Underestimation of metabolic rates owing to reincorporation of $^{14}$CO$_2$ in the perfused rat liver

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$^{14}$CO$_2$ production by perfused rat livers was simulated by infusing NaH$^{14}$CO$_3$ into the perfusate. Recovery of label as $^{14}$CO$_2$ gas + perfusate bicarbonate was 45–85%. Rates of $^{14}$CO$_2$ exchange in the liver are 3–70 times greater than net rates of CO$_2$ production. Therefore $^{14}$CO$_2$ reincorporation can lead to significant underestimations of rates of oxidation of $^{14}$C-labelled substrates in liver.

Metabolic rates measured in a variety of experimental models are frequently expressed as rates of oxidation of $^{14}$C-labelled substrates to $^{14}$CO$_2$. Although it is well known that $^{14}$CO$_2$ can be reincorporated by exchange reactions, it is generally assumed that these processes are quantitatively negligible. As far as we know, the extent to which $^{14}$CO$_2$ exchange in the liver might underestimate rates of substrate oxidation has not been evaluated. We have investigated this question by using rat liver perfusions. Production of $^{14}$CO$_2$ was simulated by a constant infusion of a tracer of $[^{14}$C]bicarbonate. We report that a substantial fraction of $^{14}$CO$_2$ is converted in the liver into compounds non-volatile in acid. This suggests that many reported metabolic rates calculated from the production of $^{14}$CO$_2$ are, in fact, underestimated.

Experimental
Liver perfusions
Livers from schedule-fed Sprague–Dawley rats were perfused with recirculating buffer (Krebs & Henseleit, 1932) containing 4% dialysed bovine serum albumin and glucose (15 mM or 4 mM in perfusions of livers from fed or starved rats respectively). The surgical technique and the perfusion apparatus have been described previously (Brunengraber et al., 1973). Where indicated, a 1 mM concentration of oleate was maintained by a primed-constant infusion (Brunengraber et al., 1978).

After an equilibrium period of 30 min, NaH$^{14}$CO$_3$ was infused into the effluent line of the perfusion reservoir at a rate of $2.5 \times 10^3$ d.p.m./min for 90 min. The effluent CO$_2$ from the oxygenator and the CO$_2$ present in the perfusate bicarbonate pool at 120 min were trapped as described previously (Endemann et al., 1982). The flow rate of the gas mixture (O$_2$/CO$_2$, 19:1) passing through the oxygenator was kept at 150 ml/min in all experiments to avoid variations in the rate of CO$_2$ exchange. During the experiment, duplicate samples of perfusate (0.25 ml) were taken at 5 min or 10 min intervals for counting of radioactivity. One set of samples was counted without processing. The second set of samples was incubated with one drop of acetic acid for 30 min to eliminate $^{14}$CO$_2$ before adding the counting fluid. The rate of NaH$^{14}$CO$_3$ infusion was measured at the end of each experiment. In control experiments, livers were perfused for 30 min and were then removed from the apparatus before starting the infusion of NaH$^{14}$CO$_3$.

Liver glycogen was isolated after alkaline digestion (Good et al., 1933) and counted for radioactivity. Labelling of perfusate glucose was measured as described by Mallette et al. (1969). Counting efficiencies for all samples were determined by recounting the radioactivity of each vial after addition of an internal standard of $[^{14}$C]toluene.

Tracer kinetics
In control experiments without livers, the rate of $^{14}$CO$_2$ exchange in the oxygenator was calculated from the profile of the specific radioactivity of bicarbonate in the perfusate, by using the following reasoning. It is assumed that, in the perfusate, bicarbonate and CO$_2$ behave as a single pool. As a tracer of H$^{14}$CO$_3^-$ is continuously infused at a rate of 1 d.p.m./min into the perfusate, the radioactivity

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present in the bicarbonate pool, $A(t)$, increases with
time in relation to its specific radioactivity, $S(t)$:

$$\frac{dA(t)}{dt} = I - kS(t) = I - \frac{kA(t)}{M}$$

(1)

where $k$ is the rate of CO$_2$ exchange in the oxy-
genator ($\mu$mol/min) and $M$ is the constant total
amount of CO$_2$ + HCO$_3^-$ present in the perfusate
($\mu$mol). Integration of this expression and division
by $M$ yield the specific radioactivity of the bicarbon-
ate pool:

$$S(t) = \frac{I}{k} \left(1 - e^{-kt/M}\right)$$

(2)

Experimental values of $S(t)$ were fitted to a general-
ization of eqn. (2) which permitted a non-zero inter-
cept, by using the non-linear regression procedure
NLIN of SAS Computer System (Hellwig &
Council, 1979) running on an IBM 370/168
computer.

The use of eqn. (2) to analyse data from experi-
ments conducted in the presence of a liver assumes
that constant $k$ combines all processes of CO$_2$
exchange (in the oxygenator and in the liver). The
model fitted the data well in general [the standard
error of fit was within experimental errors of $S(t)$;
there was no pattern in the residuals], allowing the
use of the extrapolated plateau $[S(t) \text{ for } t = \infty]$ in
later calculations. However, parameter identification
with eqn. (2) where two independent estimates of $k$
are possible suggests that this model is too simple
and the data are too few to describe precisely the
rise of $S(t)$ to a plateau. As discussed below, the
rate of $14$CO$_2$ fixation by the liver increases during
the course of the perfusion.

### Results

In model perfusions without liver, the total radio-
activity recovered in the effluent gas of the oxygen-
ator and in the perfusate bicarbonate at 120 min
was 95% of the label infused (Table 1). The rate of
CO$_2$ exchange in the oxygenator, calculated from
the extrapolated specific radioactivity of bicarbonate
in the perfusate $[S(t) \text{ for } t = \infty]$ by using eqn. (2),
was $40 \pm 8 \mu$mol/min (mean $\pm$ S.E.M.; $n = 5$). In
the presence of a liver, recovery of $14$CO$_2$ varied from
45 to 85% depending on the metabolic status of the
liver and the substrate present in the perfusate. In
other words, 10–50% of the label infused was incor-
porated into compounds non-volatile in acid.
(Note that all calculations take into account the
recovery of label in control experiments.) Incorpora-
tion of $14$CO$_2$ was significantly increased by starva-
tion and decreased by diabetes. Ethanol and oleate
significantly decreased the incorporation of $14$CO$_2$
in livers from starved, but not from fed, rats. The
specific radioactivity of bicarbonate in the perfusate
(Fig. 1) varied roughly in parallel with the percent-
age recovery of $14$CO$_2$.

Apparent total rates of CO$_2$ fixation were cal-
culated by two methods. First, the rate of label in-
fusion was divided by the extrapolated specific
radioactivity of perfusate bicarbonate $[S(t) \text{ for } t = \infty]$ to yield turnover rates that comprise $14$CO$_2$
exchange in both the oxygenator and in the liver.
The rate of $14$CO$_2$ exchange in the oxygenator was
deducted from the latter turnover rates, yielding
rates of $14$CO$_2$ fixation at equilibrium in the liver
(Method A, Table 1). Second, the total H$14$CO$_3^-$
incorporated (d.p.m.) was divided by the integrated
specific radioactivity of bicarbonate in the perfusate,
yielding an average rate of $14$CO$_2$ fixation over the

### Table 1. $14$CO$_2$ fixation in the isolated perfused rat liver

The data are presented as means $\pm$ S.E.M. for five observations in each group. *Recovery of $14$CO$_2$ is the percentage of label infused recovered as the sum of CO$_2$ evolved during the experiment and perfusate bicarbonate plus CO$_2$ at 12 min. Method A is based on the turnover rate of perfusate H$14$CO$_3^-$ and Method B on the total radioactivity (d.p.m.) incorporated during 90 min (see the Experimental and Results sections).

<table>
<thead>
<tr>
<th>Group</th>
<th>Recovery of $14$CO$_2$ (%)</th>
<th>Apparent total CO$_2$ fixation ($\mu$mol/min per g dry wt.)</th>
<th>Urea synthesis ($\mu$mol/min per g dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method A</td>
<td>Method B</td>
</tr>
<tr>
<td>Model without liver</td>
<td>95.0 ± 1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fed control</td>
<td>63.1 ± 3.9</td>
<td>53.5 ± 12</td>
<td>31.3 ± 6.0</td>
</tr>
<tr>
<td>Fed + ethanol</td>
<td>67.2 ± 4.4</td>
<td>86.4 ± 13</td>
<td>36.4 ± 7.2</td>
</tr>
<tr>
<td>Fed + oleate</td>
<td>68.0 ± 6.9</td>
<td>21.3 ± 1.8*</td>
<td>21.2 ± 5.6</td>
</tr>
<tr>
<td>Starved control</td>
<td>45.2 ± 4.7</td>
<td>63.9 ± 9.3</td>
<td>29.9 ± 5.2</td>
</tr>
<tr>
<td>Starved + ethanol</td>
<td>72.5 ± 3.9†</td>
<td>42.4 ± 4.0</td>
<td>22.3 ± 3.5</td>
</tr>
<tr>
<td>Starved + oleate</td>
<td>66.9 ± 4.2†</td>
<td>25.4 ± 8.2†</td>
<td>12.2 ± 4.3†</td>
</tr>
<tr>
<td>Fed diabetic‡</td>
<td>84.5 ± 5.5*</td>
<td>29.5 ± 1.5</td>
<td>8.5 ± 3.9</td>
</tr>
</tbody>
</table>

* Differs significantly from fed control ($P < 0.05$, by two-sided t test).
† Differs significantly from starved control ($P < 0.05$, by two-sided t test).
‡ Diabetes was induced by streptozotocin (65 mg/kg) 2 weeks before the experiment.
carboxylic acid cycle. Lastly, $^{14}$CO$_2$ can be incorporated by exchange via the reversal of reactions catalysed by phosphoenolpyruvate carboxykinase and malic enzyme (Chang et al., 1966; Utter & Wood, 1951; Hsu, 1970). Mühlofer et al. (1977) have shown that, in rat livers perfused in open circuit with $[^{14}$C]bicarbonate, isotopic equilibrium is reached within minutes between perfusate bicarbonate, tricarboxylic acid-cycle intermediates and glucose.

Our data show that processes by which $^{14}$CO$_2$, or its equivalent $^{14}$CO$_3^-$, is incorporated by the liver into compounds non-volatile in acid are significant and vary with the metabolic status of the organ. Comparison between the rate of $^{14}$CO$_2$ fixation at equilibrium calculated from the extrapolated specific radioactivity of perfusate bicarbonate (Table 1, Method A) and the integrated rate of $^{14}$CO$_2$ fixation (Method B) reveals that $^{14}$CO$_2$ fixation increases with time under our experimental conditions. This may be accounted for in part by the increasing accumulation of pyruvate and malate, the substrates of pyruvate carboxylase and malic enzyme. The non-steady-state character of $^{14}$CO$_2$ fixation probably explains the lack of agreement between the two estimates of $k$ that come from the fitting of eqn. (2) (in which $k$ would include a term of $^{14}$CO$_2$ fixation by the liver).

The rates of $^{14}$CO$_2$ fixation are much larger than the net rates of CO$_2$ production by the liver. The range of oxygen uptake by perfused livers from normal rats is 6–10 μmol/min per g dry wt. (Williamson et al., 1969; Brunengraber et al., 1973). Assuming a respiratory quotient (RQ) of 0.7 for livers perfused without ethanol (Forsander, 1968), this amounts to a net production of 4.2–7.0 μmol of CO$_2$/min per g dry wt. Comparing these values with the apparent total rates of $^{14}$CO$_2$ exchange (Table 1, Method A), it appears that the rate of $^{14}$CO$_2$ exchange in the perfused liver is 3–15 times the net rate of CO$_2$ production. In the presence of ethanol, the rate of oxygen uptake by the perfused liver is not altered, but the RQ is markedly decreased to almost zero in livers from starved rats (Forsander, 1968). If the RQ is 0.1, this would result in a rate of $^{14}$CO$_2$ exchange in livers from starved rats perfused with ethanol about 70 times the net rate of CO$_2$ production.

There is good evidence in the literature to ascribe the bulk of $^{14}$CO$_2$ exchange in the perfused liver to the reversal of reactions catalysed by malic enzyme and phosphoenolpyruvate carboxykinase. Hsu (1970) has shown that the rate of malate formation by pigeon liver malic enzyme is about 40% of the rate of pyruvate production. Chang et al. (1966) have reported that the relative rates of carboxylation, decarboxylation and oxaloacetate–$^{14}$CO$_3^-$ exchange by pig liver phosphoenolpyruvate carb-
oxykinase are in the proportions 1.0:11.3:34 respectively. Rognstad (1981) has shown that, in hepatocytes converting lactate into glucose in the presence of H\(^{14}\)CO\(_3\)\(^{-}\), activation of phosphoenolpyruvate carboxykinase by Mn\(^{2+}\) (Colombo et al., 1978; Brinkworth et al., 1981) increases labelling but not production of glucose.

One can question the validity of using a tracer of exogenous \([^{14}\text{C}]\)bicarbonate to estimate the rate of reincorporation of \(^{14}\text{CO}_2\) generated inside the cell. In another study (S. B. Weinstock, R. R. Kopito & H. Brunengraber, unpublished work), we found that, in two livers from fed rats perfused with 40 \(\mu\text{M} R\)-\[^{1-14}\text{C} \) mevalonate, the production of \(^{14}\text{CO}_2\) was 58% and 60% of the amount of label taken up by the liver. In the present study, the corresponding recovery of \(^{14}\text{C}\) bicarbonate label was 63 ± 3.9% (Table 1). As C-1 of R-\[^{1-14}\text{C} \) mevalonate is quantitatively released as CO\(_2\) by pyrophosphomevalonate decarboxylase, we conclude that, as far as recovery of label is concerned, a tracer of \(^{14}\text{C}\) bicarbonate reflects the fate of intracellularly generated \(^{14}\text{CO}_2\).

Our data show that not taking into account the processes of \(^{14}\text{CO}_2\) incorporation in the perfused liver can underestimate rates of oxidation of \(^{14}\text{C}\)-labelled substrates by up to 50%. Proper evaluation of \(^{14}\text{CO}_2\) production from a \(^{14}\text{C}\)-labelled substrate requires duplicate experiments with unlabelled substrate and a tracer of \(^{14}\text{C}\) bicarbonate to assess reincorporation of labelled CO\(_2\).

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References


