technical note

International Journal of Applied Radiation & Isotopes, Vol. 30, pp. 647 to 650

0020-708X/79/1001-0647$02.00/0

Apparent and Actual ^{14}C Retention in the Slower Turnover Bicarbonate Pool in Man When Using Liquid Scintillation Counting

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(*Received* 8 *March* 1979)

The use of liquid scintillation counting to determine $^{14}CO_2$ expiration without correct calibration of the apparatus suggests that about 25% of the label is retained within the slower turnover bicarbonate pool. Calibration reduces this figure to 13% and is in agreement with the figure obtained using a calibrated ionisation chamber. The discrepancy is due to reduction in the specific radioactivity in the vial, the mechanism involved being unknown, but it may be a characteristic of certain liquid scintillators under certain conditions.

Introduction

^{14}C-LABELLED substrates have been used in man for metabolic studies for several decades. Selection of the site of the label can result in the almost direct release of the label, during oxidation, into the bicarbonate pool from where it is proportionately exhaled as $^{14}CO_2$. Measurement of the rate of $^{14}CO_2$ expired after equilibration during a constant rate infusion, with allowance for the label retained within a slower turnover bicarbonate pool, permits determination of the oxidation rate of the infused substrate.

The rate of $^{14}CO_2$ exhalation in man can be determined either by the combination of measurements of CO_2 production and specific radioactivity of $^{14}CO_2$ in expired air by liquid scintillation counting, or simply by the use of a calibrated ionisation chamber. Use of the latter has several drawbacks because of the relatively small dose of ^{14}C-labelled substrate that can be safely administered in man.[1,2] Liquid scintillation counting, on the other hand, is easier to apply in the clinical situation but presents difficulties in accurate calibration.[3,4]

Conflict appears to exist in the figure obtained for the proportion of label retained within the slower turnover bicarbonate pool using each technique. Liquid scintillation counting suggests that 20–30%[3] is retained whereas the result obtained using a calibrated ionisation chamber puts the figure at only 13%.[5]

An apparatus was used, incorporating liquid scintillation counting, to determine the likely retention of ^{14}C within the slower turnover bicarbonate pool and some consideration given to the possible causes of the above suggested discrepancy.

Materials and Methods

The apparatus is depicted diagrammatically in Fig. 1. The patient lies at rest with the upper half of his body enclosed in a transparent tent, breathing room air which enters the tent around its tucked in edges. Air is drawn from the tent at a known constant rate (49.5 l. min^{-1}) and initially passes into a CO_2 analyser (Noyons Diaferometer MG 4, Kipp and Zonen) capable of measuring accurately small changes in the concentration of CO_2 between the diluted expired air and room air (4–5 ml $l.^{-1}$). This permitted calculation of the CO_2 production rate and, by a small modification, the concentration of CO_2 in room air. Most of the air leaving the apparatus is drawn directly through the pump and vented to the exterior of the building to prevent recirculation of ^{14}C-contaminated air. However, about 10 l. passes through either a CO_2 trap or a by-pass adjusted to an identical flow rate (depicted in Fig. 2) so that the CO_2 trap may be brought into circuit without disturbing the flow rate or equilibrium within the tent. Air entering the trap is initially dried by condensation and passage through regenerated silica gel prior to being drawn down a stainless steel tube and bubbled through the CO_2 trapping solution, before finally passing up the outer cylinder to the pump and thence the exterior.

The CO_2 trapping solution, hyamine hydroxide, is contained in a glass scintillation vial which can readily be

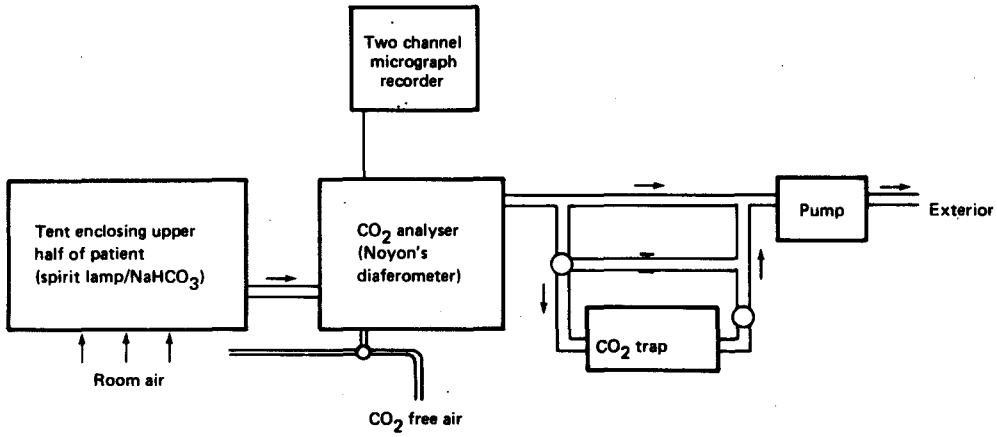

FIG. 1. Diagrammatic representation of the apparatus used to measure $^{14}CO_2$ expiration by patients.

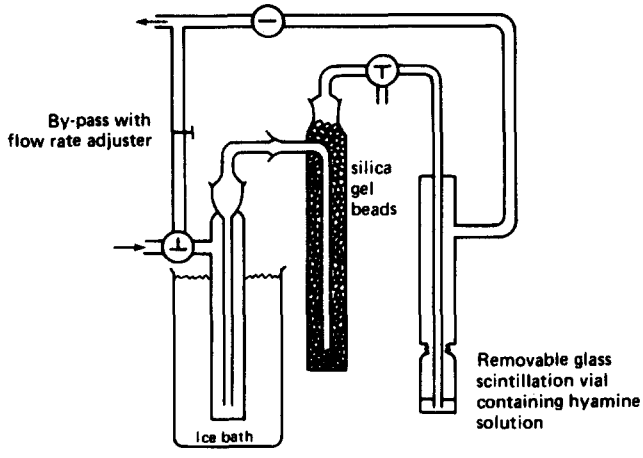

FIG. 2. Details of the $^{14}CO_2$ trap and by-pass.

screwed into and removed from the outer stainless steel cylinder. Ethanol was added to overcome the problem of reduced efficiency of CO_2 collection due to increased viscosity from vaporisation of the solvent. The optimal mixture was found to be 2 ml 1 M hyamine in methanol (scintillation grade—Nuclear Enterprises Ltd), 3 ml ethanol (A.R. quality—99.8%) with 2 drops of 1% solution of phenolphthalein in propan-2-ol as an indicator. Trapping of CO_2 in the hyamine solution until the indicator changed colour was carried out with simultaneous measurement of CO_2 production and room CO_2. The vial was then removed and the stainless steel tube flushed with 2.5 ml ethanol to remove any residual hyamine solution, and this together with 10 ml liquid scintillator NE 233 (Nuclear Enterprises Ltd) was added immediately to the vial. This was capped and stored at 4°C until counted on a Packard Tri-carb scintillation counter. The molarity of the hyamine solution was determined by titration against 0.1 N HCl (BDH Chemicals Ltd).

$^{14}CO_2$ exhalation could be calculated, allowance being made in the calculations for the room CO_2 trapped by the hyamine solution.

Calibration and Experiment

The absolute accuracy of CO_2 concentration measurement using calibration tables supplied with the analyser is claimed by the manufacturers to be $\pm 2\%$. The calibration factors were verified with an experimental accuracy of not better than $\pm 3.5\%$ by supplying CO_2 (99.98%—Distillers Company) to the tent at a measured rate (rotameter $\pm 3\%$), producing a readily calculable concentration of CO_2 in the tent, and by measurement of the respiratory quotient of ethanol burnt in a spirit lamp in the tent. Liquid scintillation counting was carried out until at least 10^4 counts had been accumulated. The efficiency of counting was determined using the internal standard method. Counting accuracy was estimated to be $\pm 2\%$.

The ability of the apparatus to determine the rate of $^{14}CO_2$ production within the tent was assessed in two ways. [1-^{14}C]Ethanol (code CFA, 44, The Radiochemical Centre, Amersham—specific radioactivity 27.1 mCi mmol^{-1} diluted to 1.88 nCi mmol^{-1}) was burnt in a spirit lamp inside the tent and the calculated and measured $^{14}CO_2$ production rates determined after the air in the tent had equilibrated (about 20 min). Similarly, the calculated and measured $^{14}CO_2$ production rates were determined when hydrochloric acid of known molarity (BDH Chemicals Ltd) was infused, at a known constant rate, into a solution of $NaH^{14}CO_3$ (code CFA, 431, The Radiochemical Centre, Amersham—specific radioactivity 0.1 mCi mmol^{-1} diluted to 410 pCi mmol^{-1}). The CO_2 production rate was adjusted in each case to produce 6–8.5 mmol min^{-1} at a specific radioactivity of 340–810 pCi mmol^{-1}, equivalent to values expected in expired air on infusing 2.25–9.0 nCi min^{-1} of $NaH^{14}CO_3$ into patients.

Five patients were then infused with a solution of normal saline containing $NaH^{14}CO_3$ (specific radioactivity 0.1 mCi mmol^{-1}) to a total dose not exceeding 5 μCi. The estimated average absorbed radiation dose to the whole body was less than 0.02 mrad. Approval was granted by the Isotope Advisory Panel and the local ethical committee. Informed consent was obtained from each patient. A priming dose was given followed by a constant rate infusion of the remaining solution, 9–27 nCi min^{-1}, over a period of 90–120 min using a Harvard infusion pump (rate constant to $\pm 1\%$). Plateau values for $^{14}CO_2$ expiration within the tent were achieved within 45–60 min. Measurements after this time permitted calculation of the percentage of the infused $NaH^{14}CO_3$ that is expired as $^{14}CO_2$ per unit time.

Results

Table 1 shows the results obtained with the release of $^{14}CO_2$ by titrating hydrochloric acid onto $NaH^{14}CO_3$ within the tent. Only 87% of the calculated $^{14}CO_2$ released by the reaction can be accounted for by measurement

TABLE 1. Calibration of the apparatus by infusing hydrochloric acid on to $NaH^{14}CO_3$, showing a highly significant ($p > 0.001$) reduction in the measured compared with the calculated $^{14}CO_2$ production due to reduction in the measured specific radioactivity

	Calculated	Measured
Specific radioactivity (pCi mmol^{-1})	393±1 (n = 2)	347±5 * (n = 8)
CO_2 production (mmol min^{-1})	8.20±0.01 (n = 2)	8.27±0.16 (n = 6)
$^{14}CO_2$ production (nCi min^{-1})	3.32±0.01 (n = 2)	2.88±0.07* (n = 6)

* p value < 0.001 Mean ± S D

TABLE 2. The measured percentage recovery of $^{14}CO_2$ for both methods of calibration and patients, and the corrected percentage $^{14}CO_2$ expired by patients

% recovery of $^{14}CO_2$	[1–^{14}C] ethanol	$NaH^{14}CO_3$	Patients
Measured	87.4 ± 3.5 (n = 9)	86.8 ± 2.0 (n = 6)	
	87.2 ± 2.9 (n = 15)		75.4 ± 2.1* (n = 5)
Corrected	100		86.5

* p value <0.001 Mean ± S D

using this apparatus. There was no significant difference between the calculated and measured CO_2 production rates, but there was a highly significant reduction in the measured compared with the calculated specific radioactivity of the $^{14}CO_2$. The same result was obtained when $^{14}CO_2$ was produced by burning [1-^{14}C]ethanol.

Table 2 shows the measured recovery of $^{14}CO_2$ expressed as a percentage of the calculated production rate of $^{14}CO_2$, by burning [1-^{14}C]ethanol, titrating acid on to $NaH^{14}CO_3$ and on infusing $NaH^{14}CO_3$ into patients. There was a highly significant reduction in the recovery of $^{14}CO_2$ from patients (75.4%) compared with the other two methods (87.2%). If these two methods used to calibrate the apparatus are corrected to 100%, then the patients expired 86.5% of the infused label.

Discussion

The use of liquid scintillation counting, by ourselves and others,[3] initially suggests that about 25% of the ^{14}C label is retained within the slower turnover bicarbonate pool. Accurate calibration confirms that only 13% is retained, a figure in agreement with results from a calibrated ionisation chamber.[5] The discrepancy lies in the measurement of specific radioactivity using liquid scintillation counting, the value being 12% lower than anticipated on calibration.

Assuming that the label cannot be destroyed and that the hyamine hydroxide is unable to discriminate between the labelled and unlabelled CO_2, the reduced specific radioactivity must be due to a distortion of the counting characteristics, or to there being too few counts in the solution in the vial at the time of counting. Bicarbonate adheres to glass and this could produce a distortion of the counting characteristics with uneven distribution of the label within the vial. However, this was discounted as addition of more carrier bicarbonate to glass vials and the use of plastic vials produced the same result.

Too few counts within the solution in the vial could be due to loss of some hyamine hydroxide during the bubbling of expired air through the solution, failure of CO_2 at the concentration in which it was present to form a bicarbonate with hyamine hydroxide, instability of the bicarbonate with immediate loss of CO_2 from the vial on adding the ethanol or liquid scintillator, or a slow loss of CO_2 into the atmosphere above the solution after the vial is capped. Compounds allied to hyamine hydroxide have an extremely low vapour pressure at room temperature (1 mm Hg). Bubbling 30 l. of nitrogen through hyamine hydroxide solution as prepared for trapping CO_2 produced a constant but only 2% decrease in the end point on titration with acid. This, however, was consistent with contamination of the nitrogen with carbon dioxide to the extent of only one part in 30,000. Placing the solution in a constant volume flask and observing the pressure changes on reaction with CO_2 and addition of ethanol and liquid scintillator in the concentrations used demonstrated that CO_2 and hyamine hydroxide reacted in equal molarity to form the bicarbonate which was stable to the addition of ethanol and the liquid scintillator.

Experiments at the Radiochemical Centre, Amersham (Radiochemical batch analysis sheet for $NaH^{14}CO_3$) and several other workers[6–8] have shown an appreciable (10–30%) and variable loss of activity on counting [^{14}C]bicarbonate unless alkali is added to the vial as an initial procedure. This is due to loss of the ^{14}C label as $^{14}CO_2$ into the atmosphere above the solution within the vial and may be even greater if the volume above the solution is increased, the $^{14}CO_2$ is removed from the atmosphere, or $^{14}CO_2$ is allowed to leak out of the vial due to an insecure cap or the use of plastic vials.[6–8] We are unable to add extra alkali as an initial procedure as this would distort our end-point. Addition of alkali at various stages during the preparation of the vial for counting, but after the CO_2 collection, did not prevent the reduction in specific radioactivity. The specific radioactivity in our vials was low (400 pCi $mmol^{-1}$) and more than three orders of magnitude less than those reported as giving rise to significant loss. It is well within the range of specific radioactivity suggested by the Radiochemical Centre, Amersham (Radiochemical batch analysis sheet for $NaH^{14}CO_3$) as sufficiently low to prevent any loss. To reduce the specific radioactivity within our vials by 12% would mean a loss of 5.0–5.5 ml CO_2 from the solution into the 7 ml atmosphere above the solution assuming the cap to be secure. Although this could be feasible the indicator in the solution did not revert to its original colour as would have been expected with reversal of the characteristics of hyamine hydroxide neutralisation by CO_2,[4] and venting of the vials for up to 2 h did not alter the activity in solution in the vials.

Recently it has been suggested that reduction in activity may occur in a solution in a glass vial even in the presence of excess phenylethylamine (11–16% with NE 250) and may be a characteristic of certain liquid scintillators.[8] NE 233 was not used in that publication. Use of two other liquid scintillators with organic bases (Unisolve 1 and Lipa luma) and a new batch of NE 233 failed to show any reduction in the specific radioactivity on recalibrating the apparatus at a later date using [1-^{14}C]ethanol. NE 233 and NE 250, along with some laboratory made scintillators, may under certain circumstances cause a reduction in specific radioactivity on counting [^{14}C]bicarbonate solutions even after all steps have been taken to minimise losses due to other known mechanisms. The mechanism for the reduction in specific radioactivity described here is not known but it is likely to lie in a common component of each of the incriminated liquid scintillators.

In conclusion, it may be stated that with the same materials and technique the reduction in specific radioactivity appears to be fairly constant. Whatever the explanation for this reduction, accurate calibration of apparatus and techniques incorporating liquid scintillation counting will convert an apparent 20–30% retention of ^{14}C within the slower turnover bicarbonate pool to an actual 13% retention.

Acknowledgements—We wish to thank Mr I. Smeaton of the Pharmacy Department for assistance in preparation of the solutions, and members of the Department of Bacteriology, Royal Victoria Infirmary, Newcastle-upon-Tyne, for sterility testing of the solutions.

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