Factors affecting Formiminoglutamic Acid Excretion in Vitamin B₁₂ Deficiency

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1. Formiminoglutamic acid, a product of the catabolism of histidine, is excreted in abnormally large amounts in the urines of vitamin B₁₂-deficient rats and of vitamin B₁₂-deficient sheep; the excretion is reduced to negligible amounts after administration of vitamin B₁₂. 2. After administration of certain methyl donors to vitamin B₁₂-deficient rats or sheep urinary excretion of formiminoglutamic acid is temporarily decreased. 3. Irrespective of the pteroylglutamic acid status of the animals neither vitamin B₁₂-deficient rats nor vitamin B₁₂-deficient sheep have the ability to deal efficiently with histidine. 4. In sheep, urinary excretion of formiminoglutamic acid is increased after administration of aminopterin; treatment with pteroylglutamic acid restores the ability of the animal to deal with the catabolic products of histidine. 5. The possible functions of vitamin B₁₂ and methionine in relieving a virtual deficiency of pteroylglutamic acid are discussed.

Dietary histidine may be incorporated into protein, excreted in the urine, or degraded; quantitatively, degradation is thought to be the most important. The imidazole C-2 of histidine is known to be a source of C₁ units, the metabolism of which is intimately linked with PteGlu† and its derivatives, and has been shown, by experiments *in vivo* with rats and pigeons, to be incorporated into purines, serine and choline (Tabor, 1954).

In animals and bacteria L-histidine is degraded by a series of enzymic reactions that produce HCNH-Glu (Tabor & Wyngarden, 1959). HCNH-Glu has been shown to be a very effective biological formylating agent; in the presence of H₄PteGlu and formiminotransferase 5-HCNH-H₄PteGlu is formed, which is converted first into 5,10-CH₂-H₄PteGlu and then into 10-HCO-H₄PteGlu in two enzymic reactions (Tabor & Wyngarden, 1959).

Animals suffering a real or virtual deficiency of

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‡ Abbreviations: PteGlu, pteroylglutamic acid; HCNH-Glu, formiminoglutamic acid; H₄PteGlu, tetrahydropteroylglutamic acid; 5-HCNH-H₄PteGlu, 5-formiminotetrahydropteroylglutamic acid; 5,10-CH₂-H₄PteGlu, 5,10-methylenetetrahydropteroylglutamic acid; 10-HCO-H₄PteGlu, 10-formyltetrahydropteroylglutamic acid; 5-Me-H₄PteGlu, 5-methyltetrahydropteroylglutamic acid.

PteGlu and those in which vitamin B₁₂ is limiting excrete excessive amounts of HCNH-Glu in their urine. Excessive excretion of HCNH-Glu has been reported to occur in vitamin B₁₂-deficient chicks (Fox, Ludwig, Barker & Weissbach, 1960; Fox, Ludwig & Baroody, 1961; Fox & Ludwig, 1961), in PteGlu-deficient chicks (Fox & Ludwig, 1961), in vitamin B₁₂-deficient rats (Silverman & Pitney, 1958), in PteGlu-deficient rats (Rabinowitz & Tabor, 1958; Silverman & Pitney, 1958), in vitamin B₁₂-deficient sheep (Gawthorne, 1968) and in human subjects treated with PteGlu antagonists (Hiatt, Goldstein & Tabor, 1958; Broquist & Luhby, 1959). Reports on the urinary excretion of HCNH-Glu by patients suffering from megaloblastic anaemia associated with deficiencies of vitamin B₁₂ and/or PteGlu are conflicting (cf. Lewis & Moore, 1962; Herbert & Sullivan, 1963; Villamil & McCracken, 1963; Chisholm & Sharp, 1964; Hibbard, 1964). The amount of HCNH-Glu excreted in the urine is determined not only by the degree of deficiency of vitamin B₁₂ and/or PteGlu but may also be influenced by iron deficiency (Chanarin, Rothman & Berry, 1965; Vitale, Restrepo, Velez, Riker & Hellerstein, 1966) and possibly by use of other pathways (Spolter & Baldrige, 1963) in the degradation of histidine.

The concentrations of PteGlu in the livers of vitamin B₁₂-deficient sheep are considerably decreased compared with those in normal animals (Dawbarn, Hine & Smith, 1958b); further, the decrease is due to vitamin B₁₂ deficiency itself and

not to the lowered food intake that accompanies vitamin B₁₂ deficiency in the ruminant. A similar phenomenon occurs in rats (Kutzbach, Galloway & Stokstad, 1967).

The interrelationships known to occur between the functions of vitamin B₁₂, PteGlu and labile methyl groups led us to study the catabolism of histidine in sheep and rats deficient in vitamin B₁₂ and in sheep to which the PteGlu antagonist aminopterin had been administered.

EXPERIMENTAL

Rats. Seven male Wistar rats, bred from dams depleted of vitamin B₁₂, were studied. They were fed *ad lib.* on a diet consisting of: ground wheat (11 kg), wheat germ (4 kg), powdered gluten (3.4 kg), yeast (autoclaved for 15 min at 15 lb in⁻²) (1 kg), CaHPO₄·2H₂O (200 g), CaCO₃ (200 g), NaCl (100 g), KI (15 mg) and cod liver oil (100 g) mixed to a paste with water.

Rats nos. 1–3 (B₁₂–) were vitamin B₁₂-deficient and rats nos. 4–6 (B₁₂+) had been treated since weaning with a daily oral supplement of 5 µg of vitamin B₁₂; all were 31 weeks old at the beginning of the experiment.

Rat no. 7 (B₁₂–) was about 3 months older than the other six.

Administration of histidine was by intraperitoneal injection. Methionine, choline, betaine, homocysteine and serine were administered as oral supplements.

The rats were kept in individual cages, the bottoms of which consisted of wire grids resting on polyvinyl chloride funnels. Urine was collected in flasks, in the top of each of which was set a glass bulb for separating faeces from urine. Urine samples were collected daily, the volumes were measured and a filtered portion of each was stored at –10°C. The animals were weighed each week.

Sheep. The sheep, all ewes of about 4 years of age, used in this experiment were confined in metabolism cages and offered daily a ration of 1 kg of cobalt-deficient wheaten hay chaff plus 50 g of washed gluten. Cod liver oil (5 ml) twice a week and copper (25 mg as CuSO₄ in 10 ml of solution) once a week were administered orally. Food intakes were measured daily and the animals were weighed each week.

The vitamin B₁₂-deficient animals (B₁₂–) were in a relatively advanced stage of the deficiency syndrome (Marston, 1961). The stores of vitamin B₁₂ of the paired control animals (B₁₂+) were adequately maintained by treatment with the vitamin, either 50 µg/day or 250 µg/week by intramuscular injection. The daily ration of the B₁₂– sheep was restricted to the amount eaten on the preceding day by the corresponding negative control.

Urine samples were collected daily in containers to which 5 ml of toluene had been added; the volumes were measured and a filtered portion of each was stored at –10°C. For the first week or so of the experiments the animals were catheterized daily to ensure collection of a complete 24 h sample. Later, when the animals were trained, catheterization was unnecessary as they would void at the approach of the attendant.

Administration of histidine, methionine and serine was by injection via a polythene cannula inserted into an external jugular vein. PteGlu was injected either intra-

venously or subcutaneously. Aminopterin was administered by subcutaneous injection.

Formiminoglutamic acid. This was determined by the method of Tabor & Wyngarden (1958). The material determined as HCNH-Glu was destroyed by autoclaving at pH 7. Chromatography of urine samples on paper, using for development the 2-methylpropan-2-ol-formic acid-water mixture (14:3:3, by vol.) described by Tabor & Rabinowitz (1957), showed that they contained a substance whose *R_F* value corresponded to that of a sample of pure HCNH-Glu.

RESULTS

Rats. The urines of the vitamin B₁₂-treated animals contained virtually no HCNH-Glu, and after injection of 645 µmol of histidine individuals of this group excreted no more than 2% of this abnormal load of histidine as urinary HCNH-Glu.

Urinary excretion of HCNH-Glu by individuals of the vitamin B₁₂-deficient group was usually between 20 and 77 µmol/24 h, and during the 24 h after the injection of histidine the equivalent of 30–40% of the total histidine intake for that period was excreted in the urine as HCNH-Glu. Administration to each rat on day 15, and thereafter daily, of an oral supplement of PteGlu (1 mg in 0.5 ml of solution) had no apparent effect on the metabolism of histidine as judged by the urinary excretion of HCNH-Glu (Fig. 1).

Ingestion of methionine by the vitamin B₁₂-deficient rats significantly decreased the amount of HCNH-Glu excreted in the urine during the following 24 h, and when histidine was administered by intraperitoneal injection at the same time that the animal received an approximately equimolar dose of methionine orally there was no significant increase in the urinary excretion of HCNH-Glu (Figs. 1 and 2).

Choline, administered as choline chloride and mixed with a small quantity of food, significantly decreased the urinary excretion of HCNH-Glu by vitamin B₁₂-deficient rats. The maximum effect was observed during the second 24 h period after consumption of choline. No further decrease in the amount of HCNH-Glu excreted in the urine was achieved by doubling the quantity of choline ingested (Fig. 1). The effect of betaine, administered to rat no. 7 (B₁₂–) as betaine hydrochloride and mixed with a small quantity of food, was similar to that of choline. As with choline the maximum effect was observed during the second 24 h period after treatment (Fig. 2). Rat no. 7 subsequently died from causes that were not determined.

Administration of serine or homocysteine did not significantly lower the urinary excretion of HCNH-Glu by vitamin B₁₂-deficient rats. Treatment with vitamin B₁₂, 10 µg on day 193 (5 µg orally and 5 µg

Fig. 1. Influence of various treatments on the urinary excretion of HCNH-Glu by rat no. 2 (B₁₂-). The performance of this animal was typical of that of the others in the group. Supplement doses are reported in mmol. On day 9, after administration of histidine on day 8, the urinary excretions of HCNH-Glu by rats nos. 4-6 were respectively 15, 19 and 9 $\mu\text{mol}/24\text{h}$; on day 29, after administration of histidine on day 28, the excretions were 9, 15 and 8 $\mu\text{mol}/24\text{h}$ respectively. PteGlu (1 mg) and vitamin B₁₂ (10 μg on day 193 and thereafter 5 μg) were administered daily over the periods indicated. No importance attaches to the gap in the results between days 73 and 83; between days 115 and 144 estimations of HCNH-Glu in the urines of the deficient rats were not carried out regularly as the concentrations were relatively low (cf. days 123-132) and indicative of the animals' having received a trace of vitamin B₁₂.

parenterally) and thereafter 5 $\mu\text{g}/\text{day}$ orally, decreased the excretion of HCNH-Glu in the urines of these animals to negligible amounts within 3 days (Fig. 1).

Sheep. All the vitamin B₁₂-deficient sheep (18) studied in this series of experiments excreted abnormal amounts of HCNH-Glu in their urine, and in all the urinary excretion of HCNH-Glu was very substantially decreased a day or so after treatment with vitamin B₁₂. Over the period of the observations the amounts of urinary HCNH-Glu excreted by the deficient sheep ranged from 40 to 3760 $\mu\text{mol}/24\text{h}$. Vitamin B₁₂-treated pair-fed control animals,

restricted to a very low food intake, occasionally excreted small amounts of urinary HCNH-Glu; on resumption of an adequate diet the amount excreted became negligible. During the 24 h after histidine loading the percentage of histidine excreted as HCNH-Glu ranged from 13 to 60% in deficient sheep and from <1 to 8% in vitamin B₁₂-treated animals.

Administration of methionine to vitamin B₁₂-deficient sheep temporarily decreased the excretion of HCNH-Glu. Later, however, the excretion was greater than that which occurred before administration of methionine, e.g. the daily excretion of HCNH-Glu by sheep no. 399 (B₁₂-) increased from

Fig. 2. Influence of administration of betaine and methionine on the urinary excretion of HCNH-Glu by rat no. 7 ($B_{12}-$). Supplement doses are reported in mmol.

260 to 690 μmol after the first dose of methionine and from 690 to 1060 μmol after the second dose. Excretion was decreased to 18 and 114 $\mu\text{mol}/24\text{h}$ respectively immediately after dosing (Fig. 3). Urinary excretion of HCNH-Glu was not lowered by administration of serine; cf. sheep no. 320 ($B_{12}-$) (Fig. 3). The mean daily excretions for the 5 days before and after administration of serine were respectively 473 and 645 μmol .

Some of the vitamin B_{12} -deficient animals responded temporarily to treatment with PteGlu, e.g. sheep nos. 038 and 816 (Figs. 3 and 4). As their reserves of vitamin B_{12} became further depleted, however, urinary excretion of HCNH-Glu again increased, until treatment with vitamin B_{12} decreased it to much lower or undetectable amounts (Fig. 3).

Sheep no. 371 ($B_{12}-$). This animal was severely vitamin B_{12} -deficient, as shown by the exceptionally low food intake (Fig. 3). Administration of PteGlu had little if any effect on either its appetite or the daily excretion of HCNH-Glu. At 14 days after dosing with PteGlu had started, the animal was treated with vitamin B_{12} and its food intake restricted for 3 weeks to that before treatment, namely 210 g/day. After treatment with vitamin B_{12} there was a dramatic decrease in the amount of HCNH-Glu excreted in the urine; in 48 h the amount was decreased from about 2000 to 265 $\mu\text{mol}/24\text{h}$. In less than 1 week after resumption of feeding *ad lib.* the amount of HCNH-Glu excreted in the urine was undetectable (Fig. 3).

Sheep no. 038 ($B_{12}-$). This animal, judged by the criteria of food intake and haemoglobin content of the blood, was not severely vitamin B_{12} -deficient. However, at the start of the experiment it was

excreting in its urine 750–920 μmol of HCNH-Glu/24h. Administration of PteGlu had little, if any, effect on its appetite, but for a period decreased the excretion of HCNH-Glu by about one-third. However, the animal's capacity to deal with the extra load imposed by injection of histidine remained severely impaired (Fig. 3). For the 5 days before treatment with PteGlu the mean daily excretion of HCNH-Glu by sheep no. 038 was 680 μmol ; this was decreased to 190 μmol after treatment. After the initial increase following histidine loading excretion fell to 150 $\mu\text{mol}/24\text{h}$, but then rose to a mean of 850 $\mu\text{mol}/24\text{h}$ for the 5 days before treatment with vitamin B_{12} ; 48 h after dosing with vitamin B_{12} was initiated excretion had fallen to 50 $\mu\text{mol}/24\text{h}$ (Fig. 3).

Sheep nos. 816 ($B_{12}-$), 616 ($B_{12}+$) and 239 ($B_{12}+$). The performances of these animals (Figs. 4 and 5) illustrate the effect, under different conditions of treatment and food intake, of a decrease in the effective concentration of PteGlu imposed by administration of aminopterin. Sheep nos. 816 ($B_{12}-$) and 616 ($B_{12}+$) were pair-fed; sheep no. 616 was therefore on a restricted food intake. Sheep no. 239 was fed *ad lib.* and consumed daily about 1000 g of food.

Although a partial disturbance of histidine catabolism occurred in these animals, no abnormality in the ability to metabolize acetate, as assessed by clearance tests (H. R. Marston, S. H. Allen & R. M. Smith, unpublished work), nor any decrease in the number of white cells was observed. It is probable that sheep develop at least a partial resistance to aminopterin, for neither increasing the dose nor prolonging the time of treatment appreciably altered the effect on the catabolism of histidine.

Before administration of aminopterin the urinary excretion of HCNH-Glu by sheep no. 816 had been decreased to 200 $\mu\text{mol}/24\text{h}$ by treatment with PteGlu.

Treatment of sheep no. 816 with small doses of vitamin B_{12} at the times indicated (Fig. 4) was dictated by food intake, and finally on day 161 by the anaemic condition of the animal; the haemoglobin concentration was at that time 2.6 g of haemoglobin/100 ml. The corresponding concentrations for sheep nos. 616 and 239 were 7.9 and 9.1 g of haemoglobin/100 ml respectively.

The effect of administration of vitamin B_{12} on the excretion of HCNH-Glu is obvious from the graph of sheep no. 816 (Fig. 4). However, after histidine loading, the aminopterin-treated animal receiving adequate vitamin B_{12} was no more able to deal with the products of histidine catabolism than was the vitamin B_{12} -deficient animal. This abnormality in histidine catabolism was sometimes more pronounced when food intake was restricted (Figs. 4 and 5).

Fig. 3. Influence of various treatments on urinary excretion of HCNH-Glu by vitamin B₁₂-deficient and vitamin B₁₂-supplemented sheep. Note use of logarithmic scale to denote HCNH-Glu excretion. (a) Sheep no. 038 (B₁₂-). L-Histidine (16mmol in 50ml of solution) was administered as indicated. PteGlu treatment commenced on day 19 (100mg intravenously and thereafter 20mg/day by subcutaneous injection). Vitamin B₁₂ treatment commenced on day 48 (100μg and thereafter 50μg/day by intramuscular injection). (b) Sheep no. 371 (B₁₂-). L-Histidine (16mmol in 50ml of solution) was administered as indicated. PteGlu treatment commenced on day 5 (100mg intravenously and thereafter 20mg/day by subcutaneous injection). Vitamin B₁₂ treatment commenced on day 19 (100μg and thereafter 50μg/day by intramuscular injection). For 3 weeks after the initial treatment with vitamin B₁₂ food intake was restricted to 210g/day. (c) Sheep nos. 399 (B₁₂-) and 114 (B₁₂+) were pair-fed. DL-Methionine (27mmol in 150ml of solution on day 2 and 13.5mmol in 75ml of solution on day 17) was administered to each animal. On day 29 sheep no. 399 was treated with vitamin B₁₂ (100μg and thereafter 50μg/day by intramuscular injection). (d) Sheep nos. 320 (B₁₂-) and 284 (B₁₂+) were pair-fed. DL-Serine (40mmol in 100ml of solution) and L-histidine (16mmol in 50ml of solution) were administered to each animal on the days indicated. On day 20 sheep no. 320 was treated with vitamin B₁₂ (100μg and thereafter 50μg/day by intramuscular injection). Urinary excretion of HCNH-Glu by sheep no. 284, after injection of histidine, is represented by ●; during the remainder of the period of observation urinary excretion of HCNH-Glu was undetectable.

Fig. 4. Influence of administration of aminopterin on urinary excretion of HCNH-Glu. Note use of logarithmic scale to denote HCNH-Glu excretion. Sheep nos. 816 ($B_{12}-$) and 616 ($B_{12}+$) were pair-fed. Between days 11 and 23 sheep no. 816 was treated with 300mg of PteGlu. Aminopterin (2.4mg/day) was administered to each animal from day 24 for 122 days. On day 149 PteGlu was administered to each animal (100mg intravenously and thereafter 20mg/day subcutaneously). Vitamin B_{12} was administered to sheep no. 816 by intravenous injection on days 48 (100 μ g), 66 (50 μ g), 104 (100 μ g) and 161 (250 μ g). L-Histidine (16mmol in 60ml of solution) was administered to each animal on day 85 and each excreted approx. 25% of the dose as HCNH-Glu.

DISCUSSION

Histidine catabolism, with excessive urinary excretion of HCNH-Glu as criterion, is impaired in rats bred from vitamin B_{12} -deficient dams and fed on a diet deficient in vitamin B_{12} . As there was no response to an oral supplement of PteGlu it may be concluded that the condition was due to vitamin B_{12} deficiency and not to an absolute deficiency of PteGlu.

The ability of vitamin B_{12} -deficient rats to deal with the degradation products of histidine may be restored, temporarily, by administration of the methyl-group donors methionine, betaine or choline, and completely by administration of vitamin B_{12} .

Fig. 5. Influence of administration of aminopterin on urinary excretion of HCNH-Glu. Note use of logarithmic scale to denote HCNH-Glu excretion. Sheep no. 239 ($B_{12}+$) was treated with 250 μ g of vitamin B_{12} /week by intramuscular injection and its daily food intake was approx. 1kg. Aminopterin was administered as follows: 2.4mg/day from day 4 for 200 days and 4.8mg/day for the next 100 days. HCNH-Glu (29 μ mol) was first detected in its urine on day 65. L-Histidine (16mmol in 60ml of solution) was administered as indicated on days 66, 291 and 343. Of these doses <1%, 45% and 1% respectively were excreted in the urine as HCNH-Glu during the 24h subsequent to injection. PteGlu was administered by injection (100mg intravenously on day 307 and thereafter 20mg/day subcutaneously).

Homocysteine and serine were ineffective in this respect (Figs. 1 and 2). Our results for choline and homocysteine differ from those of Silverman & Pitney (1958) and Brown, Silva, Gardiner & Silverman (1960). No explanation for this discrepancy is immediately apparent; the degree of the vitamin deficiency is, however, probably a contributory factor.

Although administration of methionine temporarily confers upon vitamin B_{12} -deficient rats the ability to deal efficiently with the products of histidine catabolism, a daily supplement of methionine, equivalent to approx. 0.4% of the diet, was found, in another series of experiments, not to be effective in this respect. The mean growth rate of the methionine-treated animals was not significantly different from that of those not supplemented with methionine.

Vitamin B_{12} -deficient sheep excrete excessive amounts of urinary HCNH-Glu; complete restoration of the animal's ability to deal with the products of histidine catabolism may be achieved by administration of vitamin B_{12} . Administration of methionine temporarily relieves the condition; however, both the results for sheep reported here, and those of Fox *et al.* (1961) for chicks, show that after the temporary relief of the condition obtained by administration of methionine the daily excretion

Table 1. *Effect of vitamin B₁₂ deficiency on concentration of pteroylglutamates in liver*

The four sheep listed had been fed on the standard cobalt-deficient ration for a considerable period, and at the time of slaughter sheep nos. 039 and 438 were severely vitamin B₁₂-deficient and their daily food intakes had fallen to 260 and 320 g/day respectively. Sheep nos. 329 and 363 were each treated with 50 µg of vitamin B₁₂/day by intramuscular injection and their food intakes restricted respectively to those of sheep nos. 039 and 438. Values are reproduced from Dawbarn *et al.* (1958b).

Sheep no.	Vitamin B ₁₂ (<i>Lactobacillus</i> <i>leichmannii</i>)*	Pteroylglutamates (<i>Lactobacillus</i> <i>casei</i>)†
	(µg/g of liver tissue)	(µg/g of liver tissue)
039 (B ₁₂ -)	0.032	0.97
329 (B ₁₂ +)†	1.97	4.5
438 (B ₁₂ -)	0.050	1.43
363 (B ₁₂ +)†	0.94	4.5

* Measured by the method of Dawbarn & Hine (1954) and Hine & Dawbarn (1954).

† Measured by the method of Dawbarn, Hine & Smith (1958a).

of HCNH-Glu is subsequently greater than it was before administration, suggesting that the animal may have been further depleted of vitamin B₁₂. Similar but less consistent results have been noted in these experiments after administration of serine to sheep and rats and of homocysteine to rats. Usually this increase in the amount of HCNH-Glu excreted following a temporary decrease is observed only in animals severely depleted of vitamin B₁₂.

Sheep to which aminopterin has been administered are unable to deal effectively with the products of histidine catabolism. Complete remission of the condition may be brought about by provision of an adequate supply of PteGlu, provided that vitamin B₁₂ is not limiting.

Kutzbach *et al.* (1967) have shown that in rats fed on a diet deficient in vitamin B₁₂ or in labile methyl groups, but adequately supplemented with PteGlu, the total pteroylglutamate concentration in the livers is decreased, and methionine alone is almost as effective as vitamin B₁₂ in maintaining normal values.

The concentrations, as assayed by *Lactobacillus casei*, of pteroylglutamates in the livers of vitamin B₁₂-deficient sheep are significantly lower than those in normal animals. Re-analysis of the values quoted by Dawbarn *et al.* (1958b), but omitting the datum for sheep no. 534, shows that the difference is significant at the 0.1% level by the *d* test. This decrease depends on the vitamin B₁₂ status of the animal and is not due to a restricted food intake (Table 1).

In patients suffering from pernicious anaemia the

ratio of conjugated to unconjugated forms of PteGlu is considerably lower than in normal subjects (Jeejeebhoy, Pathar & Noronha, 1965). If in the higher animals, as in bacteria (Guest & Woods, 1962; Griffin & Brown, 1964), H₄PteGlu is active, and the methyl derivative inactive, as a substrate for the polyglutamate-forming system, then conversion of PteGlu into the H₄-form would be of primary importance, not only for the further metabolism of HCNH-Glu, but also for storage. The experimental results presented here, as well as those from many other laboratories, all indicate that one of the functions of vitamin B₁₂ is to maintain within the tissues a supply of PteGlu in a metabolically active form, probably as H₄PteGlu.

Vitamin B_{12r} [a one-electron-reduced form of α-(5,6-dimethylbenzimidazolyl)aquo(hydroxo)cobamide having cobalt in a valence state of 2] catalyses the reductive methylation of amines with formaldehyde (Schrauzer & Windgassen, 1967), and, since the reduction of 5,10-CH₂-H₄PteGlu to 5-Me-H₄PteGlu should be possible via the same mechanism, these authors postulate a vitamin B₁₂ requirement for the reductase. They also postulate that activation of methylcobalamin by demethylation to vitamin B_{12r} occurs only at optimum homocysteine/methionine concentrations. These suppositions are supported by the observation by Kutzbach & Stokstad (1967) that excess of *S*-adenosylmethionine inhibits and *S*-adenosylhomocysteine reverses the inhibition of methylenetetrahydropteroylglutamate reductase.

These findings make understandable the role that methionine plays in ameliorating, in part at least, certain conditions brought about by a deficiency of vitamin B₁₂, and are not at variance with reports that the concentrations of circulating pteroylglutamates, be they predominantly 5-Me-H₄PteGlu (Jaenicke, 1964; Vitale, 1966) or other forms of PteGlu (Luhby & Cooperman, 1965), are elevated in vitamin B₁₂ deficiency.

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