DECREASED $^{14}CO_2$ PRODUCTION IN THIAMINE-DEFICIENT RATS GIVEN PYRUVATE-1-^{14}C AND ACETATE -1-^{14}C: A POSSIBLE MEANS FOR EARLY DIAGNOSIS OF BERI-BERI?

T. M. Ngo, H. S. Winchell, M. A. Williams, N. N. Finley and J. H. Lawrence

Donner Laboratory and Department of Nutritional Sciences, University of California, Berkeley, California.

Despite the availability of considerable biochemical information concerning the functions of thiamine, the effective early diagnosis of beri-beri awaits the development of simple, rapid techniques for screening populations for the metabolic defects attendant to this vitamin-deficiency state. The present work is directed toward development of such techniques.

Lactic-acid and pyruvic-acid concentration in blood is increased (*1–7*) and oxygen uptake in many tissues is decreased in thiamine-deficient rats (*8*). In man thiamine deficiency can also be detected by measuring the concentration of pyruvic acid or α-ketoglutaric acid in blood (*9*) or by determining the "carbohydrate index" which is based on concentration of pyruvic acid, lactic acid and glucose in blood after a standard exercise and administration of glucose (*10*). In addition, measurement of thiamine and its metabolites has been made in breath, blood, urine, feces and tissues (*11–18*).

It is known that before pyruvate can enter the tricarboxylic acid cycle, it must be decarboxylated to acetyl-CoA in the presence of thiamine pyrophosphate. Similarly, the oxidation of acetate to CO_2 depends on the presence of an intact tricarboxylic acid cycle, which in turn requires thiamine pyrophosphate to maintain decarboxylation of α-ketoglutarate to succinyl CoA and CO_2. For practical purposes both of the above reactions are irreversible.

The apparent dependency of the oxidation of the #1-carbon of both pyruvate and acetate to CO_2 upon the presence of thiamine pyrophosphate suggested that appearance of $^{14}CO_2$ in the breath following administration of pyruvate-1-^{14}C and acetate-1-^{14}C might be a measure of thiamine deficiency, and thus such measurements might be useful in the early detection of beri-beri. In the present study $^{14}CO_2$ appearance in the breath subsequent to administration of pyruvate, acetate-1-^{14}C and bicarbonate and plasma clearance of thiamine (thiazole-2-^{14}C) was measured in normal and thiamine-deficient rats.

MATERIALS AND METHODS

Preparation of experimental animals. Twenty-seven male Buffalo rats (Simonsen Laboratory, Gilroy, Calif.) weighing 110–135 gm and five male Buffalo rats weighing 350–390 gm were used in these experiments. The rats in the first group were 42 days old and the animals in the second group were about 3 months old at the start of the experiments. Each of these groups was further subdivided into control and thiamine-deficient subgroups. The diet of the control rats had the following composition expressed as percentages: vitamine-free casein, 20.0; sucrose, 67.5; cottonseed oil, 5.0; UCB-IRb salts, 3.5 (*19*); choline bitartrate, 1.0; vitamins A,D,E, 1.0; and vitamin B (thiamine, riboflavin, niacinamide, pyridoxine, folic acid, biotin, vitamin B_{12}, menadione and Ca D-pantothenate), 2.0. The deficient diet contained the same formula as above except that it was deficient in thiamine. All animals were allowed to feed *ad lib.* The experiments were conducted from 14 to 20 days after initiation of these diets when the thiamine-deficient rats showed weight loss and generalized asthenia. In these experiments the control rats weighed an average of 362 gm at the beginning and 385 gm at the end of the experimental period, while thiamine-deficient rats weighed an average of 410 gm at the beginning and 376 gm at the end of the experimental period.

$^{14}CO_2$ production studies. The experimental animals were divided into two groups. The control group consisted of six rats and the thiamine-deficient group consisted of seven rats. The appearance of $^{14}CO_2$ in the breath subsequent to the intravenous administration of pyruvate-1-^{14}C was measured in each of these animals. After the first series of experiments, four rats of the thiamine-deficient group received 20 mg of thiamine hydrochloride per day (Abbott

Received Oct. 2, 1968; revision accepted May 1, 1969.

For reprints contact: H. S. Winchell, Donner Laboratory and Donner Pavilion, University of California, Berkeley, Calif. 94720.

Laboratories, North Chicago, Ill.) intramuscularly 1–2 days prior to performance of a repeat study. In each study the rat received intravenously 2.5 μCi of sodium pyruvate-1-^{14}C (specific activity, 3.52 mCi/m*M*; New England Nuclear Corp., Boston, Mass.), after light ether anesthesia.

In a second series of studies $^{14}CO_2$ appearance in the breath was measured subsequent to i.v. administration of sodium acetate-1-^{14}C. The experimental animals consisted of three control and three thiamine-deficient rats. In each study, the rat received 2.5 μCi of sodium acetate-1-^{14}C (specific activity, 40.0 mCi/m*M*; Nuclear–Chicago, Des Plaines, Ill.). The experiments were repeated in thiamine-deficient rats 40–45 min after intravenous administration of 15 mg of thiamine hydrochloride and again a day later after a second i.v. dose of 15 mg of thiamine hydrochloride.

In a third group of animals the effect of thiamine deficiency on the HCO_3^- pool was studied. The group consisted of four controls and four thiamine-deficient rats. In each study the rat was given 1 μCi of $NaH^{14}CO_3$ intravenously (specific activity, 0.50 mCi/2.0 mg; New England Nuclear Corp.), and $^{14}CO_2$ was measured in expired air. Immediately after intravenous administration of ^{14}C-labeled materials, each control and thiamine-deficient rat was placed in an animal-holding chamber, and the expired air was passed through an ionization chamber at a constant rate of 3 liters/min in an experimental apparatus similar to that which has been described previously (*20–22*). At this gas-flow rate the mean turnover time of gas in the measuring apparatus (mean washout time) was less than 1 min. The rate and the amount of $^{14}CO_2$ excreted in the breath of rats were recorded continuously.

Plasma thiamine clearance studies. Five male Buffalo rats were assembled into two groups of two controls and three thiamine-deficient rats. The rats were studied individually. Each animal received 20 μCi of thiamine (thiazole-2-^{14}C) hydrochloride (specific activity, 25.2 mCi/m*M*; Nuclear–Chicago) intravenously under light ether anesthesia. The blood samples were obtained in heparinized capillary tubes from tail-vein venepuncture at approximately 1½, 4, 6½, 10½, 21, 30, 70 and 117 min after i.v. injection of thiamine (thiazole-2-^{14}C) hydrochloride. The plasma samples were then isolated using a semi-micro-method. Each sample consisted of 20 lambda of plasma dissolved in 0.5 ml of Nuclear–Chicago solubilizer (0.6 *N* solution in toluene) which was added to 15 ml of scintillation solution made of naphtalene, 2,5-diphenyloxazole (PPO, scintillation grade, Packard Instrument Co., Downers Grove, Ill.), 1,4 bis-(2-(5-phenyloxazolyl))-benzene (POPOP, scintillation grade, Packard Instrument Co.), 1,4 dioxane (J. T. Baker Chemical Co., Phillipsburg, N.J.), toluene and absolute ethyl alcohol. The ^{14}C activity in the solution was determined with a Nuclear–Chicago Model 725 liquid scintillation counter. Absolute content of ^{14}C in the sample was calculated from measurement of the liquid scintillation counter efficiency using an internal ^{14}C standard (C_6H_5CH3-^{14}C in toluene; specific activity, 1.59 μCi/4.96 ml; Nuclear–Chicago). Counting efficiency was generally 0.8 and background varied from 27.8 to 29.2 cpm.

RESULTS

$^{14}CO_2$ production studies: Pyruvate-1-^{14}C. Figure 1 gives representative curves showing the rate of appearance of $^{14}CO_2$ in the breath of a control rat and a thiamine-deficient rat prior to, and 1 and 2 days after daily intramuscular injection of 20 mg thiamine hydrochloride. The ordinate represents the $^{14}CO_2$ excretion rate expressed as mμCi/min and the abscissa as time in minutes following i.v. injection of pyruvate-1-^{14}C.

The control rat achieved a greater initial rate of $^{14}CO_2$ excretion which subsequently decreased more rapidly than did that of the thiamine-deficient rat. One day after intramuscular injection of 20 mg thi-

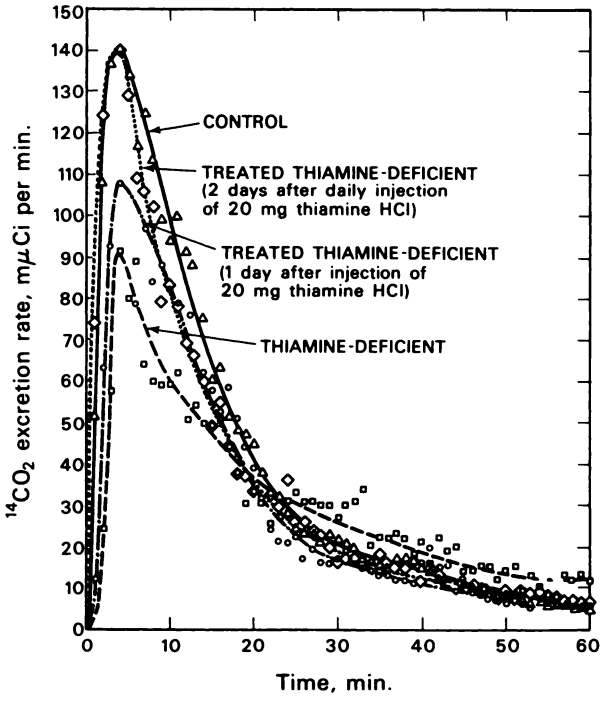

FIG. 1. Representative curves show rate of appearance of $^{14}CO_2$ in breath of control rat and thiamine-deficient rat before and 1 and 2 days after daily intramuscular injection of 20 mg thiamine hydrochloride. Ordinate shows $^{14}CO_2$ excretion rate expressed as mμCi/min and abscissa as time in minutes following i.v. injection of pyruvate-1-^{14}C.

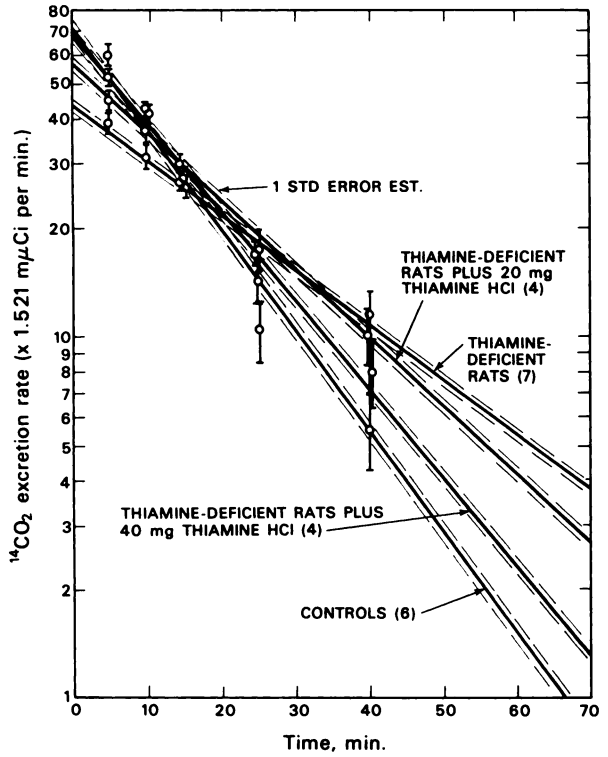

FIG. 2. Gives composite data of rate of $^{14}CO_2$ following i.v. administration of pyruvate-1-^{14}C in six control rats, seven thiamine-deficient rats and four thiamine-deficient rats on first and second day after daily intramuscular injections of 20 mg of thiamine hydrochloride. Ordinate gives $^{14}CO_2$ excretion rate expressed as × 1.521 mμCi/min and abscissa as time in minutes following i.v. injection of pyruvate-1-^{14}C. Vertical bars through each point define precision of position of mean of excretion rates of $^{14}CO_2$ for each group of rats with 95% limits based on $t_{.95}S_{y.x}$. Broken lines above and below each curve are regression lines of ±1 standard error of estimate ($S_{x.y}$).

amine hydrochloride the previously thiamine-deficient rat had a breath $^{14}CO_2$ curve intermediate between that seen in the thiamine-deficient state and that noted in the control. After two daily injections of 20 mg thiamine hydrochloride to the previously thiamine-deficient rat, the appearance of $^{14}CO_2$ in the breath is similar to that seen in the control animal.

Figure 2 gives composite data of the rate of $^{14}CO_2$ production following i.v. administration of pyruvate-1-^{14}C in six control rats, seven thiamine-deficient rats and four thiamine-deficient rats on the first and second day after daily intramuscular injection of 20 mg of thiamine hydrochloride. Each point at 5, 10, 15, 25 and 40 min represents the mean of excretion rates of $^{14}CO_2$ for each group of rats. Vertical bars through each point define precision of position of the mean with 95% limits based on $t_{.95}S_{y.x}$ ($S_{y.x}$ = 1 standard error of the estimate). The zero time intercept (A), the slope of the regression function (B) and the standard error of the slope ($S_{b(x).(y)}$) were determined by least-squares best fit of the data to the function

$$Y = A + (B \pm S_{b(x).(y)})\ X.$$

TABLE 1. SLOPE ($T_{1/2}$) AND INTEGRAL ^{14}C EXCRETION DETERMINED FROM $^{14}CO_2$ APPEARANCE IN BREATH FOLLOWING I.V. ADMINISTRATION OF PYRUVATE (#1-^{14}C) IN CONTROL AND EXPERIMENTAL RATS

Category	Half-time $T_{1/2} \pm \sigma$ (min)	^{14}C excretion in 60 min (% ± σ)
Normal rats (6*)	$10.728^{+0.193}_{-0.251}$	64.8095 ± 2.2642
Thiamine-deficient rats (7*)	$19.687^{+0.484}_{-0.080}$	42.9687 ± 4.1139
Thiamine-deficient rats 24 hr after intramuscular administration of 20 mg thiamine hydrochloride (4*)	$15.928^{+0.388}_{-0.371}$	49.2882 ± 2.8866
Thiamine-deficient rats 48 hr after intramuscular administration of 40 mg thiamine hydrochloride (4*)	$12.317^{+0.847}_{-0.973}$	57.1520 ± 3.4390

* Denotes number of animals in each group.

The broken lines immediately above and below each curve are regression lines of ± 1 standard error of the estimate ($S_{x.y}$).

In determining the significance of differences between the curves obtained in the control and thiamine-deficient groups, a p value of < 0.01 was obtained. In this analytic approach only the downslope of the $^{14}CO_2$ breath curves was analyzed (> 5 min after i.v. injection of pyruvate). Since the initial rate of $^{14}CO_2$ production from pyruvate-1-^{14}C is sufficiently rapid to be of the order of magnitude of the turnover rate of the $^{14}CO_2$ measurement apparatus, little reliable information can be extracted from the initial portion of the breath $^{14}CO_2$ curve. The slope (expressed as $T_{1/2}$) and integral amount of ^{14}C administered excreted in the breath (expressed as percent) during the initial 60 min of the study for each group of rats are presented in Table 1. It is clear that both the $T_{1/2}$ and the integral amount of ^{14}C excreted in the breath in 60 min are significantly different in control and thiamine-deficient rats. The $T_{1/2}$ in deficient rats is about double that in control rats. All parameters in deficient rats closely approached the normal range 2 days after initiation of daily administration of 20 mg of thiamine hydrochloride.

$^{14}CO_2$ production studies: Acetate-1-^{14}C. The curves describing appearance of $^{14}CO_2$ in the breath of control and thiamine-deficient rats given acetate-1-^{14}C are similar to those obtained after administra-

tion of pyruvate-1-^{14}C. Figure 3 presents representative curves describing the excretion of $^{14}CO_2$ in the breath following i.v. administration of acetate-1-^{14}C in a control, a thiamine-deficient and the same thiamine-deficient rat 45 min after i.v. administration of 15 mg thiamine hydrochloride. A significant difference in the $^{14}CO_2$ curves is noted between control and thiamine-deficient rats. Within 45 min after i.v. administration of thiamine the previously thiamine-deficient rat had a normal $^{14}CO_2$ breath curve. Figure 4, similar to Fig. 2, presents composite data describing the rate of $^{14}CO_2$ production following i.v. administration of acetate-1-^{14}C to three control, three thiamine-deficient rats and three thiamine-deficient rats given 15 mg thiamine hydrochloride i.v. 45 min prior to initiation of the study. The method of plotting the data and analysis of the least-squares best-fit single exponential regression curve with its confidence limits is identical to that described for Fig. 2. The curve defined by the data obtained in control rats is significantly different ($p < 0.01$) from that obtained in thiamine-deficient rats. However, the pattern of appearance of $^{14}CO_2$ in the breath of thiamine-deficient rats given acetate-1-^{14}C 45 min after i.v. administration of 15 mg thiamine hydrochloride was essentially identical to that noted in control animals. A repeat study on such thiamine-deficient animals 45 min after a second i.v. injection, 24 hr after the first such treatment, again yielded a normal pattern of $^{14}CO_2$ excretion. These data are presented in tabular form in Table 2 where the slope of the breath $^{14}CO_2$ curve expressed as $T_{1/2}$ and the integral percent of ^{14}C excreted in 60 min are listed for each group of animals.

TABLE 2. SLOPE ($T_{1/2}$) AND INTEGRAL ^{14}C EXCRETION DETERMINED FROM $^{14}CO_2$ APPEARANCE IN BREATH FOLLOWING I.V. ADMINISTRATION OF ACETATE(1-^{14}C) IN CONTROL AND EXPERIMENTAL RATS

Category	Half-time $T_{1/2} \pm \sigma$ (min)	^{14}C excretion in 60 min (% $\pm \sigma$)
Normal rats (3*)	$18.474^{+0.633}_{-0.553}$	52.233 ± 4.193
Thiamine-deficient rats (3*)	$27.530^{+1.571}_{-1.423}$	46.720 ± 0.683
Thiamine-deficient rats 45 min after i.v. administration of 15 mg thiamine hydrochloride (3*)	$19.184^{+0.549}_{-0.405}$	55.645 ± 3.213
Thiamine-deficient rats 45 min after second daily dose of 15 mg thiamine hydrochloride (3*)	$21.973^{+0.111}_{-0.113}$	54.640 ± 6.884

* Denotes number of animals in each group.

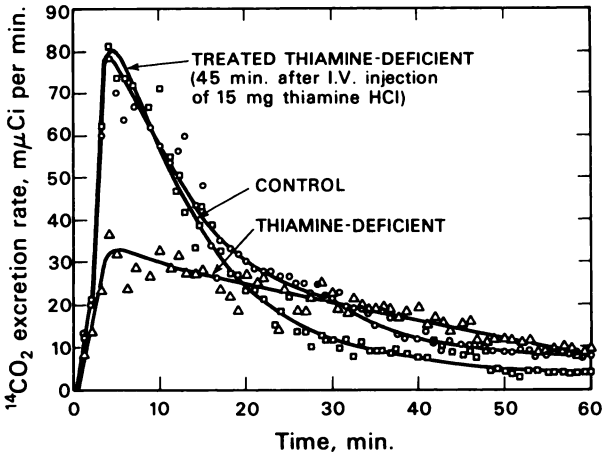

FIG. 3. Representative curves show rate of appearance of $^{14}CO_2$ in breath of control rat and thiamine-deficient rat before and 45 min after i.v. injection of 15 mg thiamine hydrochloride. Ordinate represents $^{14}CO_2$ excretion rate expressed as mμCi/min and abscissa as time in minutes following i.v. injection of pyruvate-1-^{14}C.

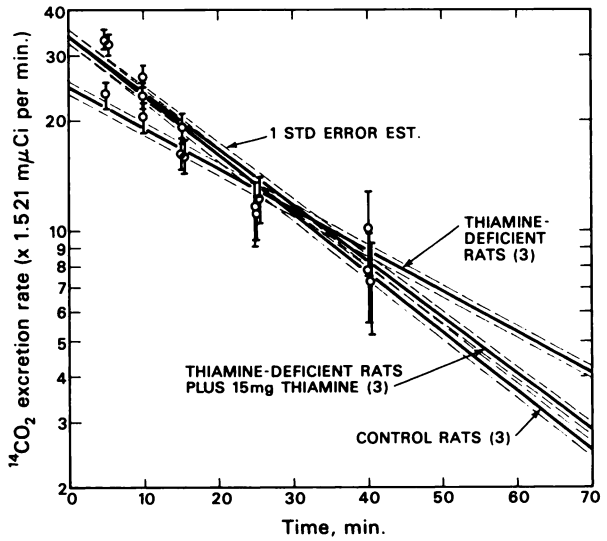

FIG. 4. Gives composite data of rate of $^{14}CO_2$ production following i.v. administration of acetate-1-^{14}C in three control, three thiamine-deficient rats and three thiamine-deficient rats given 15 mg thiamine hydrochloride i.v. 45 min before initiation of study. Ordinate represents $^{14}CO_2$ excretion rate expressed as × 1.521 mμCi/min and abscissa as time in minutes following i.v. injection of acetate-1-^{14}C. Vertical bars through each point define precision of position of mean of excretion rates of $^{14}CO_2$ for each group of rats with 95% limits based on $t_{.05}S_{y.x}$. Broken lines above and below each curve are regression lines of ±1 standard error of estimate ($S_{x.y}$).

$^{14}CO_2$ production studies: $H^{14}CO_3^-$. Since $^{14}CO_2$ produced at intracellular sites must traverse the body CO_2-$H_2CO_3^-$ pools before its excretion in the breath, it is possible that the abnormal $^{14}CO_2$ breath curves following administration of pyruvate

and acetate-1-^{14}C in thiamine-deficient animals was due to alterations in the CO_2-$H_2CO_3^-$ pools in thiamine-deficient rats rather than to alterations in specific metabolic steps related to $^{14}CO_2$ production. That this is not the case is shown in data in Fig. 5 and Table 3. This figure and table summarize data describing appearance of $^{14}CO_2$ in the breath of control and thiamine-deficient rats given 1 μCi $H^{14}CO_3$ intravenously. Presentation and analysis of the data are identical to that of Fig. 2 and Table 1. It appears from these data that thiamine deficiency does not remarkably alter CO_2-HCO_3^- pool kinetics. It may result in a small increase in integral excretion of $^{14}CO_2$ from the CO_2-H_2CO_3-HCO_3^- pool, but this effect is opposite to that noted with 1-^{14}C-labeled pyruvate and acetate. This result indicates that abnormalities in $^{14}CO_2$ appearance in the breath following administration of pyruvate-1-^{14}C and acetate to thiamine-deficient animals could not be a result of alterations in CO_2-HCO_3^- pool kinetics.

Plasma clearance of thiamine (thiazole-2-^{14}C) in thiamine-deficient rats. To determine whether significant differences are present in thiamine kinetics *per se* between control and thiamine-deficient rats, the clearance of ^{14}C activity from the plasma following i.v. administration of thiamine (thiazole-2-^{14}C) was studied. The results of such plasma thiamine clearance studies are presented for each of three thiamine-deficient and two control animals in Fig. 6. All animals in this study were of the same size and genetic background, and thus the concentration of radioactivity in the plasma following i.v. injection of a standard dose (20 μCi) is an adequate reflection of the content of radioactivity in the initial distribution compartment of intravenously administered thiamine. It can be noted from Fig. 6 that at all times following i.v. administration of labeled thiamine the ^{14}C concentration in the plasma is lower in thiamine-deficient animals than in controls but that the curves are otherwise roughly parallel. These results suggest that the initial distribution space of thiamine in thiamine-deficient animals is much larger than that in controls.

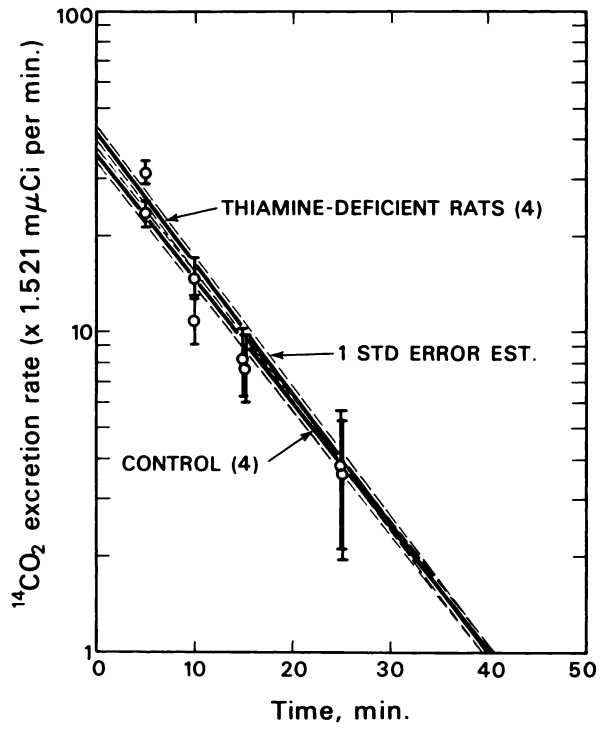

FIG. 5. Gives composite data of rate of $^{14}CO_2$ production following i.v. administration of $H^{14}CO_3$ in four control and four thiamine-deficient rats. Vertical bars through each point define precision of position of mean of excretion rates of $^{14}CO_2$ for each group of rats with 95% limits based on $t_{.95}S_{y.x}$. Broken lines above and below each curve are regression lines of ± 1 standard error of estimate ($S_{x.y}$). Ordinate represents $^{14}CO_2$ excretion rate expressed as $\times$ 1.521 mμCi/min and abscissa as time in minutes following i.v. injection of $H^{14}CO_3$.

TABLE 3. SLOPE ($T_{1/2}$) AND INTEGRAL ^{14}C EXCRETION DETERMINED FROM $^{14}CO_2$ APPEARANCE IN BREATH FOLLOWING I.V. ADMINISTRATION OF $NaH^{14}CO_3$ IN CONTROL AND EXPERIMENTAL RATS

Category	Half-time $T_{1/2}$ (min)	^{14}C excretion in 50 min (% $\pm$ σ)
Normal rats (4*)	7.858	61.1227 $\pm$ 3.0906
Thiamine-deficient rats (4*)	7.394	68.8246 $\pm$ 2.2626

* Denotes number of animals in each group.

DISCUSSION

In control rats approximately 65% of the ^{14}C administered as pyruvate-1-^{14}C appears in the breath as $^{14}CO_2$ within the first 60-min period. This result is comparable to that obtained following i.v. administration of $H^{14}CO_3$ (i.e. 61%) confirming that the largest component of pyruvate metabolism *in vivo* occurs via its decarboxylation to form acetyl CoA rather than pathways leading to glucogenesis. In control animals a smaller value of only 52% of the ^{14}C administered as acetate-1-^{14}C could be accounted for as $^{14}CO_2$ appearing in the breath during the initial 60 min following the intravenous administration of this material. Thus a small but significant amount of acetate appears to be fixed in slowly catabolized compounds (e.g. fatty acids) as opposed to direct oxidation to CO_2 in the TCA cycle.

In thiamine-deficient animals there was a delay in oxidation of both 1-^{14}C-labeled pyruvate and acetate to $^{14}CO_2$ (as evidenced by a prolonged $T_{1/2}$) as well as a diminished integral excretion of $^{14}CO_2$

in the $^{14}CO_2$ breath curves. Within 45 min after the intravenous administration of thiamine to thiamine-deficient rats the pattern of appearance of $^{14}CO_2$ in the breath subsequent to the intravenous administration of 1-^{14}C-labeled acetate was within normal limits. However, as long as 24 hr after the intramuscular injection of thiamine in thiamine-deficient rats the pattern of appearance of $^{14}CO_2$ in the breath remained abnormal and became normal only after a 48-hr period following two daily intramuscular injections of thiamine. This latter finding may be related to local binding of thiamine in the tissues surrounding the intramuscular injection site with resulting diminution in the availability of thiamine for sites elsewhere in the body. Following intravenous administration there may be more generalized distribution of thiamine throughout the body, making it more available for thiamine-dependent metabolic reactions. In particular, intravenous as opposed to intramuscular administration of thiamine may result in a greater delivery of thiamine to the liver, the site of a large fraction of pyruvate and acetate catabolism. An alternate explanation of the data is that the oxidative catabolism of the #1-carbon atom of pyruvate is more sensitive to thiamine deficiency than the oxidative catabolism of #1-carbon atom of acetate.

The fact that specific thiamine-binding sites are relatively unsaturated in thiamine deficiency as opposed to the nondeficient state is further suggested by the studies of plasma thiamine clearance curves. These curves in thiamine-deficient rats are below, but roughly parallel, to those seen in control animals. This result could be explained by postulating a larger initial distribution space for the ^{14}C-labeled thiamine in the thiamine-deficient animals as opposed to the controls. This could be accounted for by postulating an increased rate at which thiamine equilibrates across cell membranes or by a larger quantity of unsaturated thiamine-binding sites in thiamine-deficient animals as opposed to control animals.

The fact that the differences in $^{14}CO_2$ appearance in thiamine-deficient animals given acetate or pyruvate-1-^{14}C are not due to alterations of the bicarbonate pool produced by the deficiency is demonstrated by the finding of comparable $^{14}CO_2$ appearance curves subsequent to the intravenous administration of ^{14}C-labeled bicarbonate in thiamine-deficient animals and controls.

The fact that alterations in the metabolism of pyruvate and acetate in the presence of thiamine deficiency can be detected in the intact animal by measuring $^{14}CO_2$ production suggests the possible application of this approach to the early diagnosis

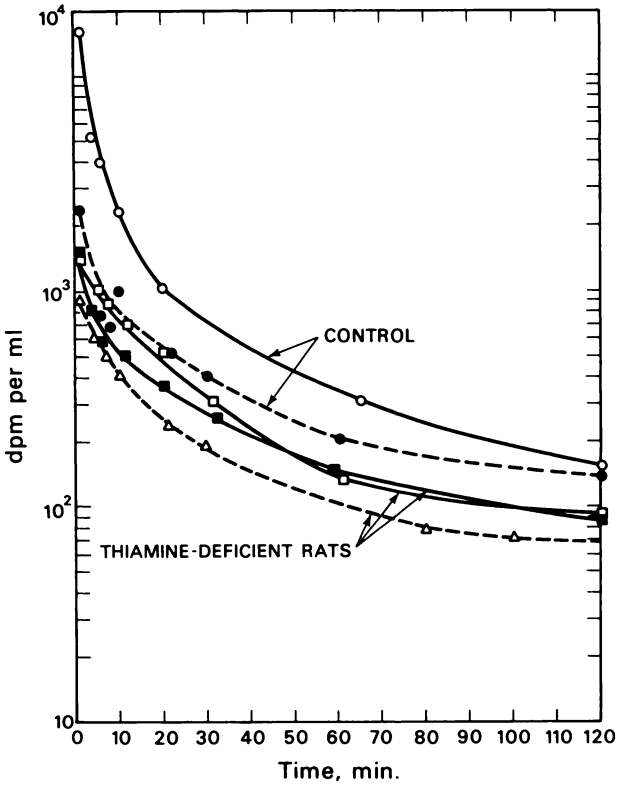

FIG. 6. Gives clearance of ^{14}C activity from plasma following i.v. administration of thiamine (thiazole-2-^{14}C) hydrochloride in two control and three thiamine-deficient rats. Ordinate represents ^{14}C activity expressed as dpm/ml of plasma and abscissa as time in minutes following i.v. injection of thiamine (thiazole-2-^{14}C).

of thiamine deficiency (beri-beri) in man. Such a human diagnostic procedure might consist of measuring $^{14}CO_2$ in the breath after the administration of 1-^{14}C-labeled acetate or pyruvate with the subsequent repetition of the study after an intravenous administration of a therapeutic dose of thiamine. A significant increase in the slope of the $^{14}CO_2$ breath curve during the second study would suggest the presence of thiamine deficiency at the time of the initial study. The validity or possible usefulness of this approach in the early diagnosis of beri-beri awaits study in human subjects.

SUMMARY

In normal rats approximately 65% of ^{14}C administered as pyruvate-1-^{14}C appears in the breath as $^{14}CO_2$ within 60 min (comparable to that obtained following administration of $H^{14}CO_3^- \approx 61\%$), providing confirmation that *in vivo* pyruvate metabolism occurs almost exclusively via decarboxylation to acetyl CoA (a metabolic step requiring the presence of thiamine pyrophosphate). In distinction only approximately 52% of ^{14}C administered as acetate-1-^{14}C appears in the breath as

$^{14}CO_2$ within 60 min, suggesting that a small but measurable amount of acetate is "fixed" in more slowly catabolized compounds (e.g. fatty acids) as opposed to direct oxidation to CO_2 in the citric acid cycle.

In thiamine-deficient rats there is a significant delay in oxidation of both pyruvate-1-^{14}C and acetate-1-^{14}C to $^{14}CO_2$. Within 45 min after i.v. injection of thiamine, the $^{14}CO_2$ appearance curve after injection of acetate-1-^{14}C is within normal limits. In thiamine-deficient animals $^{14}CO_2$ excretion following administration of $H^{14}CO_3$ is normal, but plasma clearance of ^{14}C-labeled thiamine is initially abnormally rapid, suggesting the presence in thiamine deficiency of unsaturated thiamine-binding sites in rapid equilibrium with plasma thiamine.

The $^{14}CO_2$ breath studies suggest the possibility of the diagnosis of thiamine deficiency in man by measuring $^{14}CO_2$ appearance in the breath after administration of acetate or pyruvate-1-^{14}C prior to and subsequent to the intravenous administration of thiamine. A significant increase in the rate of $^{14}CO_2$ production following administration of thiamine would suggest the presence of thiamine deficiency before thiamine administration.

ACKNOWLEDGMENT

This work was supported under U.S. Atomic Energy Commission Contract W-7495-Eng-48.

REFERENCES

1. THOMPSON, R. H. S. AND JOHNSON, R. E.: Blood pyruvate in vitamin B_1 deficiency. *Biochem. J.* **29**:694, 1935.

2. LU, G. D. AND PLATT, B. S.: Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. The effect of exercise on blood pyruvate in vitamin B_1 deficiency in man. *Biochem. J.* **33**:1,538, 1939.

3. PLATT, B. S. AND LU, G. D.: Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. The cumulation of pyruvic acid and other carbonyl compounds in beri-beri and the effect of vitamin B_1. *Biochem. J.* **33**:1,525, 1939.

4. LU, G. D. AND NEEDHAM, D. M.: The fate of injected pyruvate in the normal rabbit. *Biochem. J.* **33**:1,544, 1939.

5. LIANG, C. C.: Studies on experimental thiamine deficiency. Trends of keto acid formation and detection of glyoxylic acid. *Biochem. J.* **82**:429, 1961.

6. BANERJI, G. G. AND HARRIS, L. J.: Methods for assessing the level of nutrition. A carbohydrate tolerance test for vitamin B_1. *Biochem. J.* **33**:1,346, 1939.

7. SHILS, M. E., DAY, H. G. AND MCCOLLUM, E. V.: The effect of thiamine deficiency in rats on the excretion of pyruvic acid and bisulfite-binding substances in the urine. *J. Biol. Chem.* **139**:145, 1941.

8. BANERJI, G. G. AND YUDKIN, J.: The vitamin-B_1 sparing action of fat and protein. The oxidation of pyruvate by the tissues of symptom-free rats on diets deficient in vitamin B_1. *Biochem. J.* **36**:530, 1942.

9. BUCLE, R. M.: Blood pyruvic and α-ketoglutaric acids in thiamine deficiency. *Metabolism* **14**:141, 1964.

10. HORWITT, M. K. AND KREISLER, O.: The determination of early thiamine-deficient states by estimation of blood lactic and pyruvic acids after glucose administration and exercise. *J. Nutrition* **37**:411, 1949.

11. PEARSON, W. N. AND DARBY, W. J., JR.: Catabolism of ^{14}C-labeled thiamine by the rat as influenced by dietary intake and body thiamine stores. *J. Nutrition* **93**:491, 1967.

12. NEAL, R. A. AND PEARSON, W. N.: Studies of thiamine metabolism in the rat. Isolation and identification of 2-methyl-4-amino-5-pyrimidine carboxylic acid as a metabolite of thiamine in rat urine. *J. Nutrition* **83**:351, 1964.

13. BALAGHI, M. AND PEARSON, W. N.: Comparative studies of the metabolism of ^{14}C-pyrimidine-labeled thiamine, ^{14}C-thiazole-labeled thiamine and 35S-labeled thiamine in the rat. *J. Nutrition* **91**:9, 1967.

14. BALAGHI, M. AND PEARSON, W. N.: Metabolism of physiological doses of thiazole-2-^{14}C labeled thiamine by the rat. *J. Nutrition* **89**:265, 1966.

15. MCCARTHY, P. T., CERECEDO, L. R. AND BROWN, E. V.: The fate of thiamine-S35 in the rat. *J. Biol. Chemistry* **209**:611, 1954.

16. VERRETT, M. J. AND CERECEDO, L. R.: Metabolism of thiamine-S35 in the rabbit. *Proc. Soc. Exp. Biol. Med.* **98**:509, 1958.

17. PEARSON, W. N. AND DARBY, W. J., JR.: Catabolism of ^{14}C-labeled thiamine by the rat as influenced by dietary intake and body thiamine stores. *J. Nutrition* **93**:491, 1967.

18. IACONO, J. M. AND JOHNSON, B. C.: Thiamine metabolism. I. The metabolism of thiazole-2-^{14}C-thiamine in rat. *J. Am. Chem. Soc.* **79**:6,321, 1957.

19. WILLIAMS, M. A., CHU, L. C., MCINTOSH, D. J. AND HINCENBERG, I.: Effects of dietary fat level on pantothenate depletion and liver fatty acid composition in the rat. *J. Nutrition* **94**:377, 1968.

20. TOLBERT, B. M., KIRK, M. AND BAKER, E. M.: Continuous $^{14}CO_2$ excretion studies in experimental animals. *Am. J. Physiol.* **185**:263, 1956.

21. DOMINGUES, F. J., GILDNER, K. J., BALDWIN, R. R. AND LOWRY, J. R.: An instrument and technique for the continuous measurement of respiratory CO_2 patterns in metabolic tracer studies. *Intern. J. Appl. Radiation Isotopes* **7**:77, 1959.

22. NGO, T., WINCHELL, H. S. AND LANDAW, S.: Altered in vivo metabolism of histidine (Imidazole-2-^{14}C) in irradiated rats. *Radiation Res.* **34**:390, 1968.