Equilibration of metabolic CO_2 with preformed CO_2 and bicarbonate

An unexpected finding

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Entry of metabolic $^{14}CO_2$ into urea is shown to occur more readily than it equilibrates with the general pool of cellular plus extracellular bicarbonate plus CO_2. Since the sites of CO_2 production (pyruvate dehydrogenase and oxoglutarate dehydrogenase) and of fixation (carbamoylphosphate synthetase) are intramitochondrial, it is likely that the fixation of CO_2 is also more rapid than its equilibration with the cytoplasmic pool of bicarbonate plus CO_2. This observation may point to a more general problem concerning the interpretation of isotope data, when compartmentation or proximity of sites of production and utilisation of metabolites may result in the isotope following a preferred pathway.

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1. INTRODUCTION

When measuring metabolic CO_2 in experiments using ^{14}C-labelled substrates it is generally assumed that this intermediate is freely diffusible. However, in perfused rat liver the rates of fixation of ^{14}C, from $NaH^{14}CO_3$ added to the perfusate, into non acid-volatile compounds, is from 3–70-times greater than the net rates of CO_2 production [1]. This is taken to indicate that calculation of metabolic rates from $^{14}CO_2$ production can give substantial underestimates, up to 50%. Interpretation of data obtained in experiments using radioactive tracers requires careful consideration of all the factors involved. Instances where misinterpretation of such data has occurred are numerous and examples have been presented [2].

This paper reports experiments which show that, unexpectedly, metabolic CO_2 enters into urea synthesis more readily than it equilibrates with the general CO_2 plus HCO_3^- pool. The findings raise general questions concerning the interpretation of radioactivity data.

2. METHODS

Isolated hepatocytes were prepared from 48 h starved rats of the Wistar strain by the method in [3], with the modifications in [4]. Hyaluronidase was omitted from the perfusion medium.

Hepatocytes in 4 ml Krebs Henseleit bicarbonate saline [5] were incubated in 25 ml conical flasks fitted with a centre-well and closed with a Suba-Seal stopper. The gas space contained 95% O_2:5% CO_2.

At the end of the incubation the total bicarbonate plus CO_2 was collected by injecting 1 ml 1 N NaOH into the centre-well and 0.4 ml 20%

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(v/v) perchloric acid into the main compartment. Shaking was continued for a further 3 h to ensure complete transfer of the liberated CO_2.

The amount of CO_2 bound by the NaOH was determined, in a sample, by manometric measurement [6]. A correction was made for the carbonate contaminating the NaOH. Radioactivity in the NaOH was measured by liquid scintillation counting. From these values the specific activity (dpm/μmol) was calculated.

The urea content of the neutralised perchloric acid extract was measured as in [6]. Radioactivity in urea was measured after conversion to $^{14}CO_2$ with urease in a suba-sealed conical flask under the conditions given for the urea assay. The resulting $^{14}CO_2$ was transferred into NaOH after acidification as before. The urease used was grade VI obtained from Sigma (Poole, Dorset). The specific activity of CO_2 in the gas space at the end of the incubation was measured in a sample of the gas (~10 ml) removed with a hypodermic syringe and injected into a suba-sealed flask containing NaOH. The amount of CO_2 and radioactivity in this sample was determined as above.

3. RESULTS

The results in table 1 show that the specific activities of urea derived from [1-^{14}C]alanine, [1-^{14}C]glutamine or from endogenous ammonia in the presence of [1-^{14}C]lactate were significantly higher than the specific activity of the bicarbonate plus CO_2 pool. These findings were unaffected by the addition of carbonic anhydrase to the incubation medium. Furthermore, measurement of the specific activity of the CO_2 contained in the gas

Table 1

Comparison of the specific activities of urea and 'bicarbonate' formed from [1-^{14}C]lactate, [1-^{14}C]alanine and [1-^{14}C]glutamine, in hepatocytes from 48 h starved rats

Substrate added	Lactate	Alanine	Glutamine
Urea formed (μmol/min/g)	0.107 ± 0.06	0.75 ± 0.20	1.00
Spec. act. urea	8898 ± 1454	2431 ± 563	264
Spec. act. 'bicarbonate'	1914 ± 466	1624 ± 54	217
Spec. act. urea/spec. act. 'bicarbonate'	4.64	1.50	1.22

Each flask contained ~80 mg fresh wt hepatocytes in 4 ml Krebs–Henseleit saline. The substrates were 5 mM with 0.4 μCi radioactivity. Flasks were incubated in duplicate for 60 min at 38°C. Values are means ± SD for 3 expt. Glutamine values are for a single experiment. Specific activities are expressed as dpm/μmol. 'Bicarbonate' includes both dissolved and gaseous CO_2

Table 2

A time course of the entry of ^{14}C into urea and 'bicarbonate' from [1-^{14}C]alanine in isolated hepatocytes from 48 h starved rats

	Incubation (min)		
	20	40	60
% Total dpm found in 'bicarbonate'	5.46	10.3	16.8
Spec. act. urea	2108	1528	1072
Spec. act. 'bicarbonate'	356	650	1014
Spec. act. urea/spec. act. 'bicarbonate'	5.92	2.36	1.06
Urea formed (μmol/flask)	0.90	2.01	3.65

Each flash contained 75 mg fresh wt hepatocytes in 4 ml Krebs–Henseleit saline, 0.4 μCi [1-^{14}C]alanine and alanine to 5 mM final conc. Flasks were incubated in duplicate at 38°C. 'Bicarbonate' includes CO_2 in both the medium and the gas phase. Specific activities are expressed as dpm/μmol. Values are means of duplicates

space showed it to be in isotopic equilibrium with the bulk of the remainder of the HCO_3^- plus CO_2 pool. When lactate was the substrate and the rate of urea synthesis was low, the discrepancy between the specific activities of urea and CO_2 plus HCO_3^- was greater than when [1-^{14}C]alanine was the substrate, at which time the rate of urea synthesis was high. A time course (table 2) showed that the observed discrepancy was more marked during the early part of the incubation. As expected, the specific activity of the CO_2 plus bicarbonate rose during the course of the incubation as the substrate was metabolized.

4. DISCUSSION

The results show that the specific activities of the intracellular CO_2 and bicarbonate are higher than the corresponding specific activities in the extracellular medium and gas phase and that the difference is greater in the early stages of the incubation. The fact that the site of generation of $^{14}CO_2$ (pyruvate dehydrogenase) and its utilization as $H^{14}CO_3^-$ (carbamoyl phosphate synthetase) are intramitochondrial implies that the specific activity of the intramitochondrial bicarbonate may be even higher than that of the cytosolic bicarbonate and CO_2. If this is true of a component as freely diffusible as CO_2, assumptions concerning other 'freely diffusible' metabolites may be equally erroneous. Carbon dioxide generated in the mitochondria is likely to be in isotopic equilibrium with bicarbonate since carbonic anhydrase has been shown to be present in the mitochondrial space [7]. It is known that HCO_3^- does not cross the mitochondrial membrane at a significant rate [8] but CO_2 passes through all membranes readily because of its lipid solubility [9,10]. This may contribute to the present observations if $^{14}CO_2$ undergoes rapid hydration at the site of formation.

The observation that the discrepancy in the specific activity of urea was larger when lactate was the substrate and urea formation was at the endogenous rate, is taken to indicate that the HCO_3^- derived from lactate provides a greater portion of the HCO_3^- required for carbamoyl phosphate synthesis than when the rate of urea formation is high, relative to the rate of pyruvate dehydrogenase, as is the case when alanine is the substrate.

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