Measurement of bicarbonate turnover in humans: applicability to estimation of energy expenditure

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Elia, M., N. J. Fuller, and P. R. Murgatroyd. Measurement of bicarbonate turnover in humans: applicability to estimation of energy expenditure. *Am. J. Physiol.* 263 (*Endocrinol. Metab.* 26): E676–E687, 1992.—Bicarbonate turnover and energy expenditure were assessed in six healthy male volunteers, by the use of a constant infusion of radiolabeled bicarbonate ($NaH^{14}CO_3$) administered over 36 h, while the volunteers were confined to a whole body indirect calorimeter. Recovery and dilution of isotope were assessed from measurements made on continuous collections of CO_2, entering and leaving the calorimeter, urine, and intermittent spot breath and saliva samples. Mean recovery of infused label in gaseous CO_2 was 95.6 ± 1.1% (SD) between 12 and 36 h. Applying a 95% mean recovery of label to each subject individually enabled the use of integrated mean specific activity of CO_2 in spot breath and urine samples to predict measured net CO_2 production and energy expenditure to within about ±6%. Estimates based on urinary measurements were compromised slightly by the exchange of label through the bladder wall (this was dependent on pH and volume of urine). It is concluded that this constant-infusion labeled bicarbonate method offers a potentially useful means of assessing net CO_2 production and total energy expenditure over the short term (e.g., 1–3 days).

net carbon dioxide production; indirect whole body calorimetry; free-living energy expenditure

TRACER TECHNIQUES have been developed and refined to enable the assessment of energy expenditure in humans and animals under free-living conditions, which is beyond the scope of classical whole-body indirect calorimetry. However, a combination of calorimetry and tracer methods can be used to partition total energy expenditure into its two major components: *1*) basal metabolic rate and *2*) activity plus thermogenesis (30, 31).

The most widely used method currently available for estimating total free-living energy expenditure makes use of doubly labeled water ($H_2{}^{18}O$ and ^2H_2O) to provide estimates of energy expenditure over extended periods of time (typically 2–3 wk for adults, Ref. 31). Unfortunately, this method does not accurately assess net CO_2 production over shorter periods (e.g., 1–3 days). Attempts to use bolus administration of bicarbonate to estimate net CO_2 production over very short periods of time (e.g., 2–4 h) have been made in both animals (34) and humans (1, 5). In contrast, the constant-infusion labeled bicarbonate method (9, 14, 17, 34, 39), which has been applied to the estimation of net CO_2 production in farm animals (e.g., cattle and sheep; Refs. 9, 35, 37–39) over periods of <1 day, has yet to be applied to humans (for background and preliminary reports see Refs. 17 and 22).

The principle of the labeled bicarbonate method can be illustrated by the use of a simple model, whereby carbon labeled $^{14}CO_2$ is infused at constant rate and achieves rapid and complete equilibrium with a CO_2 pool of constant size. In this model there is also production of CO_2 but no isotopic exchange or fixation of labeled CO_2, so that the recovery of label from the CO_2 pool becomes equal to its rate of infusion. Under these circumstances the extent of isotopic dilution is dependent only on the rate of CO_2 production. Thus variability in CO_2 production rate over time will be reflected in a variable dilution of isotope.

In humans, there are certain deviations from the basic assumptions of this model shown by the variable and incomplete recovery from breath of label, that has been infused into the body at constant rate (3, 4, 6, 8, 14, 24, 26–29, 40). The reason for apparent incomplete recoveries partially lies in the short duration of the studies and the slow turnover time of CO_2 through some of the body pools. Not all of the CO_2 that enters these low flux pools has sufficient time to reemerge and be recovered in expired air (isotope exchange). The incomplete recovery, which occurs even if a priming dose is used, is also partly due to incorporation or fixation of labeled CO_2 into substances such as carbohydrate, protein, urea, and the glycerol moiety of triglycerides. The reactions responsible for fixation of CO_2 have been reviewed elsewhere (14). Because some CO_2 is produced and utilized by the body at the same time, net CO_2 production (equal to the CO_2 excreted) is less than actual CO_2 production (flux). However, if labeled $^{14}CO_2$ becomes incorporated into a substance that is subsequently oxidized to $^{14}CO_2$, then the effect is equivalent to entry of labeled CO_2 into another pool of CO_2. Because different substrates have variable turnover times, and some (such as triglycerides) turn over particularly slowly, recovery of label in breath will be incomplete during short-term periods. Some labeled $^{14}CO_2$ may also be incorporated into substrates that are not oxidized or that are poorly utilized, for example urea that is formed from CO_2. The loss of urea in urine will contribute to the incomplete recovery of labeled CO_2 in breath. If it is erroneously assumed that recovery of labeled CO_2 in breath is complete, the labeled-bicarbonate method will overestimate both net CO_2 production and energy expenditure.

Various studies, in which adult subjects were given a constant infusion of labeled bicarbonate have shown the recovery of label in breath to be in the range 70–95% (see Refs. 14, 40). This variability in recovery may be due to differing lengths of time over which the various studies were carried out (14) or to certain methodological differences between them. In some of these studies, intermittent collections of CO_2 (both labeled and unlabeled) were made, from which estimates of recovery were calculated using specific activity (for $^{14}CO_2$) or enrichment (for $^{13}CO_2$). In other studies, collections were made during apparent plateau phases in specific activity or enrichment. Some movement or physical activity

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would almost certainly have occurred between measurements, having a confounding effect on the apparent plateau and recovery of label in breath.

The fraction of observed CO_2 production relative to the rate of dilution of $^{14}CO_2$ with unlabeled CO_2 corresponds numerically to the fraction of administered label recovered in breath. This fraction reflects the combined effects of the various processes described above (e.g., fixation and isotopic exchange of CO_2). Should it be shown that this fraction is predictable and that its variability is small between individuals undergoing different activities, then a constant value may be used to calculate energy expenditure. A mean integrated estimate of the degree of isotopic dilution over the measurement period, to which a correction or "recovery factor" can be applied, is required. This may be achieved by taking repeated "spot" or "snatch" samples of either expired air or physiological fluids or, alternatively, by taking a continuous collection of urine.

No study has yet attempted to assess bicarbonate kinetics over extended periods of time (e.g., 1 day or more) in humans, using continuous collections of both labeled and unlabeled CO_2, and none has used physiological fluids other than breath samples. Therefore, this human study was undertaken in a whole body indirect calorimeter to provide more comprehensive information regarding the recovery of labeled CO_2 over at least 24 h (to include periods of sleep, rest, activity, and food ingestion). Furthermore, the potential use of the labeled bicarbonate method (from measurements of isotope dilution of CO_2 in samples of end expiratory air, saliva, blood, or urine) for the assessment of both net CO_2 production and 24-h total energy expenditure is evaluated.

SUBJECTS AND METHODS

Subjects. The characteristics of the six healthy male subjects who volunteered for the study are summarized in Table 1. The weights of all subjects had remained stable over the few weeks immediately before the study. Body fat, as a percentage of body weight, was estimated from the sum of four skinfold thicknesses (biceps, triceps, subscapular, and suprailiac), as described by Durnin and Womersley (12). Each subject entered the same calorimeter (internal volume 11 m^3), located at the Dunn Clinical Nutrition Centre, starting at 10:00 A.M., and remained there for the subsequent 36 h.

Infusion. The subjects were injected, over a period of 5–10 min, with a 45- to 50-ml bolus (45–50 mmol) of 8.4% (wt/vol) unlabeled sodium bicarbonate (Boots, Nottingham, UK), just before entering the calorimeter. This procedure was adopted in an attempt to ensure less acidic urine and, concomitantly, greater excretion of both labeled and unlabeled urinary bicarbonate for ease of measurement. A constant infusion (2.77 mmol/h) of 0.2 M sodium bicarbonate (obtained by mixing known volumes of 1.4 and 8.4% sodium bicarbonate; Boots), containing 500 μCi of radiolabeled sodium bicarbonate ($NaH^{14}CO_3$; Amersham, Buckinghamshire, UK), was administered for the subsequent 36 h of the study (unlabeled bicarbonate was infused along with the labeled bicarbonate in an attempt to prevent production of acidic urine over the full course of the study). The continuous infusion was administered through a peripheral forearm vein at a fixed rate, governed by a volumetric infusion pump (922E, IMED, Milton Trading Estate, Oxon, UK). The accuracy (±1%) of this pump had been established previously, by measuring the rate of delivery of both distilled water and bicarbonate solutions. In addition, the total amount of infusion administered to each subject was determined gravimetrically at the beginning and end of the study (the density of the infusate had previously been determined to within 1 part in 1,000). The specific activity of aliquots of the infusate were measured before and after infusion, to ensure that no loss of label had occurred from the infusate container (plastic). Previous experience had shown that no loss of label occurred while the infusate passed through the administration set to the subject. In this study, a priming dose of labeled bicarbonate was not used so as to gain a better insight into bicarbonate kinetics.

Table 1. *Details of the 6 male subjects in the study*

Subject No.	Age, yr	Weight, kg	Height, m	Body Mass Index, kg/m^2	Body Fat, %
1	38	80	1.78	25.2	24.4
2	36	92	1.89	25.8	19.2
3	29	71	1.85	20.7	16.5
4	29	87	1.72	29.4	23.1
5	41	77	1.91	21.1	17.6
6	30	73	1.83	21.8	13.0
Means ± SD	35.5±5.1	80.0±7.4	1.83±0.1	24.0±3.4	19.0±4.2

For calculation of the exposure of the body to radiation, it was assumed that 95% of the administered label was expired as gaseous CO_2 and another 2% was lost in urine (see RESULTS), so that the effective whole body dose equivalent (assuming that the remaining radioactivity was in bone) was ~5 μSv (0.5 mrem). This is equivalent to ~1 day of natural background radiation. However, if some of the expired CO_2 had cycled through bone, then the value may have been slightly higher than this.

Calorimeter protocol. The calorimeter was maintained at a temperature of 26 ± 0.5°C for the duration of the study. The subjects were required to follow a set calorimeter protocol, involving periods of rest, sleep, exercise on a bicycle ergometer (cycling rate 50/min at 50 and 100 W, in 2 separate hourly sessions), and meals (Fig. 1). Food presented to the subjects was prepared and weighed in a metabolic kitchen, to ensure an approximate energy content of 1.5 times the estimated basal metabolic rate (BMR) (prediction equations, Ref. 32). Any food left uneaten was weighed, to calculate actual dietary intake. The mean energy intake of the subjects was 10.52 ± 0.43 MJ/day, which was divided among breakfast (24 ± 3%), lunch (38 ± 2%), and supper (38 ± 1%). Of the total dietary energy, 45 ± 1% was derived from carbohydrate, 36 ± 1% from fat, and 19 ± 1% from protein (the diet was intended to reflect the subjects' normal eating habits, not the proportions recommended for good health of the population in general). Food was passed into the calorimeter, and samples were passed to observers outside the calorimeter via specially built airlocks.

Calorimetry and continuous collections of CO_2. Atmospheric air was pumped through the calorimeter at a constant rate of 200 l/min, with an accuracy of ±1%, and on repeated occasions the setting was found to be within ±0.25%. The CO_2 and O_2 content of gases both entering and leaving the chamber were measured using infrared and paramagnetic analyzers (Servomex, Crowborough, Sussex, UK), respectively. The calorimeter calibration procedure was executed at 2-h intervals, starting 20 min before the hour and lasting for 20 min. Calibration was achieved with pure nitrogen representing the zero value for both CO_2 and O_2, atmospheric air for the 20.94% O_2 span, and 1% CO_2 in air for the CO_2 span. The 1% CO_2 in air (BOC, London, UK) was certified α-tested to an accuracy of ±1% of the stated concentration, which was confirmed to within ±0.5% by three

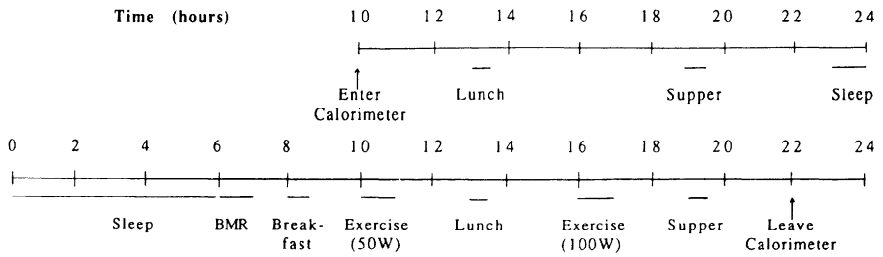

Fig. 1. Details of experimental protocol.

independent techniques performed in our laboratory; these included chemical analysis by the Lloyd-Haldane procedure and measurement of the increase in O_2 concentration resulting from removal of CO_2 by soda lime. The third method of confirmation of CO_2 concentration was by trapping of CO_2 and determination of CO_2 by titration (for preliminary study and precautions, see APPENDIX 1). The test gas was passed through an accurately (±0.5%) calibrated flowmeter (Alexander Wright, London, UK) and via a sintered glass filter into an accurately weighed solution of hyamine-methanol (~200 ml containing between 80 and 100 mM hyamine, with phenolphthalein as indicator) and then passed through a second solution to ensure complete trapping of CO_2. Barometric pressure and gas temperature within the flowmeter were recorded to calculate gas volumes at standard temperature and pressure. The total CO_2 trapped by this procedure was determined by difference between titrations with 0.2 M HCl, in aliquots of the hyamine-methanol solution, before and after trapping of CO_2 (see APPENDIX 1).

This trapping and titration procedure (described above) was also adopted for collection and measurement of CO_2 passing out of the calorimeter chamber. For the full duration of the study, a system that sampled calorimeter exhaust gases [dried through a column of 8–16 mesh calcium chloride (19); Analar grade, BDH, Essex, UK] at a flow rate of ~1 l/min, from the 200 l/min calorimeter outflow, allowed for continuous trapping and determination of CO_2. Again, the barometric pressure and temperature were recorded to allow calculation of gas volumes at standard temperature and pressure. At 3-h intervals the measuring cylinders containing trapping agent were changed, and the solutions were analyzed for CO_2. The trapped CO_2 was estimated by difference between titrations of aliquots obtained before and after the trapping process, using 0.2 M HCl (see APPENDIX 1). Also, duplicate aliquots were taken for scintillation counting. Hence, the total CO_2 and labeled $^{14}CO_2$ leaving the calorimeter were assessed. Because the CO_2 concentration and the amount of label in atmospheric air entering the calorimeter were also measured, the production rate and specific activity of CO_2 of the subject were calculated.

Energy expenditure by calorimetry. Energy expenditure in the calorimeter was calculated from measurements of CO_2 and O_2 entering and leaving the chamber using infrared and paramagnetic analyzers, respectively, by the application of a fast-response algorithm (7). The use of this algorithm overcomes any delay in response imposed by the size of the chamber. In contrast, the continuously collected CO_2 that was trapped in the hyamine-methanol from the gas leaving the calorimeter was subject to a delay (washout time constant 55 min). Therefore, trapped CO_2 (collected continuously over 3-h periods) was compared with the infrared measurements of CO_2, which had not been subjected to analysis by the fast response algorithm. The specific activity of the overall 24-h CO_2 collections trapped continuously in hyamine-methanol was calculated from the 3-h collection periods. Over this extended period of time, possible errors associated with short-term delay in washout were considered to be negligible. This was partly because the protocol was designed to allow for similar activity periods over the few hours leading up to and following on from the 24-h study period and partly because the measured CO_2 concentrations within the chamber (and the specific activity of air and breath) were essentially identical for both of these periods. Also the washout time constant was relatively short, compared with the study period.

Breath tests. Specific activity of CO_2 in end-expiratory air was determined from spot breath tests as described previously (22). Spot breath tests were conducted every hour for the duration of the study, except during the period of sleep. Additionally, these tests were carried out at 15-min intervals during the exercise period and for 1 h subsequent to this.

Urine collections. Continuous, complete collections of urine were obtained every 3 h, except during sleep when a 9-h collection was obtained. Each subject was required to empty the bladder immediately before entering the calorimeter, to obtain a control or prestudy sample of urine and to ensure a true first 3-h collection. All samples were collected into graduated cylinders containing 5 ml of 5 M sodium hydroxide (NaOH; Analar grade, BDH, essentially CO_2 free, tested by measuring the release of CO_2 on acidification). Cylinders were sealed to prevent possible trapping of ambient CO_2 by the NaOH. Aliquots of urine were stored at −20°C in a tightly sealed container until analysis. A small midstream sample of each urine collection was obtained, untreated with NaOH, to measure pH. The volume of the midstream sample was accounted for in all calculations regarding CO_2 recovery.

Random samples of urine had previously been obtained from a group of normal healthy subjects for the purpose of establishing the relationship between pH and the concentration of acid labile CO_2 in the urine. In a separate study, described in APPENDIX 2, the transfer of label across the bladder was investigated.

Blood tests. A blood sample was taken from a peripheral arm vein at the beginning and at the end of the study for all subjects. It proved possible for only one subject to provide samples of his own arterialized venous blood (every 3 h, except during sleep, to coincide with the sampling regimen of breath and saliva) from a dorsal hand vein that had been cannulated immediately before the subject entered the calorimeter. This vein, which was kept patent by slow infusion (1 ml/h) of 0.9% saline, was heated to a temperature of 65–70°C in a specially built hand box for 10–15 min before sampling. Two 10-ml lithium-heparinized tubes were filled virtually to the top with the arterialized blood to minimize exchange of ambient and labeled CO_2. The tubes were sealed and kept on ice until collected via the calorimeter airlock. These samples were frozen at −20°C, and analyzed the following day. This same subject also completed 5 min of forearm exercise, using a hand dynamometer at 30% maximal grip strength for 5 s and relaxing for the alternate 5 s. At the end of this period the wrist circulation was occluded by inflating a sphygmomanometer cuff (200 mmHg for 1 min), and a deep venous blood sample was obtained from the antecubital vein of the exercising arm. Immediately after this (within seconds) a sample of arterialized venous blood was taken from the contralateral hand, as above.

Saliva sampling. All subjects were required to produce saliva samples (3–5 ml) at hourly intervals except during sleep. All

washed their mouths at least 30 min before sampling and were not allowed food or drink during this period. This way any CO_2 present in food and drinking water (subjects were provided with uncarbonated tap water only) did not interfere with CO_2 specific activity of saliva. Salivation was induced by the use of fruit-flavored chewing gum. Although impregnated with artificial sweeteners, it was shown to be free of acid-labile CO_2 and to be of neutral pH. Saliva samples were collected in tubes containing 0.1 ml of 5 M NaOH (also shown to be free of acid-labile CO_2, BDH) and stored at −20°C.

Analysis of urine, blood, and saliva samples. Measurements of labeled and unlabeled CO_2 in urine, blood, and saliva were made within 1–2 days after sampling. Details regarding the procedures used and their validity, especially with respect to the stability and specific activity of CO_2, have been described previously (13, 22). The recovery of acid-labile CO_2 from urine and blood (~95 and 98%, respectively) was accounted for in the calculations. Saliva was analyzed in the same manner as that for blood (22). The concentration and specific activity of urinary urea were assessed using the untreated urine sample (for details see Ref. 22).

CALCULATIONS

Energy expenditure. Energy expenditure was calculated from the equation of Elia and Livesey (18): energy equivalent of 1 liter oxygen (kJ/l O_2) = 15.818 + 5.176 RQ, where, RQ is the respiratory quotient. This equation assumes that protein oxidation accounts for 15% of energy expenditure and that the energy equivalents of O_2 for protein (RQ 0.835), fat (RQ 0.71), and carbohydrate (RQ 1.0) are 19.48, 19.61, and 21.12 kJ/l, respectively (18). When protein oxidation contributes as little as 5% or as much as 25% to energy expenditure then the resultant error is only ~1%.

Observed individual recoveries of $^{14}CO_2$. Although, in the labeled bicarbonate method, it may be more appropriate conceptually to apply the fraction of CO_2 production relative to the rate of dilution of $^{14}CO_2$, the term *recovery* of infused labeled CO_2 in breath is used. This is partly because it is numerically equal to the above fraction and partly for comparative purposes with a variety of other studies, which have assessed recoveries of CO_2 during substrate oxidation. In addition, shortly after the start of an unprimed constant infusion where there is a large retention of label in the body pool, the term *recovery* may be more appropriate.

Recovery of infused label as gaseous CO_2 between 12 and 36 h (including the period of sleep) was calculated from the amount of label leaving the calorimeter (see *Calorimetry and continuous collection of CO_2*) relative to the amount infused. The radioactivity of the bicarbonate ($H^{14}CO_3$) in the infusate at the end of the study was found to be 99.9 ± 0.2% of that at the beginning. The concentration and specific activity of CO_2 in the calorimeter were both found to be to virtually identical at 12 and 36 h, and, although accounted for in the calculations, differences in these concentrations were considered to have a negligible influence on the results. The observed individual recoveries of infused label between 0 and 12 h were calculated from the cumulative hourly recoveries, which were calculated from the product of the hourly CO_2 production rates (μmol; fast response algorithm; Ref. 7) and the mean specific activity of CO_2 [disintegration·min^{-1} (dpm)·μmol^{-1}] in spot breath samples obtained at the beginning and end of each respective hour.

Prediction of CO_2 production. Total CO_2 production in the 12- to 36-h period was estimated from the mean integrated specific activity of CO_2 produced by each subject, according to the following equation (*method A*): daily CO_2 production (μmol/day) = f × $^{14}CO_2$ infused as bicarbonate (dpm)/integrated mean specific activity (dpm/μmol), where $^{14}CO_2$ infused as bicarbonate is the disintegration per minute of total bicarbonate infused over the course of 1 day, f is the observed fraction of CO_2 production relative to the rate of dilution of $^{14}CO_2$ and is numerically the fraction of infused label (pooled mean of the 6 individual values) recovered as gaseous CO_2 (lost via breath and skin) between 12 and 36 h (the recovery factor), and the integrated mean specific activity for individual subjects represents the arithmetic mean specific activity of the individual samples (e.g., breath or urine) collected over equal periods of time. The value f used in the prediction is the pooled mean value obtained from six individual studies, and represents the average correction factor from studies in all subjects. The factor f cannot be measured under normal field conditions and therefore has to be assumed.

Because some samples were not calculated at equal time intervals, appropriate weighting factors were applied. For example, mean specific activity of the urine samples (5 3-h samples during waking hours, SA_1 to SA_5, plus one 9-h overnight or nocturnal sample, SA_{noct}) was calculated as (SA_1 + SA_2 + SA_3 + SA_4 + SA_5 + 3 SA_{noct})/8 (dpm/μmol). The specific activity of the nocturnal (9-h) sample was weighted by a factor of three times that of the 3-h samples (collection interval 3 times longer).

Similar calculations were applied to the estimation of mean specific activity of breath samples, except that the time interval was 1 h instead of 3 h. Estimation of the mean specific activity was calculated from spot breath samples taken during the 1-h period of exercise (4 tests at 15-min intervals) and during the subsequent 1-h period (also 4 tests), in an attempt to give these equal weighting compared with the other samples. During the period of sleep, estimation of the hourly specific activities was also attempted, using the results of samples obtained immediately before and after sleep (1 sample each). The estimations were made assuming both a linear change and an exponential change in specific activity of CO_2 between the first and last sample.

Daily CO_2 production was also calculated from consecutive collections during the 12- to 36-h infusion period according to the following equation (*method B*): daily CO_2 production (μmol/day) = f [(infused dpm, *period 1*/SA_1) + (infused dpm, *period 2*/SA_2) + (infused dpm, *period 3*/SA_3) + ·····], where, SA_1, SA_2, and SA_3, etc., are values of specific activity (dpm/mol) obtained during *periods 1, 2,* and *3*, etc., which were not necessarily of equal duration. Each component of the equation aimed to represent CO_2 production during the specified infusion period.

Methods A and *B* have their own peculiar advantages

and disadvantages. *Method A* used a recovery factor considered to be applicable over the entire study period, but it applied an equal weighting to all specific activities collected over equal time intervals, irrespective of whether they were obtained at a time when CO_2 production rate was high (as in exercise) or low (as in sleep or rest). Therefore, samples with a high specific activity (collected when CO_2 production was low) may have had a greater influence on the overall estimate of net CO_2 production than samples with a low specific activity (the latter being collected when CO_2 production was high). Although *method B* made some allowance for this, by essentially attempting to summate the CO_2 produced during individual periods of time, it used the incorrect assumption that the fractional recovery of labeled CO_2 (f) was constant at different times of the day (see RESULTS).

Results are expressed as means ± SD.

Ethical approval for the study was obtained from the Ethical Committee of the Dunn Clinical Nutrition Centre, Cambridge, UK, and informed consent was obtained from all subjects.

RESULTS

The coefficient of variation for measurement of gaseous concentration of CO_2 (in a gas mixture) using the hyamine-methanol titration system was found to be ≤1%. The coefficient of variation for repeated measurement of specific activity of CO_2 in breath and physiological fluids was between 1.0 and 3.5%.

Observed recovery of labeled CO_2. Recovery of infused labeled CO_2 was found to rise rapidly after commencement of the infusion, reaching 85–90% after 4 h (see Table 2; 3- to 6-h period). Intersubject variation in recovery of infused label as gaseous CO_2 was remarkably small for the 12- to 36-h period (mean 95.6%, range 94.7–97.2%; Table 2), and remained small after accounting for the amount of label recovered in urine (mean of bicarbonate plus urea 1.85%) over this same period (total mean recovery 97.4%, range 96.0–98.7%). Of the label recovered in urine between 12 and 36 h, 84.2 ± 3.3% was present as urea (Table 3).

The amount of infused label recovered in gaseous CO_2 was found to vary during the course of the study. In particular, the recovery of infused CO_2 was high during the 1-h exercise periods. At 50 W the recovery was 128% (~140% during the first 0.5 h and 115% in the second 0.5 h), and at 100 W the recovery was 139% (>160% during the first 0.5 h and <120% during the second 0.5 h). However, the overall balance was redressed in the hours immediately subsequent to this (71 and 67%, respectively).

Table 2. *Recovery of infused [^{14}C]bicarbonate between 0 and 36 h*

Time Period, h	Gaseous CO_2	As Total Urinary Label	Gaseous CO_2 + Urinary Label
0–3	49.9±2.4	0.4±0.2	50.3±2.6
3–6	86.0±4.0	0.8±0.5	86.8±4.3
6–12	87.4±4.1	1.4±0.6	88.8±3.9
12–36	95.6±1.1	1.9±0.4	97.4±0.8

Values are means ± SD in percent.

Table 3. *Distribution of label in urine*

Time Period, h	As Urea	In Acid-Labile CO_2	Present in Other Substances
0–3	46.5±15.0	48.0±14.8	5.6±3.1
3–6	72.1±10.1	20.7±10.7	7.2±2.1
6–12	78.8±7.5	18.2±7.0	4.0±1.5
12–36	84.2±3.3	10.0±2.3	5.8±2.0

Values are means ± SD in percent.

CO_2 production and energy expenditure. A strong relationship ($r = 0.998$) was demonstrated between the measurements of CO_2 made every 3 h by the infrared analyzers and by the titration method. The bias and 95% limits of agreement (±2 SD) between the 3-h measurements obtained by the two methods were 0.03 and ±0.098 mol (the titration method being higher by 0.9 ± 1.0%).

Total energy expenditure, based on measurements of CO_2 production and O_2 consumption, was found to be 11.87 ± 0.93 (SD) MJ/day, and the ratio of total energy expenditure to BMR was 1.54 ± 0.08. The energy balance was found to be −1.24 ± 0.51 MJ/day, and the 24-h RQ (estimated between 12 and 36 h) was 0.832 ± 0.019.

The pattern of CO_2 production during the course of the day, which was similar to the pattern of energy expenditure (data not shown), is indicated in Fig. 2. Mean energy expenditure for the periods of 50 and 100 W exercise was found to be 2.75 and 4.15 times BMR, respectively. Similarly, CO_2 production was found to be, respectively, 2.81 and 4.00 times greater than basal levels. Smaller increments in energy expenditure and CO_2 production were observed after the ingestion of meals (Fig. 2). Both energy

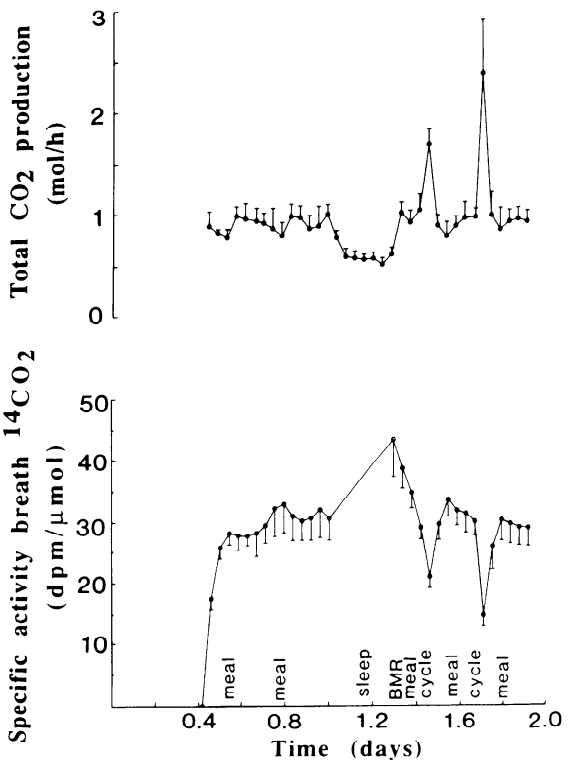

Fig. 2. Pattern of CO_2 production (fast response algorithm) and associated changes in CO_2 specific activity (spot breath samples) during 12 to 36 h period. Results are means ± SD.

expenditure and CO_2 production were found to be at their lowest during periods of sleep (~3–5% below the BMR levels).

Concentration and specific activity of CO_2 in breath, urine, blood, and saliva. The concentration of acid-labile CO_2 in whole blood was shown to increase from 20.1 mM (range, 17.8–22.8 mM) at the beginning of the study to 23.1 mM (range 19.8–25.2 mM) at the end, and the concentration in plasma rose from 23.6 to 27.0 mM.

The concentration of urinary acid labile CO_2 was shown to rise rapidly above a pH of 6.5 (Fig. 3), both in the subjects infused with bicarbonate in the calorimeter and in the separate group of normal subjects (not infused). Despite the infusion of bicarbonate, changes in urine CO_2 concentration and pH were small and always within normal physiological ranges. The concentration of acid labile CO_2 in urine showed a tendency to rise between the beginning (range 2.1–27.2 mM, before the bicarbonate infusions) and end of the study (15.9–30.4 mM). Furthermore, over the course of the study, variations in urinary CO_2 concentrations reflected variations in urinary pH (the mean values for the 3-h samples collected during different times ranged from pH 6.8–7.2, while the mean value for the sample collected overnight was pH 6.5).

The specific activity of CO_2 in breath (Fig. 2), urine (Fig. 4), and arterialized venous blood (data not shown) was found to vary inversely with CO_2 production. In general, the specific activity of CO_2 in urine was similar to the mean value obtained from spot breath samples, for equivalent time periods. However, some of the individual samples demonstrated substantial deviation from the line of unity (Fig. 5). A strong relationship was demonstrated between specific activity of CO_2 in spot end-expiratory air and that of arterialized venous blood, in the one subject where this comparison was possible. The specific activities of CO_2 in arterialized blood were found to be

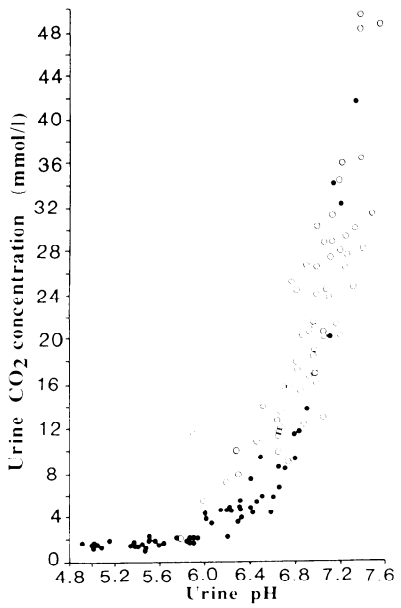

Fig. 3. Relationship between urine pH and acid-labile CO_2 in urine. Open circles, results obtained from subjects participating in study; closed circles, results from random samples of a distinct group of normal subjects.

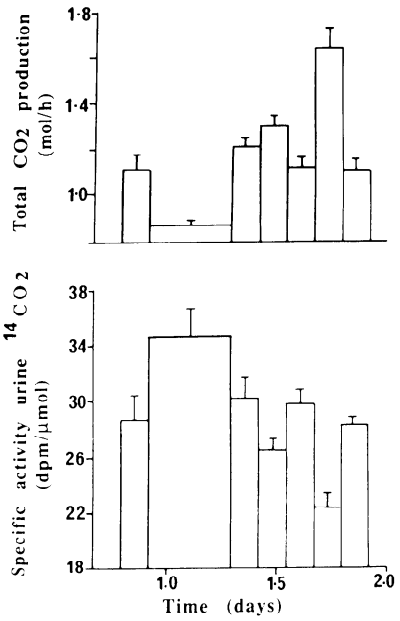

Fig. 4. Changes in CO_2 production (fast response algorithm) and specific activity of acid-labile CO_2 in urine during course of study. Results are means ± SD.

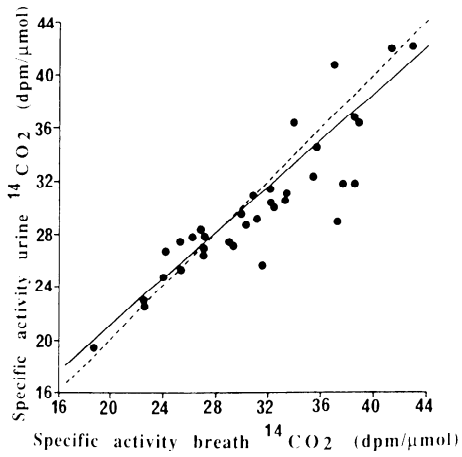

Fig. 5. Relationship between specific activity of urine $^{14}CO_2$ (3 h samples) and end-expiratory breath $^{14}CO_2$ [mean results over 3 h, $r = 0.89$, $y = 5.23 + 0.79x$; bias and 95% limits of agreement (2 SD) between methods = 1.18 and 5.36 dpm/μmol, respectively]. Line of identity denoted by dotted line.

98.3 ± 6.4% of the values in breath samples obtained at essentially the same time.

The lowest specific activities in the spot breath samples were obtained during periods of physical activity (1 h exercise periods, see SUBJECTS AND METHODS). Concomitantly, the highest levels were found during periods of low physical activity (at the end of sleep, and at the beginning and end of the BMR measurement period). Local CO_2 production would be expected to increase isotopic dilution, and this is consistent with results obtained in the one subject in whom it was possible to follow the effect of forearm muscle exercise, by the difference in CO_2 concentration and specific activity between arterialized (17.8 mM and 26.6 dpm/μmol, respectively) and deep venous blood (24.9 mM and 20.0 dpm/μmol). Before exercise the corresponding values in arterialized blood were 18.0 mM and 26.9 dpm/μmol, and in venous blood, 19.5

mM and 24.8 dpm/μmol. Leg exercise in the same subject (cycling at 50 and 100 W for 1 h) affected the arterialized blood concentration by <1.5 mM.

Specific activity of salivary CO_2 followed a similar daily pattern as the other samples, but the actual values were consistently lower. For example, the overall values for the specific activities of salivary CO_2 were 83 ± 7% of those obtained from breath CO_2 at comparable times (integrated mean specific activity of saliva samples were 26.9 ± 4.0 dpm/μmol, compared with 32.4 ± 3.0 dpm/μmol for the spot breath samples, *method A*). The mean integrated specific activity of arterialized blood, in the single subject (*subject 1*) in whom sequential samples were taken, was found to be 34.7 dpm/μmol (*method A*).

Prediction of CO_2 production and energy expenditure from measurement of specific activity in breath and urine. The prediction of CO_2 production rate was generally found to be 2–4% lower using *method A* than *method B* (Tables 4 and 5). Applying exponential functions to the spot breath measurements over certain periods resulted in differences in estimated 24-h CO_2 production of ≤1%. Thus, for simplicity, only results derived from the methods that include linear functions are reported (Tables 4 and 5).

Although there was only small intersubject variation in net CO_2 production, 24-h CO_2 production rates were strongly related to the mean specific activities of CO_2, measured over the same time period by continuous collection of CO_2 (Fig. 6 and Table 4, which also includes integrated mean specific activities of spot breath samples for comparison).

Based on assumptions that a constant 95% of the infused label was recovered in gaseous CO_2, the estimated CO_2 production was close to measured CO_2 production (e.g., within ±6%). Furthermore, it was assumed that the energy equivalent of CO_2 was 23.85 kJ/l or 534.6 kJ/mol (applicable to those individuals consuming a "Western" type diet close to energy-nutrient balance, Refs. 13 and 15); it was found that errors associated with estimation of energy expenditure from spot breath samples were all <7% and those based on urine measurements <9% (Table 5) for each subject individually. However, the use of saliva to predict energy expenditure gave results that were systematically greater (14.40 ± 2.85 MJ/day, $P < 0.01$) than those obtained with other physiological fluids. The values were 121 ± 14% of actual measurements obtained by indirect calorimetry. In the single subject in whom calculations based on blood measurements were possible, energy expenditure was estimated to be 10.81 MJ/day (*method A*) or 95.4% of measured energy expenditure.

DISCUSSION

Recovery of ^{14}C label. Several studies that have investigated recovery of $^{14}CO_2$ or $^{13}CO_2$ in expired air, from a constant infusion of labeled bicarbonate (3, 6, 8, 17, 24, 27–29, 40), have generally shown recoveries of 80–90% toward the end of 12 h of infusion. In this study, with exercise the recovery was >90% beyond the 12 h, as it was in another study, without periods of formal exercise (8), which is probably a reflection of the reappearance of CO_2 from pools that have relatively slow turnover time or oxidation of substrates that had previously been formed from fixation of labeled carbon. In addition, the present study reports a somewhat higher recovery of label during the 3- to 12-h period (87%), as opposed to other studies that report a recovery in the fed and fasted states of between 56 and 87% over similar time periods (28, 29), or 70–82% at 4 h (24).

Several possible factors may lead to these differences. The first concerns the infusion, in this study, of unlabeled CO_2 (as sodium bicarbonate). However, the ingestion of organic salts in vegetarian or fruit diets may result in production of bicarbonate within the body, at a rate that exceeds the amount of bicarbonate infused in this study. Furthermore, in the subjects infused with bicarbonate, the blood bicarbonate concentration and urine pH remained within the normal range. Second, because this study was conducted in a whole body calorimeter, there was complete (continuous) collection of all labeled and unlabeled CO_2 from the body, and this included the 1–2% of CO_2 lost through the skin under normal circumstances (2, 20, 21). This loss has not been accounted for in previous human studies, because only breath samples were collected for analysis. A third consideration involves the measurement, in previous studies, of CO_2 specific activity while the subjects were at rest. Lower recoveries may have been experienced if measurements were made between periods that included a certain degree of physical activity (this extra CO_2 production may not have been included in the calculations). Furthermore, the lower specific activity of CO_2 created by increased CO_2 production does not necessarily return to the status quo immediately

Table 4. *Total CO_2 production and integrated mean specific activity of CO_2 in 12 to 36 h period for each subject*

Subject No.	CO_2 Production, mol/day	Specific Activity of CO_2, dpm/μmol				
		Continuous Gas Collection	Spot Breath Samples		Urine Samples	
			Method A	*Method B*	*Method A*	*Method B*
1	20.39	34.77	35.28	34.73	36.00	34.88
2	25.65	27.88	27.15	26.35	25.89	25.62
3	21.62	32.36	33.37	31.34	31.13	30.42
4	23.12	31.15	31.00	29.65	31.82	31.01
5	20.16	34.66	34.37	33.04	32.26	31.75
6	21.26	33.24	33.35	31.55	33.14	31.87
Means ± SD	22.033±2.061	32.34±2.58	32.42±2.95	31.11±2.64	31.71±3.32	30.92±3.02

CO_2 production measured by biochemical method. Continuous gas collection values represent ratio of total radioactivity to total gaseous CO_2 produced by the subjects and collected over 24 h. dpm, disintegration/min.

Table 5. *Energy expenditure (24 h) predicted from isotope dilution**

Subject No.	Measured 24 h Energy Expenditure, MJ/day	Energy Expenditure (24 h) Predicted From CO_2 Specific Activity									
		Continuous O_2 Collection		Spot Breath Samples				Urine			
				Method A		*Method B*		*Method A*		*Method B*	
		MJ/day	%Measured	MJ/day	%Measured	MJ/day	%Measured	MJ/day	%Measured	MJ/day	%Measured
1	11.33	10.90	96.2	10.65	94.0	10.82	95.5	10.44	92.1	10.77	95.2
1	13.68	13.71	100.2	13.85	101.2	14.26	104.2	14.51	106.1	14.67	107.2
3	11.69	11.56	98.9	11.26	96.3	11.99	102.6	12.07	103.3	12.36	105.7
4	12.43	12.36	99.4	12.12	97.5	12.67	101.9	11.81	95.0	12.12	97.5
5	10.95	10.77	98.4	10.93	99.8	11.38	103.9	11.65	106.4	11.84	108.1
6	11.25	11.37	101.0	11.27	100.2	11.91	105.9	11.34	100.8	11.80	104.9
Means	11.88	11.78	99.0	11.68	98.2	12.17	102.3	11.97	100.6	12.26	103.1
± SD	±0.9	±1.01	±1.5	±1.07	±2.5	±1.09	±3.2	±1.37	±5.4	±1.2	±4.9

MJ/day, values calculated from specific activity of all gaseous CO_2 produced by subjects in 24 h; %Measured, value represents predicted estimate as percentage of measured 24 h energy expenditure. * Assumes that energy equivalent of CO_2 is 23.85 kJ/l CO_2 (534.6 kJ/mol) and that recovery of infused label in gaseous CO_2 is 95% in all subjects.

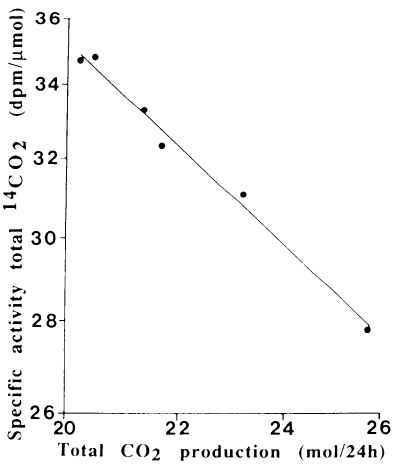

Fig. 6. Relationship (logarithmic) between total CO_2 production and overall specific activity of CO_2 produced (continuous collection) for 6 subjects ($r = 0.995$, $\log y = 2.74 - 0.92 \log x$).

after the physical movement has ceased or indeed when CO_2 production has returned to normal.

The variation between subjects in this study (range for the 12 to 36 h period 94.5–96.5%), which was remarkably small and considerably lower than that reported in other studies, may have been due to the continuous nature of the measurements.

It may be suggested that only a relatively small proportion of label is incorporated into stores of fat and protein (these have a very slow turnover time with respect to bicarbonate), because of the high total recovery of label during the 12 to 36 h period (97.4%, Table 2). This suggestion is consistent with conclusions drawn from studies involving fed and fasted rats, whereby only 1.8–2.8% of a bolus injection of labeled bicarbonate were retained in carbohydrate, fat, and protein after 6 h (33).

Although a bolus injection of labeled CO_2 (given as bicarbonate) has been used to estimate net CO_2 production in both animals (34) and humans (1, 5, 23), this method is limited by a short period of study (e.g., 2–4 h, imposed by the rapid multiexponential loss of label from the body; Ref. 5), and a variable recovery in breath of labeled CO_2 as the result of different physical activities. The apparent increase in the recovery of label during exercise is partly due to accelerated oxidation of organic compounds, such as carbohydrate, that had been previously labeled with carbon (fixation of CO_2). This is equivalent to increased entry to the body pool of $^{14}CO_2$; with stable isotopes there is also the added complication that ^{13}C abundance in carbohydrate is higher than that in fats. The apparent increase in recovery is also partly due to loss of label from the body pool of CO_2. The loss of label from the circulating pool, which is reflected in the specific activity of breath CO_2, is not associated with large changes in the size of the CO_2 pool (most forms of exercise produce little change in the circulating CO_2 concentration, and this is consistent with the small change of <1.5 mM in the arterialized blood, during 1 h of exercise, in the one subject in whom blood sampling was possible). The reduction in the specific activity of circulating CO_2, which is caused by increased net production and excretion of CO_2, may also have secondary effects in reducing the specific activity of CO_2 in other body pools, which are in equilibrium with the circulating pool of CO_2. It is also theoretically possible that the rate constants that describe such an exchange between the CO_2 in the central pool and other more peripheral pools may increase during exercise (5). Finally, the increase in ventilation, which prevents acidosis from developing during exercise, may also contribute to the loss of intravenously infused label via breath, before the infused label has had sufficient time to reach other tissues and other CO_2 pools of the body. However, this last effect is considered to be small, partly because the loss of CO_2 via breath is small relative to the flow of CO_2 through the vasculature of the lungs and partly because most of the label reaching the lungs has already circulated through the arterial blood.

The above observations, if taken together, suggest that in studies of short duration (e.g., 2–3 h; Refs. 1, 5, and 23), as in those involving bolus infusions of labeled bicarbonate (1, 5), different recovery factors should be applied in subjects undertaking different physical activities. Indeed, substantial fluctuation in CO_2 recovery (above 95% during exercise and below 95% during the rest period immediately after exercise) indicates that it may be inappropriate to use the labeled bicarbonate method (with a

constant recovery factor) for estimating mean energy expenditure over short periods of time, especially where physical activity is expected to be variable (5). The greater the length of the study, the less is the likelihood that the results will be influenced by either transient changes in the quantities of "stored" label in the body pools (including labeled substrates such as glycogen/glucose) or a variable rate of cycling of label through the various body pools of CO_2 that have different turnover times. This is because, during longer studies, the recovery of $^{14}CO_2$ fluctuates around the eventual mean value during the period of the study. The increased rate of loss of label in breath during exercise can only transiently be expected to exceed the rate of infusion (see RESULTS and Ref. 36 for patterns of change with $H^{13}CO_2$). However, short-term studies may give important qualitative information regarding changes in energy expenditure because of changes in physical activity and thermogenesis.

The majority of whole body studies in adults, involving constant infusion of labeled bicarbonate, has shown a 70–85% recovery of label during the first 3 h of infusion, whereas ~50% were recovered over this same period in the present study. This may be due to the use of a priming dose in some previous studies (e.g., 3, 24), which results in the failure of retention of label in the body pool. No such prime was used in the present study to gain some further insight into bicarbonate kinetics. Furthermore, the recovery of ~50% in this study represents the mean recovery over the entire 3 h and not the progressively increasing recovery, which reached a value of ~70% between the 2nd and 3rd h.

Isotopic dilution of CO_2. The remarkably small intersubject variability in recovery of labeled CO_2 (as continuously collected gas) between 12 and 36 h, suggests that it may be appropriate to incorporate a constant value for this into equations for estimating net CO_2 production and energy expenditure from the labeled bicarbonate method (at least in subjects with similar characteristics and undergoing similar regimens). However, if the labeled bicarbonate method is to be applied to free-living conditions over extended periods of time, it is necessary to first establish the extent of variability in recovery factors. Should this variability prove to be small, as in this study, it may then be possible to apply a constant recovery factor to all study conditions. Clearly, this variability is likely to be dependent on the length of study (see above and Table 2).

The calculation of net CO_2 production in free-living circumstances requires an estimate of the integrated mean specific activity of CO_2 over a period of time. The integrated mean specific activity of CO_2 in spot breath samples was 98.2 ± 2.5% for *method A* and 102.3 ± 3.2% for *method B* (Table 5) of the continuously collected gaseous CO_2; therefore, frequent and repeated measurements of breath samples ensured prediction of net CO_2 production to within 5–6%.

Isotopic dilution of CO_2 between the lung and arterial blood is considered to be small, because the production of CO_2 by the heart is <1% of the amount of CO_2 that passes through it over the same period of time (16). This explains why the specific activity of CO_2 in arterial blood was essentially the same as that in end-expiratory CO_2 and is consistent with a recent study (11) in which a small number of dogs were infused with labeled bicarbonate, either into a peripheral vein (to pass through the lungs before the systemic circulation) or into the aorta (to bypass the lungs). Samples taken from the same site (femoral vessels) showed no significant difference between the two routes of infusion, suggesting that the rate of CO_2 production by the lungs and heart is small, compared with the rate at which circulating CO_2 passes through these tissues. However, in all cases, blood sampled from a limb vein had a higher CO_2 content (by ~10%) than arterial blood; concomitantly, the estimated CO_2 flux was higher when calculations were based on measurements of venous blood (lower enrichment) than arterial blood (higher enrichment). These findings are consistent with those of the subject in the present study, in whom lower specific activity of CO_2 was demonstrated in peripheral venous blood than arterial blood. Such observations serve to emphasize the importance of sampling site when calculating CO_2 flux.

The tendency for specific activity of CO_2 in saliva to be lower than in blood, urine, or breath samples, taken coincidentally, implies that the use of saliva will result in erroneously high estimates of net CO_2 excretion. The cause of this low specific activity in saliva is uncertain but may originate from isotopic dilution by locally produced CO_2 (e.g., in the salivary glands or other tissues in the mouth) or possibly from exchange of labeled CO_2 with ambient CO_2 of lower specific activity than blood. Isotopic dilution by the CO_2 present in drinking (tap) water is not considered to have been important, partly because of low concentration in tap water and partly because no water was permitted for the 30 min immediately before saliva sampling.

Measurement of the specific activity of CO_2 in urine may confer important advantages for the estimation of net CO_2 production, over and above other physiological fluids. Because urine is continuously formed by the kidneys, its specific activity may be considered to represent an integrated mean value, similar to that in air expired by the lungs over the same time period. Should this prove to be the case, measurement of urine would not only offer a theoretical advantage for prediction of net CO_2 production but also a practical advantage in that measurements could be made on fewer samples. In addition, the kidneys are not subject to the same extent of isotope dilution, at a local level, as some other tissues (e.g., exercising forearm muscle, as shown here in the study of one subject, or liver; Ref. 22); because, blood perfuses the kidney with a relatively greater amount of CO_2 (~20 mmol acid labile CO_2/min) than is produced through the metabolic activity of the kidney itself (10, 16, 25). However, despite these theoretical advantages, the use of urinary measurements to predict the specific activity of CO_2 produced by the body, collected continuously, was less accurate than spot breath measurements (Table 5). Of the possible factors underlying this observation, two are considered here. First, although the collection-time intervals may be the same, there may be substantial variation in both content and concentration of CO_2 present in the urine at different

times of day. For example, greater amounts of CO_2 tend to be excreted in the urine after meal ingestion than before it (17); greater amounts are also excreted in subjects who tend to drink larger volumes. Should such fluctuations occur during a certain collection interval, the result would tend to be influenced more by periods (within this time) associated with excretion of urine of high CO_2 content than those of low CO_2 content. The second factor involves the possible exchange, or transfer, of CO_2 across the bladder wall. Assuming that complete and instantaneous equilibration occurs between urinary CO_2 in the bladder and blood CO_2, then the specific activity of urinary CO_2 should equal that of a spot measurement of either blood or breath. Alternatively, assuming that no exchange of CO_2 occurs between blood and urine in the bladder, then the specific activity of urinary CO_2 would represent an average of the CO_2 entering the bladder over that whole collection period.

Evidently, the fractional transfer of labeled CO_2 (introduced directly into the bladder; see APPENDIX 2) across the bladder is dependent on urine pH and volume (APPENDIX 2 and Fig. 7). At high pH a greater proportion of acid-labile CO_2 is present as bicarbonate and fractional transfer of CO_2 across the bladder wall is low, so that the recovery of label in breath is low. In contrast, at low pH a greater proportion of the acid-labile CO_2 exists in the free state, so the fractional transfer across the bladder (and subsequent expiration in breath) is greater. The transfer of CO_2 across the bladder wall is greater (by ~50%) when the bladder contains a low volume of urine (50–100 ml) than when the volume is higher (150–250 ml). Presumably, the exchange of CO_2 occurs both from the bladder to the blood (and possibly other adjacent tissues) and vice versa, and the particular direction in which the CO_2 specific activity changes will depend on the CO_2 specific activity gradient. However, in studies where labeled CO_2 is infused over extended periods of time, it is likely that any gradient in CO_2 specific activity between blood and urine in the bladder will fluctuate and largely cancel out.

Estimation of energy expenditure. Essential to the estimation of energy expenditure by the labeled bicarbonate method are values for the fractional recovery of labeled CO_2 and the energy equivalent of CO_2. In this study, a constant value of 0.95 for the fractional recovery of labeled CO_2 in breath has been adopted because, between 12 and 36 h, the recoveries from all subjects were found to fall within a relatively narrow range (94.7–97.2%). Although the energy equivalent of CO_2 may vary according to the relative proportions of fat, carbohydrate, protein, and alcohol oxidized (for detailed discussion, see Ref. 15), a value of 23.85 kJ/l CO_2 may be adopted for subjects such as those studied here, who are close to nutrient and energy balance, consuming a "Western" type of diet. If these values are used to estimate energy expenditure by isotope dilution, then the estimated mean value for energy expenditure is close to the measured mean value, irrespective of whether spot breath (98.2 ± 2.7% with *method A* and 101.3 ± 3.2% with *method B*) or urine samples (100.6 ± 5.9% with *method A* and 103.1 ± 4.9% with *method B*) are used for the assessment.

Practical aspects of labeled bicarbonate method. Administration of labeled bicarbonate over a long period of time should not prove to be a major problem in patients already receiving intravenous infusions. In normal subjects the most feasible method of achieving continuous infusion is by minipumps, similar to those used for delivering insulin to diabetics. Possibilities include the use of subcutaneous infusions (as in some animal studies; see Ref. 14), and nasogastric or oral delivery (e.g., Ref. 24). Oral or nasogastric routes of administration raise the theoretical possibility of eructation of labeled CO_2 before equilibration within the body pool as shown in some ruminants (e.g., Ref. 9). However, this was not considered to be a problem in a human study, which reported similar recoveries of labeled CO_2 in breath (assessed by spot breath tests) after intravenous and nasogastric administration (24).

The most simple and practical method of assessing the integrated CO_2 specific activity over a period of time is to make a single estimate using a continuous collection of urine. Although urine collections have the theoretical advantage of continuous production, they are also susceptible to the disadvantages discussed previously. Alternatively, the use of the specific activity of urea (which is formed from CO_2) may overcome some of the drawbacks of using urinary acid labile CO_2. The excretion of urea is known to be less variable than that of CO_2; important changes in urea specific activity are much less likely to occur than with acid labile CO_2 in the bladder, and the concentration of urea in urine may be one to two orders higher than the magnitude of the urinary CO_2, which implies a lower requirement of radioactive label in the infusion. Preliminary work (22) suggests that the specific activity of urea is less than that of expired air, due to a predictable level of isotopic dilution from locally produced CO_2 (from splanchnic tissues, especially the liver where urea is formed). The use of urea as a tool for the estimation of net CO_2 production and energy expenditure over several days is currently under investigation in humans.

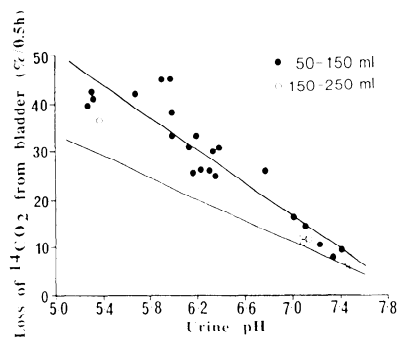

Fig. 7. Relationship between urine pH and fractional loss of label (^{14}C) across bladder. Label instilled directly into bladder by urinary catheter. Solid circles, results obtained with 50–150 ml of urine in bladder; open circles, results with 150–250 ml.

APPENDIX 1

Titration. A known weight of hyamine (BDH)-methanol solution containing phenolphthalein as indicator was titrated with 0.2 M HCl (Analar, BDH) to the end point, when the color (pink) disappeared. The volume of HCl was determined from

the density of HCl and the weight used in titration. All titrations were carried out to completion within ~1 min, and in triplicate over ice to prevent any loss of CO_2 from occurring should the temperature of solution have been allowed to rise significantly. Because of this precaution, the heat produced by the titration process did not cause the temperature of the solution to rise above 20°C, nor was there any significant loss of methanol through evaporation.

The end point of the titration was found to be dependent on the total amount of water present; the greater the proportion of water in the final solution, the greater was the amount of acid required to achieve loss of indicator color at the end point. However, although the titration curve (pH curve) of a hyamine-methanol solution containing CO_2 was found to be virtually identical to one that did not contain CO_2, a very slight deviation of the curves was evident close to the end point of the titration. An additional, albeit minute, amount of acid was required to achieve the end point in hyamine-methanol solutions containing CO_2, and the extent of this deviation was found to depend on the amount of CO_2 present in solution. The small, and yet opposing, effects of water (present in the HCl used for titration) and CO_2 were accounted for when calculating the amount of CO_2 trapped in solution.

Furthermore, although the effect of methanol on the end point of the titration was found to be almost negligible, variation in the amount of methanol present was also taken into account. However, even if all of these effects had been ignored completely, the maximum combined error in the estimate of CO_2 trapped in solution would have been less than ±1.5%.

APPENDIX 2

Bladder study. Eight patients (age 36–78 yr, wt 57–90 kg) admitted to Addenbrooke's Hospital, Cambridge, had been catheterized as part of the treatment for either cerebrovascular accident or elective abdominal surgery (inflammatory bowel disease or carcinoma of the colon). The absence of urinary tract infection had been confirmed by catheter specimen of urine, just before commencement of the study.

The urinary catheter of each subject was clamped for variable periods of time to allow for urine volumes of between 50 and 250 ml to accumulate in the bladder. Three of the eight subjects, 0.5 h before the study, received an intravenous infusion of 30 mmol sodium bicarbonate (30 ml, 8.4% solution; Boots), over a period of 5–10 min, to alkalinize the urine. Urine was withdrawn, with a 60-ml bladder syringe, from the bladder of all subjects and reintroduced several times to ensure adequate mixing and to obtain a sample aliquot. Then 2 μCi of ^{14}C-labeled sodium bicarbonate (Amersham) and 7 μCi of ^{3}H-labeled dextran (Amersham) were injected into the bladder and mixed, as described above. A second aliquot of urine was taken. The bladder was clamped for a further 30 min, before the mixing and sampling procedure was repeated. This occurred at 30-min intervals for 2 h. The aliquots were subdivided into two, one for the measurement of pH and the other placed in a preweighed, tightly sealed, tube containing NaOH for the measurement of concentration of label (^{3}H and ^{14}C) and acid-labile CO_2. At the end of the 2 h period, all urine present in the bladder was withdrawn for assessment of ^{3}H-labeled dextran.

The amount of urine present in the bladder at any time point was calculated from the isotopic dilution of urinary ^{3}H-labeled dextran. Previously, it had been established that the recovery of ^{3}H-labeled dextran, under these circumstances, was close to 100%. The specific activity of CO_2 in expired air was measured in five of the subjects, both before (0 time) and after injection of tracer into the bladder (at 15, 45, 75, and 105 min).

Results. The amount of [^{3}H]dextran recovered from the urine was 100.8 ± 2.5% of that injected by bladder syringe directly into the bladder. In contrast to this, the urinary recovery of labeled bicarbonate, injected in the same way, was found to be substantially lower and to be dependent on both urine pH and volume. The proportion of labeled CO_2 lost via the bladder wall (Fig. 7) was lower when in urine of alkaline pH (alkaline urine is known to be associated with increased CO_2 concentrations) and large volume, than in more acid urine and smaller in volume. Furthermore, direct evidence of transfer of CO_2 across the bladder wall is provided by the detection of labeled CO_2 (introduced directly into the bladder, as above) in breath. The specific activity of CO_2 detected in breath was severalfold higher in the two subjects in whom a low pH was evident (pH 5.2–5.4 and 5.9–6.0) than in the three subjects with the slightly more alkaline urine (pH 7.3–7.5, 7.1–7.2, and 6.8–7.2). The integrated mean specific activity of breath CO_2 (dpm/μmol) over the 2 h of the study correlated well with the measured transfer of CO_2 (dpm) through the bladder wall: $r = 0.98$, standard error of estimate (SEE) 0.18×10^6 dpm, $y = -262 + 903x$. The specific activity of breath CO_2 at the end of the study (reflecting the specific activity of the central pool of CO_2) also correlated well with the measured loss of labeled CO_2 through the bladder: $r = 0.92$, SEE 0.24×10^6 dpm, $y = 144 + 606x$.

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