given intraperitonially at 1 mg./mouse/day.

*In addition to that present in the basal diet, the animals here received 1 mg./day each nicotinamide during the urine collection period of 48 hours following an equilibration period of 2 days.

Urine samples were collected on the 8th and 9th days following the grouping after the onset of folic acid deficiency on the basal diet as shown by a hæmogram; this preparatory period was 5 weeks.

The fuller data and related observations will be published by P. Fatterpaker, U. Marfatia and A. Sreenivasan.

TABLE III

Probable non-involvement of Folic Acid in Methyl synthesis from Precursors

	Folic acid deficient		Folic acid replete	
	Choline	Methionine	Choline	Methionine
	(mg. per g. of liver)			
No precursors	5·08	5·44	6·05	5·77
Methanol + serine	5·40	5·60	6·42	6·00

Basal ration and administration of folic acid as in the previous series. Precursors (1 mg. methanol and 2 mg. of *dl*-serine) were given in solution intraperitonially on the 8th day after grouping following overnight fasting; animals were killed 6 hours later following access to