SHORT COMMUNICATION

Recovery of infused [^{14}C]bicarbonate as respiratory $^{14}CO_2$ in man

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(*Received 12 May 1982; accepted 13 September 1982*)

Summary

1. Nine adult subjects (eight obese, one normal) were infused with $NaH^{14}CO_3$ for up to 36 h.

2. The recovery of label as respiratory $^{14}CO_2$ was close to 90% throughout and did not change with feeding or with dietary composition.

Key words: carbon dioxide, [^{14}C]bicarbonate, leucine, oxidation, protein turnover.

Introduction

Studies of the metabolism of carbon-labelled metabolites in man frequently require measurements of the rate of production of labelled CO_2 in the breath. A complicating factor, however, is the apparent recapture by other metabolic pathways of the labelled CO_2 released by oxidation, which necessitates the use of a correction factor in such calculations. By giving $NaH^{14}CO_3$ and following the production of $^{14}CO_2$ over periods between 2 h and 10 h, a number of groups have reported recoveries of 80–87% [1–3].

Our recent studies of the response of protein metabolism to feeding involved continuous infusion of subjects with [1-^{14}C]leucine for periods of 24–36 h [4, 5]. Because the previous studies of labelled CO_2 retention were of relatively short duration, we have examined whether the fraction of label retained depends on length of infusion up to 36 h. Also, previous measurements did not compare fed and fasted states, while in our studies of leucine metabolism subjects were fed hourly for the first 12 h, fasted between 12 and 24 h and, when infusions were prolonged further, fed hourly between 24 and 36 h. The present study was therefore performed in parallel with the measurements of protein turnover to give a practical estimate of the magnitude of the correction for $^{14}CO_2$ retention while using the same equipment and experimental protocol. Nine subjects were given continuous infusions of [^{14}C]bicarbonate and the recovery of $^{14}CO_2$ in the breath was monitored for 24–36 h.

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Methods

Eight obese subjects were admitted to the hospital ward for weight reduction and were given a normal diet consisting of approx. 2000 kcal and 75 g of protein daily. Starting on the third morning, a group of four (three female, one male) were given infusions of $NaH^{14}CO_3$ (diet N group). This group also contained one normal subject (male) who ate *ad libitum* until the day of the infusion. After 3 days on the normal diet the other four subjects were transferred to low-energy diets for 3 weeks, after which they were also given infusions of $NaH^{14}CO_3$. Of these subjects, one was given 500 kcal and 50 g of protein (diet P) and the other three, 500 kcal, zero protein (diet O) (see ref. [6]).

Commencing at approx. 09.00 hours on the day of infusion, subjects were given continuous infusions of $NaH^{14}CO_3$ as described earlier for [1^{14}C]leucine infusion [4–6]. The infusion solution contained 10–20 μCi of $NaH^{14}CO_3$ (The Radiochemical Centre, Amersham, Bucks, U.K.) dissolved in unlabelled $NaHCO_3$ (0·25 mol/l) and was sterilized by Millipore (0·22 μm) filtration. The ^{14}C content of 50 μl of this solution

0143-5221/83/020231-03$2.00

was assessed by scintillation counting in 10 ml of a xylene-based scintillant [7], with correction for efficiency by the external standard method. The appropriate daily intake of food (i.e. diet N, P or O) was given as 12 equal, hourly portions during the first 12 h of infusion. No food was given for the period 12–24 h, and when applicable equal hourly portions of the appropriate diet were also given between 24 and 36 h.

The rate of output of $^{14}CO_2$ in the breath was assessed by multiplying the total rate of CO_2 production by the specific radioactivity of respiratory CO_2. Total CO_2 production was estimated at regular intervals from the increase in CO_2 concentration of air drawn at a rate of 70 l/min through a large plastic tent (volume approx. 1000 l) placed around the bed for the duration of the infusion [4–6]. Calibration of the system by burning ethanol in the tent showed the absolute accuracy to be 99·7%, with a standard deviation of 0·22% on three determinations. The specific radioactivity of $^{14}CO_2$ was measured at approx. hourly intervals by absorption in 1 mmol of Hyamine hydroxide [8], followed by addition of 10 ml of scintillation fluid (4 g of 2,5-diphenyloxazole/l in toluene). This gave approx. 2×10^3 c.p.m./vial, which was counted for 5 min.

These studies were approved by the Ethical Committee of the Hospital and by the Isotope Advisory Panel of the Department of Health and Social Security.

Results and discussion

The production of $^{14}CO_2$ in the breath rose rapidly in the first 2 h of infusion and in all subjects had become constant by 6 h, as was reported previously [1, 2]. The recovery of ^{14}C was quite variable both between and within subjects, ranging from 80% to 104% (Table 1). In eight out of nine subjects there was a small increase between the 0–12 h and 12–24 h periods. However, the mean increase was quite small and there was little further increase in the interval 24–36 h. This low level of recycling of the label means that it must have been lost mostly by irreversible processes (e.g. into urine) or into a pool with a very slow rate of turnover (e.g. by fixation into amino acids and hence into protein). Furthermore, the fixation of CO_2 did not appear to be affected by feeding/fasting or by the intake of low-energy and protein-free diets.

The mean rate of recovery of 90% is higher than that reported by Issekutz *et al.* [1], who obtained 80% during 8 h after a single intravenous dose of $NaH^{14}CO_3$ and also during an 8 h constant infusion of $NaH^{14}CO_3$. James *et al.* [2] also reported values close to 80% during 10 h constant infusion. With primed continuous infusions lasting up to 2 h, Clague *et al.* [3] obtained a recovery of only 75%, but after calibrating their apparatus they revised this value to 87%. The calibration, by infusing $NaH^{14}CO_3$ into acid or by burning [^{14}C]ethanol, revealed a 13% loss of counts, which they attributed to a problem with scintillation counting of CO_2 with certain scintillators. We have also infused $NaH^{14}CO_3$ into acid and collected the CO_2 evolved in Hyamine under conditions similar to those we used for collecting expired CO_2. This showed that the same loss of counts did not occur with our system for counting CO_2 radioactivity, or if it did, it occurred equally for the infusion solution and the expired CO_2.

Clague *et al.* [9] have suggested a method for calibrating each subject based on the relative concentration of [^{14}C]bicarbonate in plasma during sequential infusions of $NaH^{14}CO_3$ and the ^{14}C-labelled metabolite under study. However, this method requires that the CO_2 production, respiration rate and plasma bicarbonate concentration, as well as other metabolic processes, are identical during the two infusions; conditions that may be difficult to achieve in many experiments. In our study with infusions of [1-

TABLE 1. *Percentage recovery of infused* [^{14}C]*bicarbonate as respiratory* $^{14}CO_2$

Rates of $^{14}CO_2$ production were expressed as percentages of the rate of infusion of ^{14}C. In each period mean values were calculated from an average of six repeated determinations, with an average coefficient of variation of 6%. For the 0–12 h period the mean included only those values obtained after constant labelling was judged by eye to have been achieved. Values in parentheses indicate the total (unlabelled) CO_2 output in the periods 12–24 h and 24–36 h expressed as percentages of the values during 0–12 h. There were no significant differences in any of the time periods between subjects given diet N and diet O, as judged by Student's *t*-test. The rise between 0–12 h and 12–24 h was significant ($P < 0.02$, paired *t*-test), but there was no significant change between 12–24 h and 24–36 h.

Diet	Sex	Obese/ normal	0–12 h (feeding)	12–24 h (fasting)	24–36 h (feeding)
N	F	O	87	91 (84)	93 (102)
N	F	O	91	97 (83)	—
N	M	N	90	94 (82)	—
N	F	O	96	104 (75)	—
N	M	O	80	91 (89)	93 (117)
P	F	O	87	88 (76)	87 (96)
O	F	O	90	99 (81)	94 (94)
O	F	O	81	92 (83)	—
O	F	O	91	86 (85)	92 (101)
Mean ± SD			88 ± 5	94 ± 6	92 ± 3

^{14}C]leucine, to measure the effects of feeding and diet on whole body protein turnover and leucine oxidation, we chose to correct for CO_2 fixation by measuring directly the $^{14}CO_2$ production during parallel infusions of $NaH^{14}CO_3$ into groups of similar subjects under the same conditions. Because the correction factor for CO_2 fixation was obtained with the same apparatus with both bicarbonate and leucine infusions, any errors resulting from the method of assessing respiratory $^{14}CO_2$ production would tend to cancel out.

Although the value for $^{14}CO_2$ recovery that we obtained did not appear to vary appreciably with time of infusion or with feeding or dietary treatment, we should not assume it will always be constant until we know more of the metabolic reactions responsible. Furthermore, the appropriate value may depend on the experimental plan as well as the equipment used. We suggest that all studies involving the conversion of metabolites to labelled CO_2 *in vivo* should be accompanied by parallel experiments with labelled bicarbonate, using the same equipment and experimental protocol. A single value for labelled CO_2 recovery, such as the 90% obtained in the present investigation or the 80% obtained previously [1], should not be used as a universal correction factor.

Acknowledgments

We are grateful to the Medical Research Council for financial support and to Professor J. C. Waterlow for his encouragement.

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