722102

PRIVACY ACT MATERIAL REMOVED

Lack of Effect of L-Methionine Ingestion on $^{14}CO_2$ Excretion from L-Histidine (Imidazole-2-^{14}C) in Folic Acid and Vitamin B_{12} Deficient Humans

By Hannes B. Stanbury, H. S. Winchell and N. Kusubov

A DIET CONTAINING ONE TO TWO PER CENT L-METHIONINE normalizes increased urinary excretion of formiminoglutamic acid (FIGLU) and decreased $^{14}CO_2$ excretion from L-histidine (imidazole-2-^{14}C) in folic acid and vitamin B_{12} deficient rats.[1-3] If such findings could also be found in man, and if they reflect biochemical effects of methionine on monocarbon fragment metabolism rather than competition between amino acids for intestinal absorption and cell membrane transport, then this finding could provide further insight into the metabolic abnormalities attendant to B_{12} and folic acid deficiency in man. Herbert and Sullivan have demonstrated a suppression in the amount of FIGLU excretion in folic acid and B_{12} deficient human subjects following a loading dose of 20 Gm. histidine when 20 Gm. of methionine a day were ingested prior to the loading dose.[3] There appear to be no observations in the present literature concerning the effect of methionine ingestion on the metabolism of the imidazole number two carbon atom of histidine to CO_2 following its parenteral administration in human subjects with folic acid or B_{12} deficiency. The present study was undertaken to provide such data.

Materials and Methods

Measurement of excretion of $^{14}CO_2$ in the breath subsequent to the intravenous administration of histidine (imidazole-2-^{14}C) was identical to that previously published (specific activity 57.8 μCi/mM., Nuclear Chicago Corp., Chicago, Ill.).[4] Each patient received 11 to 18 μCi. of histidine (0.03–0.04 mg.) intravenously at the initiation of each study.

All patients showed megaloblastic changes in the bone marrow aspirate. The pertinent peripheral red blood cell determinations for these patients at the time of hospital admission are as follows.

Patient	Hct.	Hbg.	Red Blood Cell	MCV
[redacted]	17	5.3	1.53	112
	13	3.2	0.75	160
	27.5	8.9	2.17	127
	30	10.9	2.57	116

From Donner Laboratory, University of California, Berkeley, Calif.

First submitted February 17, 1969; accepted for publication July 22, 1969.

Supported by Atomic Energy Commission Contract W-7495-Eng-48.

Hannes B. Stanbury, M.D.: *Postdoctoral Fellow under N.I.H. Training Grant 5-T01-GM01753-03, Donner Laboratory, University of California, Berkeley, Calif.* H. S. Winchell, M.D., Ph.D.: *Associate Research Physician, Do[illegible] Berkeley, Calif.* N. Kusubov: *Technician, [illegible] Berkeley, Calif.*

PRIVACY ACT MATERIAL REMOVED

These patients, [redacted], and [redacted] had folic acid deficiency, [redacted] and [redacted] had dietary folic acid deficiency, and additionally, H.B. had severe alcoholic hepatic cirrhosis. The breath $^{14}CO_2$ excretion in [redacted] and [redacted] following injection of L-histidine (imidazole-2-^{14}C) was characteristic of folic acid deficiency.[4] The reticulocytosis ([redacted] 8.5%, [redacted] 5%) and elevation of hematocrit ([redacted] 25%, [redacted] 31%) and hemoglobin concentration ([redacted] 7.5 Gm. %, [redacted] 6.0 Gm. %) within two weeks after initiation of a normal hospital diet alone further supported this diagnosis. However, serum folic acid concentration was not measured in these two patients. [redacted] had a malabsorption syndrome and a consequent serum folic acid concentration of 0.7 ng./ml. Despite the low serum folic acid level in this patient the breath excretion of $^{14}CO_2$ subsequent to administration of histidine (imidazole-2-^{14}C) was within low normal limits.[4] An additional patient, [redacted] was found to have vitamin B_{12} deficiency, with a serum folate level of 7.8 ng./ml. Intestinal absorption of ^{57}Co-Cyanocobalamin was 1.33 per cent (normal 20–30%) as measured by use of the whole body counter.[5] This patient subsequently responded with a reticulocytosis of 13.8 per cent within one week following parenteral administration of 1000 μg. vitamin B_{12}. $^{14}CO_2$ excretion in this patient ([redacted]) was within normal limits as is characteristically seen in vitamin B_{12} deficiency.[4]

Results

Oral ingestion of L-methionine (400 mg./kg. body weight to [redacted] and [redacted] and 800 mg./kg. body weight to [redacted]) given in three equal doses prior to repeat administration of L-histidine (imidazole-2-^{14}C) failed to alter the pattern of appearance of $^{14}CO_2$ in the breath (Table 1). [redacted] and [redacted] received the last oral dose of methionine three hours prior to the study. [redacted] and [redacted] received the last oral dose of methionine 18 hours and six hours prior to the study, respectively.

Discussion

In folic acid deficiency, and possibly in vitamin B_{12} deficiency, the number of formimino carbon atoms from FIGLU which are metabolized through the monocarbon pool to CO_2 per unit time is fixed by the availability of reduced forms of folic acid.[6-8] When large quantities of histidine are absorbed from the intestinal tract and delivered to sites of metabolism in subjects with a limited ability to metabolize FIGLU, only a small fraction of the FIGLU arising from the metabolism of such histidine can be metabolically accom-

Table 1.—Time at Which Maximum Rate of $^{14}CO_2$ Occurred (T max), and Cumulative Percent of Administered ^{14}C Which Was Excreted in Breath as $^{14}CO_2$ During Initial 60 Min. After I.V. Administration of Histidine (Imidazole-2-^{14}C) Before and After Ingestion of L-methionine in Patients with Folic Acid or vitamin B_{12} Deficiency.

Patient	Weight (Kg.)	T max (min.) L-Methionine Ingestion (Normal 30–60 min.) Before	After	$^{14}CO_2$ Excretion in 60 min. (%) L-Methionine Ingestion (Normal 0.1–0.5%) Before	After
[redacted]	78	*	*	*	*
	66	*	*	*	*
	60	40	34	0.88	[illegible]
	75	36	38	1.31	1.18

* ^{14}CO excretion too small for accurate determination (i.e., indeterminate T max and cumulative 60 min. $^{14}CO_2$ excretion < 0.10%).

BEST COPY AVAILABLE

1160163

modated and a large quantity of the excess FIGLU is excreted in the urine. When tracer quantities of histidine labeled with ^{14}C at the imidazole number two carbon atom site are administered *parenterally* to such subjects we would expect that the fraction of the ^{14}C label excreted in the urine on FIGLU would be large and the fraction excreted as $^{14}CO_2$ in the breath would be proportionately smaller. When sufficiently small quantities of histidine are absorbed from the intestinal tract and delivered to sites of metabolism in subjects with a limited ability to metabolize FIGLU such that the amount of reduced folic acid available is sufficient to metabolize much of the FIGLU arising from the metabolism of histidine, little excess FIGLU accumulates and little FIGLU is excreted in the urine. When tracer quantities of histidine labeled with ^{14}C at the imidazole number two carbon position is administered parenterally under these latter conditions we would expect little excretion of ^{14}C labeled FIGLU in the urine and proportionately greater $^{14}CO_2$ excretion in the breath. Thus, any perturbation which decreases absorption of histidine into the body of such patients would result in a decrease in total FIGLU excretion in the urine, as well as an increase in $^{14}CO_2$ excretion in the breath and a decrease in ^{14}C labeled FIGLU excretion in the urine following parenteral administration of histidine (imidazole-2-^{14}C). It is to be expected that large oral doses of methionine would result in decreased delivery of histidine to its site of metabolism by virtue of its established inhibition of intestinal absorption and cell membrane transport of histidine.[9-12] Herbert and Sullivan[3] discussed this mechanism as a possible explanation of the methionine effect on FIGLU excretion. In addition to this the results of Brown et al.[2] can also be explained on the basis of methionine inhibition of intestinal absorption and cell membrane transport of histidine alone, and a direct effect of methionine on histidine metabolism need not be invoked.

Since the rate-limiting step in the metabolism of the imidazole number two carbon atom site of histidine in the normal nonfolic acid deficient nonvitamin B_{12} deficient animal is not known, the above arguments are not applicable. Indeed, in the normal animal with sufficient stores of folic acid and vitamin B_{12} the availability of large quantities of methionine as a monocarbon fragment donor might be expected to suppress the entrance of other monocarbon fragments (such as the imidazole number two carbon atom of histidine) into the monocarbon pool. This is consistent with findings in normal rats.[2] It is proposed that in the present studies the failure to demonstrate alteration in $^{14}CO_2$ excretion following the intravenous administration of histidine (imidazole-2-^{14}C) to folic acid and vitamin B_{12} deficient subjects was due to failure of methionine given in this dosage and for this duration to significantly alter body histidine pool size. The delay between the administration of the last methionine dose and the injection of histidine may have contributed to the lack of effect of methionine in patients [redacted] and [redacted]. However, we did not increase the methionine dose or prolong the duration of treatment because of apparent induction of undesirable side effects by methionine in these patients.[13]

Summary

Three human subjects with folic acid and one with vitamin B_{12} deficiency

PRIVACY ACT MATERIAL REMOVED

failed to show any effect of ingestion of L-methionine (400–800 μg./kg. body weight) on the production of $^{14}CO_2$ from parenterally administered high specific activity L-histidine (imidazole-2-^{14}C). It is suggested that the inhibitory effect of methionine on intestinal absorption and cell membrane transport of histidine can explain previously published effects of methionine on the oxidation of the imidazole-2 carbon atom of L-histidine and that a direct effect of methionine on histidine metabolism need not be invoked.

References

1. Silverman, M., and Pitney, A. J.: Dietary methionine and the excretion of formiminoglutamic acid by the rat. J. Biol. Chem. 233:1170, 1958.

2. Brown, D. D., Silva, O. L., Gardiner, R. C., and Silverman, M.: Metabolism of formiminoglutamic acid by vitamin B_{12}- and folic acid-deficient rats fed excess methionine. J. Biol. Chem. 235:2058, 1960.

3. Herbert, V., and Sullivan, L. W.: Formiminoglutamicaciduria in humans with megaloblastic anemia: Diminution by methionine or glycine. Proc. Soc. Exp. Biol. Med. 112:304, 1963.

4. Fish, M. B., Pollycove, M., and Fechtneir, T. B.: Differentiation between vitamin B_{12}-deficient and folic acid-deficient megaloblastic anemias with ^{14}C-histidine. Blood 21:447, 1963.

5. Heyssel, R. M.: Absorption and excretion of vitamin B_{12} measured by whole-body counting. Proceedings of a panel on the clinical use of whole-body counting. International Atomic Energy Agency, Vienna, 1966.

6. Miller, H., and Waelsch, H.: The transfer of the formimino group of formamidinoglutaric acid to tetrahydrofolic acid. Arch. Biochem. Biophys. 63:263, 1956.

7. Miller, H., and Waelsch, H.: Formimino transfer from formamidinoglutaric acid to tetrahydrofolic acid. J. Biol. Chem. 228:397, 1957.

8. Lubby, A. L., Cooperman, J. M., and Teller, D. N.: Histidine metabolic loading test to distinguish folic acid deficiency from Vitamin B_{12} in megaloblastic anemias. Proc. Soc. Exp. Biol. Med. 101:350, 1959.

9. Wiseman, G.: Preferential transference of amino acids from amino-acid mixtures by sacs of everted small intestine of the golden hamster (mesocricetus auratus). J. Physiol. 124:412, 1954.

10. Wiseman, G., and Chadially, F. N.: Studies in amino-acid uptake by RD_3 sarcoma cell suspension in vitro. Brit. J. Cancer 9:480, 1955.

11. Hagihira, H., Ogata, M., Takedatsu, N., and Suda, M.: Intestinal absorption of amino acids, III. Interference between amino acids during intestinal absorption. J. Biochem. 47:139, 1960.

12. Nakamura, R.: The transport of histidine and methionine in rat brain slices. J. Biochem. 53:314, 1963.

13. Stabelin, H. B., and Winchell, H. S.: Induction of psychotic behavior in folic acid deficient patients by ingestion of L-methionine. Unpublished.

1160764