SHORT COMMUNICATION

Development of a technique for measuring the oxidation rate of a $^{14}$C-labelled substrate from $^{14}$CO$_2$ production without the need for collection of expired air

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Summary

1. A method is proposed for the determination of the rate of oxidation of $^{14}$C-labelled substrates in man from blood sampling only.

2. The method was verified by simulating release of $^{14}$C into the bicarbonate pool of patients by using a constant-rate infusion of sodium $[^{14}\text{C}]$bicarbonate, the plasma level of total $^{14}$CO$_2$ (d.p.m. ml$^{-1}$ 70 kg$^{-1}$) correlating well ($r = 0.878$, $P = 0.001$) with the infusion rate ($\mu$Ci/h).

3. Techniques involved in employing this method are discussed, particularly in the light of errors involved.

Key words: carbon dioxide ($^{14}$CO$_2$), $^{14}$C-labelled substrates, oxidation.

Introduction

$^{14}$C-labelled substrates can be used to increase our knowledge of metabolism in man under various conditions. Oxidation of the substrate will, at some stage, release $^{14}$CO$_2$ into the body's bicarbonate pool from where it is proportionally exhaled, some being retained within a slower-turnover bicarbonate pool (Tolbert, Kirk & Upham, 1959). Careful selection of the site of the label with measurement of the rate of $^{14}$CO$_2$ expiration will permit determination of the oxidation rate of that substrate, after allowing for the proportion of label retained within the slower-turnover bicarbonate pool.

$^{14}$CO$_2$ expiration can be measured by several techniques (Tolbert et al., 1959; Kinney, Morgan, Domingues & Gildner, 1964; Issekutz, Paul, Miller & Bortz, 1968; O'Keefe, Sender & James, 1974; Clague, Keir & Clayton, 1979). However, all necessitate the use of a mouthpiece, hood or tent and these are often poorly tolerated by traumatized or postoperative patients. A technique was therefore developed to enable the oxidation rate of the $^{14}$C-labelled substrate to be determined by blood sampling only.

In man the fraction of a bolus injection of sodium $[^{14}\text{C}]$bicarbonate expired with time fits best to three exponential functions (Tolbert et al., 1959). However, the half-times of the two fastest components are so similar that they can be considered as a single pool during constant-rate infusions. Thus there appears to exist a large rapid-turnover bicarbonate pool, of which plasma constitutes a part, and a smaller, slower-turnover pool. $^{14}$CO$_2$ entering this latter pool can be considered as not re-entering the rapid-turnover pool during a study of a few hours' duration (Issekutz et al., 1968).

This rapid-turnover pool is of a constant size in normal man relative to body weight (Winchell, Stahelin, Kusubov, Slinger, Fish, Pollycove & Lawrence, 1970). It enlarges after major surgery, trauma and sepsis (Long, Spencer, Kinney & Geiger, 1971) but may remain unchanged after uncomplicated elective surgery. Carbon dioxide production in man is also fairly constant in the resting steady state (8--10 mmol/min) but rises slightly after major surgery (Long et al., 1971).
There therefore appears to exist a single relevant bicarbonate pool (the rapid-turnover bicarbonate pool) that remains fairly constant in size with a single input (carbon dioxide production) and two outputs (carbon dioxide expiration and entry into a slower-turnover bicarbonate pool), the input and outputs being equal and constant in the resting steady state. Into this rapidly turning-over pool is introduced the $^{14}$CO$_2$ produced by oxidation of the $^{14}$C-labelled substrate. If the $^{14}$C-labelled substrate is infused at a constant rate the $^{14}$CO$_2$ will pass through the bicarbonate pool, after a period of equilibration, at a constant rate. However, doubling the rate of oxidation of the substrate will not only double the rate of $^{14}$CO$_2$ expiration but will also double the specific radioactivity of $^{14}$CO$_2$ (the concentration of labelled to total carbon dioxide) within the bicarbonate pool, provided that the pool size and carbon dioxide turnover do not change. Also if there is no redistribution of carbon dioxide within the bicarbonate pool the $^{14}$CO$_2$ present in a single portion of that pool must double. It would therefore appear that the radioactivity present in plasma as $^{14}$CO$_2$ should be proportional to the rate of entry of $^{14}$CO$_2$ into the rapid-turnover bicarbonate pool of an individual from oxidation of an infused $^{14}$C-labelled substrate.

**Materials and methods**

To test this concept a total of 19 sodium $^{14}$C bicarbonate infusions were given before and after surgery. The infusion was given as a predetermined priming dose followed by a constant-rate infusion. Preliminary studies indicated that plateau values were obtained in expired air at 30–45 min and so three samples of blood were taken at 60, 75 and 90 min to determine the radioactivity in plasma. No patients displayed evidence of any acid–base imbalance. Sodium $^{14}$C bicarbonate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. (code CFA, 431; specific radioactivity of 0-1 mCi/mmoll) and prepared in batches by Millipore filtration of about 5 μCi portions into 50 ml bottles of sterile sodium chloride solution (150 mmoll/l). The solution was drawn up into a sterile plastic syringe and a measured volume removed in duplicate for liquid scintillation counting to determine the activity of the infusate (μCi/g). No loss of radioactivity occurred provided the solution was not exposed to air. The infusion was given by using a Harvard infusion pump (constant rate ±1%) and the infusion rate determined by subsequent calibration of the pump, syringe and needle (g/h). The rate of entry of $^{14}$C bicarbonate into the rapid-turnover bicarbonate pool could then be accurately calculated (μCi/h).

Blood samples were collected into heparinized tubes without the use of a tourniquet. Tubes were completely filled and firmly capped before storage at 4°C for up to 4 h, no loss of radioactivity occurring under these conditions. The blood was centrifuged and 1 ml portions of plasma were immediately removed on uncapping the tubes and added in duplicate to vials containing 200 μl of ethanolamine/methoxyethanol solution (1:2) and 14-8 ml of NE260 [Nuclear Enterprises Ltd (G.B) Sighthill, Edinburgh, U.K.] and stored at 4°C before counting. Counting was considered accurate to 2%.

Results were expressed as $^{14}$CO$_2$ radioactivity in plasma (d.p.m./ml), the mean of the six measurements being taken for each patient and corrected for varying pool size with body weight. They were then plotted as $^{14}$CO$_2$ radioactivity (d.p.m. ml$^{-1}$ 70 kg$^{-1}$) against the rate of $^{14}$C bicarbonate infusion (μCi/h) and subjected to linear regression analysis.

**Results**

Fig. 1 shows the results. The plasma total $^{14}$CO$_2$ correlated well with the infusion rate although the scatter of results about the line produced a standard deviation of about 15% of the mean. There were insufficient numbers to draw separate conclusions from the pre- and postoperative data.

**Discussion**

The concept put forward that the $^{14}$CO$_2$ radioactivity in 1 ml of plasma is directly proportional...
Measurement of $^{14}$CO$_2$ production with plasma

Measurement of $^{14}$CO$_2$ production with plasma appears to be valid. In the case of a suitably labelled infused substrate, the $^{14}$CO$_2$ input into the pool is a measurement of its oxidation rate. The concept not only obviates the need to collect expired air to determine the oxidation rate but, as it measures entry rates and not exit rates from the rapid-turnover bicarbonate pool, it also does away with the need to allow for loss of label into the slower-turnover bicarbonate pool.

The standard deviation of 15% in the relationship shown in Fig. 1 is clearly too high to permit accurate measurements of substrate oxidation simply from plasma total $^{14}$CO$_2$ radioactivity, even when this is corrected for body weight. Several factors can explain this variability including low count rates, variations in pool size even after adjustment for body weight and variations in carbon dioxide turnover between individuals and within the same individual pre- and post-operatively. In addition there will be errors in the technique of handling.

These errors can be minimized by calibrating each patient with a sodium $[^{14}\text{C}]$bicarbonate infusion immediately before infusion of the $^{14}$C-labelled substrate. The patient receives an infusion of sodium $[^{14}\text{C}]$bicarbonate as above with blood sampling at plateau (60–90 min) and the radioactivity (A d.p.m./ml) correlated with the infusion rate (L μCi/h). The patient is then primed with the $^{14}$C-labelled substrate followed by a constant-rate infusion (M μCi/h). Further blood samples are taken at plateau and the $^{14}$CO$_2$ radioactivity in plasma (B d.p.m./ml) applied to the values obtained during the preceding sodium $[^{14}\text{C}]$bicarbonate infusion. If the turnover (Q mmol/h) of the substrate is known then $^{14}$CO$_2$ production from the $^{14}$C-labelled substrate = $B/A \times L\,\mu\text{Ci}/\text{h}$ and therefore the percentage of the $^{14}$C-labelled substrate oxidized = $B/A \times L/M \times 100\%$ and the oxidation rate of the substrate = $B/A \times L/M \times Q \,\text{mmol/h}$.

The assumption is made that the pool size and carbon dioxide production remain unchanged in the period between establishment of both plateaux. This can be short (90–150 min) as the half-time of the pool is only 35 min and allows for rapid redefining of the plateau. The preliminary sodium $[^{14}\text{C}]$bicarbonate infusion has primed the pool and plateaux can readily be defined if the radioactivity is not rising or falling over three samples (mean ± 8%).

A technique has been described and verified as valid that permits determination of the oxidation rate of a suitably labelled substrate from plasma sampling only, without having to subject an individual to one of the methods used to collect expired air. The technique is relatively simple and accurate enough to produce meaningful results.

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**References**


