

The potential use of the labelled bicarbonate method for estimating energy expenditure in man

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Two tracer methods have been used to assess energy expenditure in animals and humans: the doubly-labelled-water method (for animals Lifson *et al.* 1955; for humans Prentice, 1988; Coward, 1988) and the labelled bicarbonate method (for humans present paper; for animals Corbett *et al.* 1971). Whereas the doubly-labelled-water technique attempts to provide information about the energy expenditure over long periods of time (2–3 weeks in adult human subjects), the bicarbonate method attempts to provide information about shorter periods of time (hours to days). Both techniques assess carbon dioxide production, and therefore it is necessary to relate CO₂ production to energy expenditure. These aspects will be discussed separately.

Assessment of CO₂ production

The bicarbonate method is essentially an isotopic-dilution technique involving either a radioactive isotope of carbon (¹⁴CO₂, as in the human study described on pp. 250–255) or a stable isotope of C (¹³CO₂). When labelled bicarbonate is infused into the body at a known rate, it will mix with and be ‘diluted’ by endogenous CO₂ produced by the tissues of the body, before it is expired in breath. The extent of this ‘dilution’ (the specific activity of ¹⁴CO₂ or enrichment of ¹³CO₂) is used to calculate CO₂ production. When a constant infusion of labelled bicarbonate is given, CO₂ production can be estimated from the mean specific activity in equation 1 (Corbett *et al.* 1971).

$$\text{CO}_2 \text{ produced} = \frac{\text{label infused}}{\text{specific activity of CO}_2} \tag{1}$$

This simple equation assumes that the labelled CO₂ (bicarbonate) infused into the body is totally recovered in expired air after mixing in a single pool of CO₂ with unlabelled CO₂ produced by the tissues of the body (Fig. 1).

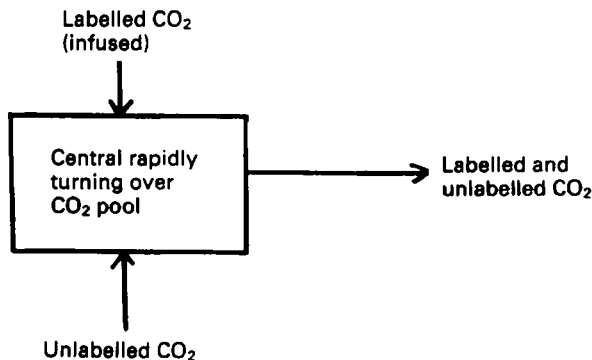
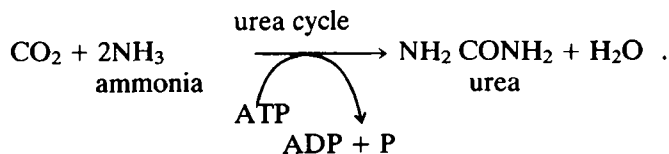


Fig. 1. A simple one-compartment model of carbon dioxide kinetics.

However, in reality the recovery of CO_2 is not complete and the CO_2 in the body does not exist in a single pool. In addition the specific activity of CO_2 does not remain constant. It oscillates about a mean value because of fluctuations in CO_2 production.

Recovery of infused CO_2 . The recovery of infused bicarbonate in man has generally been reported to range from 80 to 94% (Issekutz *et al.* 1968; Winchell *et al.* 1970; James *et al.* 1976; Allsop *et al.* 1978; Clugston & Garlick, 1983). In one study a recovery of only 51% was reported (Irving *et al.* 1983). This incomplete recovery of labelled bicarbonate is largely due to the operation of two processes. The first involves trapping or fixation of CO_2 by certain metabolic processes. For example, CO_2 is utilized in the formation of urea which may accumulate in body fluids or be excreted in urine



CO_2 may also be 'fixed' by other reactions including those catalysed by pyruvate carboxylase (EC 6.4.1.1) and malate dehydrogenase (EC 1.1.1.37; NADP-dependent, also known as 'malic' enzyme)

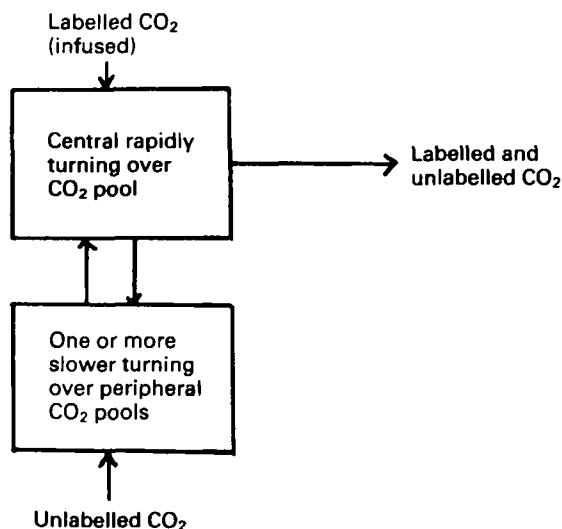
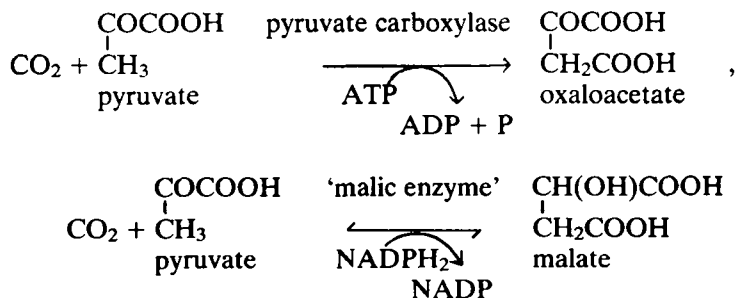


Fig. 2. A two-or-more compartment model of carbon dioxide kinetics.

These last two reactions form products which are intermediates in the citric acid cycle. Their C skeleton may be used to form amino acids, lipids and glycogen due to the distribution to ^{13}C or ^{14}C on both of the carboxyl groups of citric acid cycle intermediates (Krebs *et al.* 1966; Brosnan, 1982), and to the operation of anaplerotic reactions that feed into and out of the citric acid cycle.

Another process that may lead to apparent 'trapping' of CO_2 is the entry of CO_2 into pools that turn over slowly. Clearly the fractional recovery of $^{13}\text{CO}_2$ or $^{14}\text{CO}_2$ in expired air is likely to be less complete when the period of collection is short and close to the start of the infusion of labelled CO_2 . Although the details of bicarbonate kinetics are somewhat controversial (for references, see Irving *et al.* 1983), it appears that CO_2 in the body exists in a central rapidly turning over pool which includes CO_2 in the circulation, (free CO_2 , bicarbonate, carbonic acid and carboxyhaemoglobin), and one or more other pools (e.g. muscle) which turn over more slowly (Fig. 2). The pool of CO_2 in bone turns over particularly slowly. Thus although the daily CO_2 production in adult man may be fifteen to twenty times greater than the total freely-exchangeable CO_2 of the body (0.8–1.0 mol; Allsop *et al.* 1978; Irving *et al.* 1983), some of the pools have a turnover of more than fifteen to twenty times per d, whilst others a turnover rate of less than fifteen to twenty times per d.

The previously-mentioned observations have two important implications for the labelled bicarbonate method for assessing energy expenditure. First, a more appropriate equation than equation 1 for calculating CO_2 production is equation 2.

$$\text{CO}_2 \text{ production} = \frac{\text{labelled infused} \times f}{\text{specific activity of CO}_2}, \quad (2)$$

where f is the fractional recovery of $^{14}\text{CO}_2$ of $^{13}\text{CO}_2$ in breath. Second, if the value of f varies considerably, either between individuals or in the same individual at different times, then substantial error will arise in the estimate of CO_2 production when a constant value of f is used in equation 2. Furthermore, since the specific activity of CO_2 may vary with time, it is important to measure the specific activity of CO_2 over a sufficiently long period of time (e.g. 12–24 h) so that a more representative mean value of specific activity can be used in equation 2.

Animal studies. The labelled bicarbonate method has been used to assess the energy expenditure of free-living animals, (for example, see White & Leng, 1969; Young & Corbett, 1969; Young 1970; Corbett *et al.* 1971). The estimates based on the labelled technique were found generally to agree to within 15–20% of those obtained by direct measurements of gaseous exchange. The estimate of CO_2 production by the tracer technique depends to some extent on the sample that is used for measuring specific activity (e.g. breath, urine or blood; Young, 1970; Corbett *et al.* 1971). For example, in one study (Corbett *et al.* 1971) the specific activity of CO_2 in breath was found to be about 7% lower than that in jugular venous blood. This difference was considered to be due to eructation of unlabelled or weakly-labelled CO_2 from the rumen into expired air. However, it must also be remembered that the specific activity of CO_2 in jugular venous blood is influenced by the CO_2 produced locally in the head. Corbett *et al.* (1971) concluded that the specific activity of CO_2 in urine is a more reliable indicator of the overall specific activity of CO_2 in the body, partly because urine measurements were considered to reflect mean values over a period of time, and partly because urinary CO_2 was considered to be largely derived from arterial blood, rather than from the kidneys. However, when there are no major fluctuations in CO_2 production, as in many of the

sheep studied by Corbett *et al.* (1971) spot measurements of specific activity in urine, blood or breath may be used to obtain an approximate estimate of energy expenditure.

CO₂ entry rates have also been assessed in studies involving a single injection of bicarbonate into the circulation. The results were found to correlate satisfactorily with those obtained by the constant infusion technique (White & Leng, 1969). However, with the single-injection method it is essential to make frequent measurements of CO₂ specific activity and to subject the results to detailed mathematical analysis (White & Leng, 1969; Irving *et al.* 1983). This is because there is a rapid multi-exponential loss of label from the central compartment into other CO₂ compartments and into breath.

Studies in man. We have recently evaluated the potential value of the constant-bicarbonate-infusion technique in six healthy lean male subjects in studies carried out over 36 h in whole-body indirect calorimeters. During the study breath tests were carried out frequently and the results compared with those obtained from the analysis of 3-hourly urine samples. In addition a portion of the air leaving the calorimeter was dried with calcium chloride, which is free of CO₂ adsorption effects (Elia *et al.* 1986), and passed continuously through a CO₂-trapping agent for the duration of study (twelve 3-hourly collections). This unique approach in humans allowed continuous measurements of specific activity to be compared with a series of spot measurements.

The mean recovery of the ¹⁴CO₂ which was trapped continuously between 12 and 36 h was 95.6 (SD 1.1) %, range 94–97% (*n* 6). The variation in recovery was considerably less than that reported in any other study. This implies that if a value for *f* of 0.95 is used in equation 2, CO₂ production in our subjects will be predicted to within 2%. It should be noted that the 95% recovery of ¹⁴CO₂ observed between 12 and 36 h was greater than that observed earlier between 3 and 8 h (approximately 85%). It was also higher than the mean values obtained in most other human studies (80–85%) which involved intermittent spot measurements of breath samples of expired air between 2 and 8 h. The higher recovery in our study was probably due to the prolonged period of collection which allowed sufficient time for labelled CO₂ to recycle through slowly turning over pools.

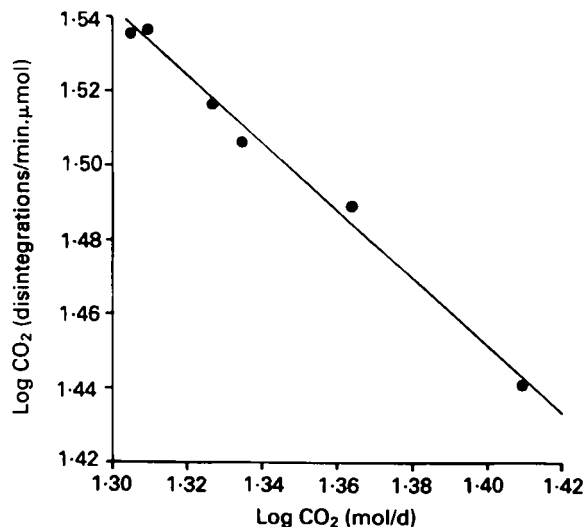


Fig. 3. The relationship between log carbon dioxide production over 24 h (12–36 h) and log specific activity of CO₂ (calculated for continuously collected samples) (*r* 0.994).

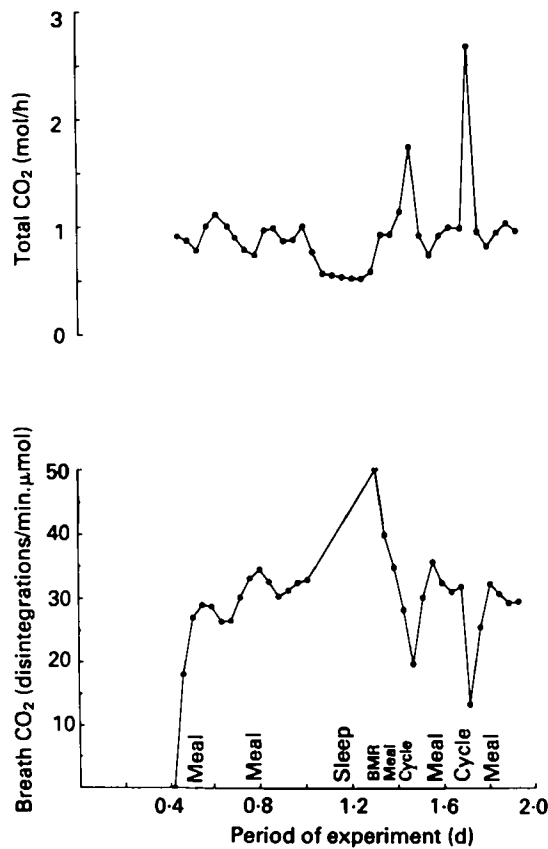


Fig. 4. The change in carbon dioxide production and in specific activity of CO₂ in spot breath samples during a 36 h calorimeter run in one subject.

The only other human study involving a 36-h infusion of bicarbonate concluded, on the basis of spot breath tests, that the mean recovery of labelled CO₂ in breath was about 93% (Clugston & Garlick, 1983).

Since the recovery of labelled CO₂ in our study was remarkably constant over the period 12–36 h, the inverse correlation between CO₂ production and CO₂ specific activity was also remarkably good ($r=0.994$; Fig. 3). However, these mean 24-h values give no indication of the short-term changes which occur as a result of sleep, exercise and food ingestion. The short-term changes in the spot breath samples of a typical subject are indicated in Fig. 4. Initially the specific activity of CO₂ was low as the label equilibrated with the bicarbonate pools. After 3 h there were small fluctuations in specific activity and in CO₂ production. During sleep CO₂ production decreased whilst the specific activity of CO₂ increased (Fig. 4). During a 1-h period of mild exercise (1 kp) there was a modest increase in CO₂ production and a reciprocal decrease in specific activity. After the exercise period both CO₂ specific activity and CO₂ production returned towards normal. The changes that occurred during more severe exercise (2 kp for 1 h) were more marked (Fig. 4). It therefore appears that there is an inverse relationship between CO₂ production and CO₂ specific activity in breath during periods as short as 1 h.

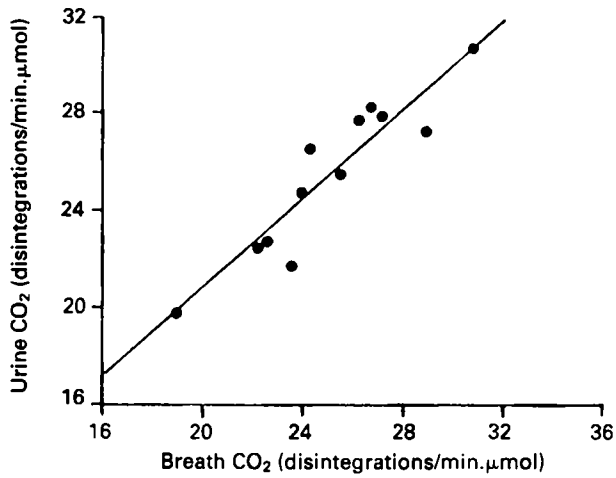


Fig. 5. The relationship between carbon dioxide production and specific activities of CO₂ in the urine samples of one subject.

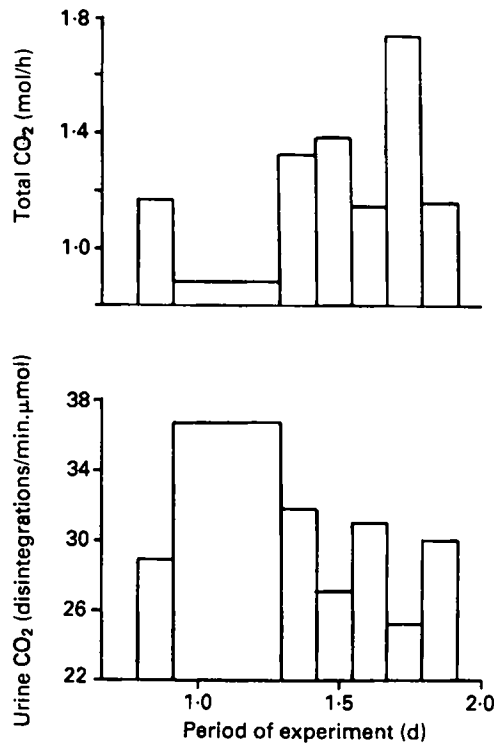


Fig. 6. The relationship between the specific activity of carbon dioxide in urine samples collected over 3 h and the specific activity in spot breath tests (aggregate values over 3 h). The samples were collected during the periods that included 1 h of cycling.

The specific activity of CO_2 in urine correlated with the specific activity of CO_2 in spot breath samples (Fig. 5) and varied inversely with CO_2 production (Fig. 6). The composite mean values of specific activity in urine samples collected over 24 h were 98 (sd 4.5)% of those in the expired CO_2 which was collected continuously over the same period of time. The composite mean values for spot breath tests were 100 (sd 2)% of the continuously collected samples. The larger variation in the urine results (sd more than twice that for spot breath samples), may be partly related to the wide variation in the CO_2 content of the urine that enters the bladder (see below). Fig. 7 shows the wide variation in the concentration of acid-labile CO_2 in samples of urine obtained from a variety of normal subjects. There may also be major fluctuations in pH and CO_2 concentration of urine obtained from the same individual at different times of day (Macy, 1942). For example, meal ingestion in normal subjects may be associated with a rise in urine pH (Fig. 8) and urinary CO_2 concentration (the 'alkaline tide' of feeding). During total starvation urine pH may decrease to a value between 4.5 and 5 and increase again on refeeding. The diurnal changes in pH and CO_2 concentration in the urine of one of the subjects involved in the bicarbonate tracer study are indicated in Fig. 9.

These variations in urine pH and CO_2 concentration have important implications for the estimation of mean specific activity in bicarbonate studies. When a urine sample with a high CO_2 content mixes with one that has a low CO_2 content, the resulting specific activity of CO_2 will not be half-way between the two individual specific activities, but

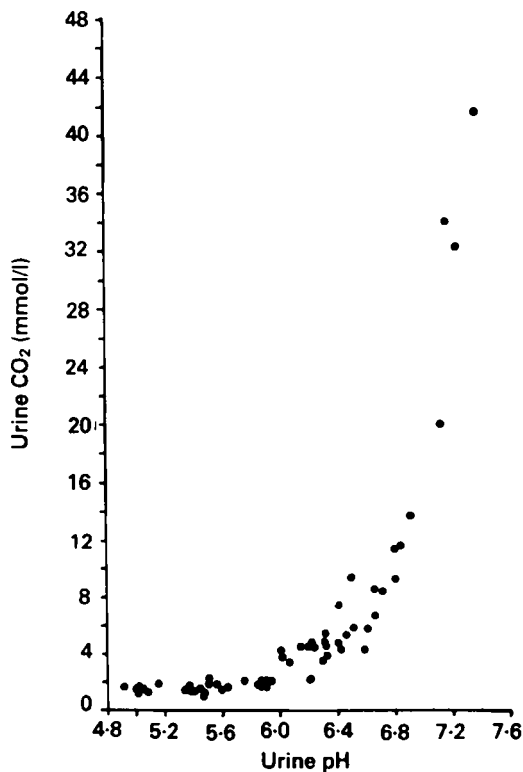


Fig. 7. The relationship between pH and the concentration of acid-labile carbon dioxide in urine samples obtained from normal subjects.

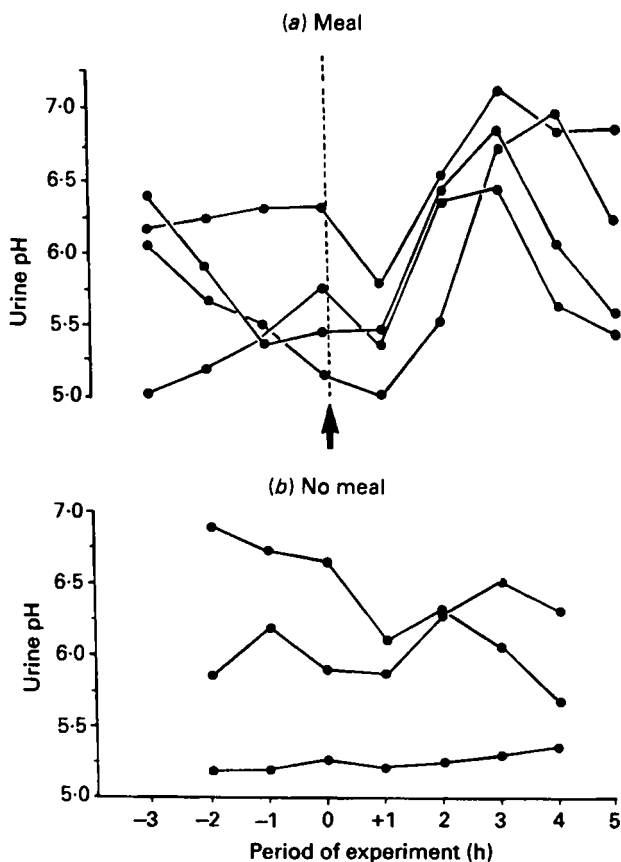


Fig. 8. The effect of a single meal (\uparrow) on urine pH (a). The meal, which consisted of 3724 kJ (890 kcal) was taken at 12.30 hours following an overnight fast. The control subjects missed breakfast as well as the midday meal (b).

heavily displaced towards the one with the high CO_2 content. Such an effect may also occur when urine produced by the kidney mixes with urine already in the bladder.

A second problem that may arise during storage of urine in the bladder, is the exchange of labelled CO_2 with blood CO_2 . If such an exchange occurred rapidly and completely, urine specific activity would be equivalent to that of a spot blood or breath test. If no exchange of label occurred, the specific activity of urinary CO_2 would depend on the total labelled and unlabelled CO_2 excreted by the kidney over a period of time. The truth almost certainly lies somewhere between these two extremes. In studies involving direct instillation of labelled bicarbonate (together with a marker for assessing urine volume) into the bladders of catheterized post-operative patients, it was found that a substantial transfer of CO_2 occurred through the bladder wall (up to 30–40% every 0.5 h). The label entered the circulation and was subsequently detected in breath. The fractional transfer of labelled CO_2 across the bladder was found to depend on both the concentration of acid-labile CO_2 in urine (pH related) and on the volume of urine in the bladder (M. Elia and N. Fuller, unpublished results). Presumably the transfer of CO_2 across the bladder is bi-directional so that the net change in urine specific activity will

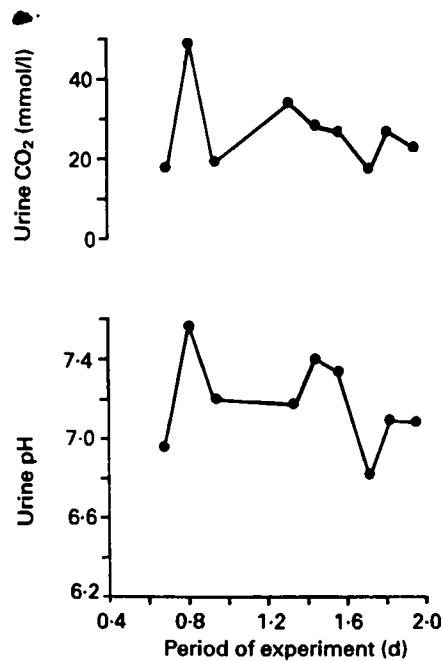


Fig. 9. The daily variation (12–36h) in urine pH (3 h urine samples) in a subject participating in a bicarbonate-infusion study. A constant low-dose infusion of sodium bicarbonate was given between 0 and 36 h.

depend on the differences in specific activity between blood and urine. If no such difference exists, no change in specific activity will occur. If there is a difference, the two compartments would tend to equilibrate. In practice many of the above effects may cancel out, particularly in studies carried out over long periods of time, e.g. 24 h.

The energy equivalent of CO₂

Once the estimated CO₂ production is established, it is necessary to relate it to energy expenditure. Unfortunately the energy equivalent of CO₂ is not constant since it depends on the type of fuels being oxidized. When a glucose polysaccharide is oxidized the following equation applies (Elia & Livesey, 1988):



The respiratory quotient is 1 and the energy equivalent of CO₂ is 21.12 kJ/l (473.3 kJ/mol CO₂). When the triglyceride dioleypalmityl triglyceride is oxidised, the following equation applies (Elia & Livesey, 1988):



The respiratory quotient (RQ) is 0.7097 and the heat equivalent of CO₂ is 27.48 kJ/l CO₂ or 615.9 kJ/mol CO₂. These values are 30% higher than that for polysaccharide oxidation. The energy equivalents of CO₂ production rates over a range of non-protein RQ values, including those above 1, which are associated with net lipid synthesis, are illustrated in Table 1. It is clear that the variation in the non-protein-energy equivalent of CO₂ is greater than the variation in energy equivalents of O₂. Protein oxidation usually

Table 1. *The non-protein energy equivalent of oxygen and carbon dioxide (values from Elia & Livesey, 1988)*

Non-protein respiratory quotient	Heat equivalent (kJ/l)	
	O ₂	CO ₂
0.7097	19.50	27.48
0.75	19.73	26.30
0.80	20.00	25.00
0.85	20.28	23.86
0.90	20.56	22.87
0.95	20.84	21.94
1.00	21.12	21.12
1.05	21.40	20.38
1.10	21.68	19.71

makes little difference to the overall energy equivalents of CO₂, partly because protein oxidation accounts for only a small proportion of total energy expenditure (about 15%) and partly because the value for the energy equivalent of CO₂ for protein oxidation is intermediate between that for fat and carbohydrate oxidation (Livesey & Elia, 1988).

In subjects who are in energy balance over long periods of time, the mean RQ should be close or equal to the food quotient (i.e. the RQ associated with the oxidation of dietary constituents). The food quotient of Western societies and of individual subjects in these societies is about 0.85. The value is often higher in Third World countries where the diet contains a large proportion of carbohydrate. It may, however, vary considerably in subjects who are not in energy balance. For example, during starvation the RQ may be close to 0.70 (Elia *et al.* 1984, 1987) whereas in hospitalized patients receiving a hyperenergetic parenteral nutrition regimen with glucose as the main energy fuel, the RQ may be 1 or more. Furthermore, there may be variation in the RQ of the same subject at different times of the day, e.g. a meal containing 3350 kJ (800 kcal) may increase the RQ by 0.05–0.010, whilst prolonged exercise may be associated with a progressive reduction of RQ as glycogen stores become depleted and fat is used increasingly as an energy source. The variation in the respiratory exchange ratio of a typical subject participating in a 36-h calorimetry study is indicated in Fig. 10. The lowest values were observed during sleep and the highest after meal ingestion and during exercise. In the bicarbonate method it is necessary to estimate the mean RQ which is likely to prevail over the period of study so that an appropriate value for energy expenditure can be calculated (Table 1). If a value for RQ of 0.85 is chosen to calculate energy expenditure in conjunction with the 24 h measurements of specific activity in urine and spot breath samples, the results are 100.8 (SD 5.9) % (range 92–107%) and 98.4 (SD 2.9) % (range 94–101%) respectively of those calculated from gaseous exchange.

Some practical considerations

A disadvantage of the bicarbonate method is that the labelled compound cannot be given orally because of acidity in the stomach which may release gaseous CO₂. This CO₂ may be eructated without entering the central bicarbonate pool. Therefore the labelled compound has to be given intravenously or subcutaneously (or intraperitoneally, as in some animal studies). Although intravenous administration of bicarbonate is not a major difficulty in hospitalized patients, particularly those that are already receiving an intravenous infusion, in free-living subjects it can pose major difficulties. However, a

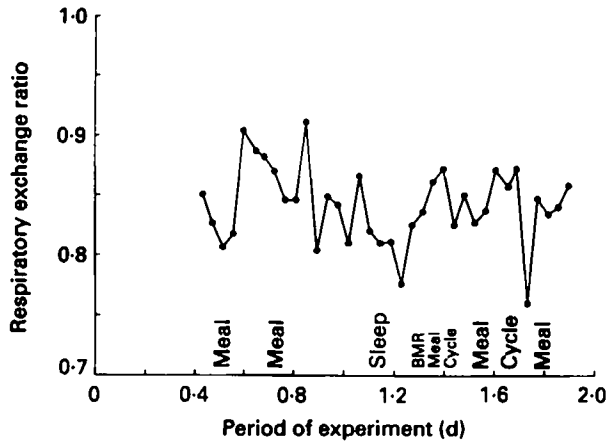


Fig. 10. The change in the respiratory exchange ratio in a subject participating in a bicarbonate-infusion study.

continuous subcutaneous infusion of labelled bicarbonate via a mini pump is feasible since many diabetics use this method for administering insulin to control their blood sugar concentration over 24-h periods.

A second practical consideration concerns sampling. Spot breath, blood, or salivary samples may have to be taken frequently during periods of altered activity, in order to ensure that important changes in CO_2 -specific activity are not overlooked. The sampling procedure may therefore interfere with the normal pattern of daily activities. Urine samples can be more conveniently used for assessing mean specific activities over a period of time but the results in normal subjects may be only approximate for reasons already discussed. A further error may be introduced, especially in short-term studies, because the energy equivalent of CO_2 (Elia & Livesey, 1988) has to be predicted. Nevertheless, in normal subjects, energy expenditure during 24-h periods could be predictable to within about 10%.

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