Alterations in Histidine Catabolism in Normal Rats Given Pharmacological Doses of Folic Acid and Cyanocobalamin[1] (34173)

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The imidazole no. 2-carbon atom of histidine is uniquely catabolized to CO_2 by passage through the "monocarbon pool" attached to a reduced form of folic acid (1). The rate of oxidation of this carbon atom site to CO_2 has been shown to be markedly diminished in both folic acid and cyanocobalamin (Vitamin B_{12}-deficient rats (2), and in folic acid-deficient human subjects (3). In cyanocobalamin-deficient human subjects the rate of CO_2 production from the imidazole no. 2-carbon atom site of histidine appears to be normal (3). The present communication demonstrates significant alterations in such catabolism in normal rats given pharmacological doses of either folic acid or cyanocobalamin.

Methods and Materials. Normal inbred Buffalo rats (Simonsen Laboratory, Gilroy, California) weighing 240–245 g were divided into control, folic acid-treated and cyanocobalamin-treated groups. The animals were fed S-L white diets consisting of protein, 24.00%; fat, 6.85%; fiber, 3.14%; calcium, 0.73%; phosphorus, 0.52%; ash, 4.69%; vitamins A, D, riboflavin, pantothenic acid, niacin, and choline. The control group consisted of 10 rats which received no prior treatment. The group receiving cyanocobalamin consisted of 4 rats which were given 250 μg of cyanocobalamin intravenously 60 min prior to the study (Rubramin, cyanocobalamin 1000 μg/ml in isotonic saline, pH adjusted to 5–6.5 with HCl, 1.5% benzyl alcohol, E. R. Squibb and Sons, N. Y.). The group receiving folic acid consisted of 6 rats which were given 15 mg of folic acid subcutaneously 60 min prior to the study (Folvite, sodium folate 15 mg/ml, sodium sequestrene 0.2%, pH 9, 1.5% benzyl alcohol, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.). At the initiation of each study, each rat, under light anesthesia with diethyl ether, received 2.5 μCi of L-histidine (imidazole-2-^{14}C) intravenously (sp act: 57.8 mCi/mmole, Amersham/Searle Corporation). Immediately after such injection the animal was placed in a device which measured the $^{14}CO_2$ excretion rate (5–6).

Results. Figure 1 presents composite data of the rate of $^{14}CO_2$ production following intravenous administration of L-histidine (imidazole-2-^{14}C) in 10 control rats, 4 rats given 250 μg of cyanocobalamin intravenously 60 min prior to the study, and 6 rats given 15 mg of folic acid subcutaneously 60 min prior to the study. The ordinate represents percentage of administered ^{14}C excreted as $^{14}CO_2$ per minute and the abscissa represents time in minutes following intravenous injection of L-histidine (imidazole-2-^{14}C). Each point represents the mean of the $^{14}CO_2$ excretion rate for each group of animals at the given time and the end of the vertical bars through each point represents one standard error of the mean for each group.

As shown, qualitative differences exist between control curves and those obtained in either cyanocobalamin- or folate-treated rats. For comparison of $^{14}CO_2$ breath curves, two parameters were utilized for each curve. The first parameter is the time at which maximum rate of $^{14}CO_2$ excretion in the breath occurs (T_{max}), and the second parameter is the cumulative percentage of ^{14}C appearing as $^{14}CO_2$ within the initial 60 min subsequent to the intravenous administration of the ^{14}C-labeled histidine. Table I presents values for the mean and standard error of the mean (SE) for each of these two parameters as

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determined in each individual study. From Fig. 1 and Table I we may conclude that normal rats, pretreated with pharmacological doses of cyanocobalamin, show a greater initial excretion rate of $^{14}CO_2$ and a shorter time at which the maximum rate of $^{14}CO_2$ excretion is reached (T_{max}) than normal untreated rats, while normal rats pretreated with pharmacological doses of folic acid show a slower initial excretion rate of $^{14}CO_2$ and a longer T_{max} than normal untreated rats.

Discussion. The present results demonstrate that pharmacological doses of cyanocobalamin increase the rate and amount of oxidation of the imidazole no. 2-carbon atom site of histidine to CO_2 *in vivo* while pharmacological doses of folic acid effect the opposite results. Kinetically stated these results mean that the fractional turnover rate of the rate-limiting processes involved in the oxidation of this carbon atom site of intravenously administered histidine and the fraction of this carbon atom site oxidized to CO_2 are increased by pharmacological doses of cyanocobalamin and decreased by pharmacological dose of folic acid. It is possible that this may be the result of alterations in physical transport of administered histidine to intracellular sites of catabolism such as might occur secondary to alteration in the kinetics of cell membrane transport of histidine. This latter possibility seems unlikely since recent unpublished experiments in this laboratory failed to show any effect of cyanocobalamin or sodium folate on the *in vitro* intracellular accumulation rate of labeled histidine. It is also possible that the results obtained may be due to alterations in the biochemical kinetics involved in histidine catabolism. It has been shown previously that high levels of folic acid inhibit dihydrofolic acid reductase *in vitro*

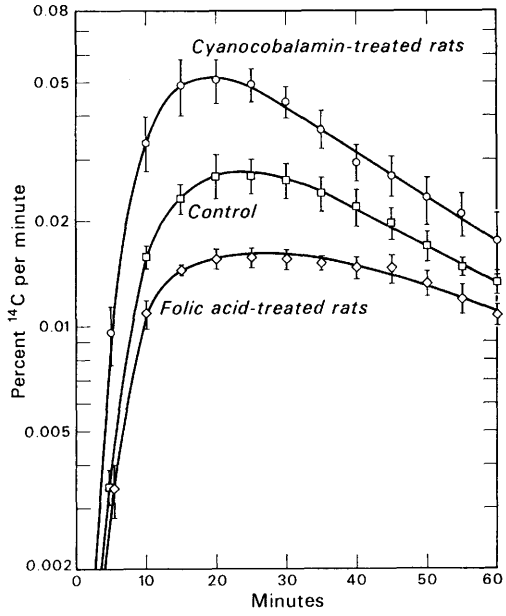

FIG. 1. Composite data of the rate of $^{14}CO_2$ production following intravenous administration L-histidine (imidazole-2-^{14}C) in 10 control rats, 4 rats given 250 μg of cyanocobalamin intravenously 60 min prior to the study, and 6 rats given 15 mg of folic acid subcutaneously 60 min prior to the study. The ordinate represents percentage of administered ^{14}C excreted as $^{14}CO_2$ per minute and the abscissa represents time in minutes following intravenous injection of L-histidine (imidazole-2-^{14}C). Each point represents the mean of the $^{14}CO_2$ excretion rate for each group of animals at the given time and the end of the vertical bars through each point represents one standard error of the mean for each group.

TABLE I. Changes in the Time at Which the Maximum Rate of Excretion of $^{14}CO_2$ in Expired Breath Occurred (T_{max}) and Cumulative Percentage of $^{14}CO_2$ Excreted in Breath during the Initial 60 min following iv Administration of L-Histidine (imidazole-2-^{14}C) in Control Rats and Rats Given Either Folic Acid or Cyanocobalamin.[a]

Category	T_{max} (min ± SE)	^{14}C excretion in 60 min (% ± SE)
Control (10)	23.10 ± 0.77	1.02 ± 0.10
Folic acid (15 mg) sc 60 min prior to study (6)	28.58 ± 1.16	0.72 ± 0.02
Cyanocobalamin (250 μg) iv 60 min prior to study (4)	19.12 ± 1.38	1.84 ± 0.26

[a] The mean value and standard error of the mean for T_{max} and percentage of ^{14}C excreted in 60 min is given. (The number of animals in each group of rats is noted in parentheses.)

(7). If the turnover rate of tetrahydrofolic acid (THF) moieties are sufficiently rapid to significantly exhaust preformed THF within 1 hr, then this may explain our results following administration of high doses of folic acid. From evidence in cyanocobalamin-deficient subjects, it has been postulated that cyanocobalamin influences the utilization of methylated tetrahydrofolic acid (8). If the present results are due to cyanocobalamin-induced acceleration of catabolism of the imidazone no. 2 carbon atom of histidine after its attachment to tetrahydrofolate, then such an effect is not maximal at physiological levels of cyanobalamin. That the present results are relatively specific is suggested by our unreported findings that $^{14}CO_2$ excretion in the breath following administration of propionate (no. 2-^{14}C) to rats is not altered by prior administration of the same cyanocobalamin preparation used in the present experiments. Similarly, $^{14}CO_2$ excretion following administration of methionine (methyl-^{14}C) was not altered by prior administration of folate.

Summary. Following administration of histidine (imidazole-2-^{14}C) to normal rats given pharmacological doses of cyanocobalamin the initial rate and amount of $^{14}CO_2$ excreted in the breath is significantly increased while in normal rats given pharmacological doses of folic acid these measurements are decreased as compared to normal untreated rats. These results indicate that pharmacological doses of cyanocobalamin increase and folic acid decrease either the rate of physical transport of histidine to intracellular sites of catabolism or the fractional rate describing the actual biochemical steps involved in such catabolism.

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