EXCRETION OF RADIOACTIVE CARBON DIOXIDE BY RATS AFTER ADMINISTRATION OF ISOTOPIC BICARBONATE, ACETATE, AND SUCCINATE*

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The rate of excretion of C\textsubscript{14}O\textsubscript{2} has been followed after the intraperitoneal injection into rats of (1) isotopic bicarbonate, NaHCO\textsubscript{14}O\textsubscript{3}, (2) isotopic acetate, CH\textsubscript{3}C\textsubscript{14}OONa, and (3) isotopic succinate, NaOOCCH\textsubscript{2}CH\textsubscript{2}C\textsubscript{14}OONa. The data so obtained yield information on the rate at which CO\textsubscript{2} participates in the metabolic processes of the body, as well as the rate at which isotopic carbon is eliminated following its administration in the three chemical forms.

Previous measurements of excretion of isotopic CO\textsubscript{2} reported in the literature have been few and, in these few, the experiments were not designed to determine the rates of CO\textsubscript{2} excretion following the administration of a single dose of isotopic material (1-3). In the present experiments, the excretion of C\textsubscript{14}O\textsubscript{2} and total CO\textsubscript{2} has been measured at frequent intervals up to 4 hours.

EXPERIMENTAL

White rats weighing 120 to 140 gm. were fasted for 24 hours prior to the experiment, given the isotopic material in about 1 ml. of solution by intraperitoneal injection, and placed in a metabolism chamber for collection of respiratory CO\textsubscript{2}.

Isotopic Solutions Used—Isotopic NaHCO\textsubscript{14}O\textsubscript{3} was prepared from a solution of isotopic Na\textsubscript{2}C\textsubscript{14}O\textsubscript{3} diluted with m/15 phosphate buffer, pH = 7.4. The final volume was approximately 1 ml. An aliquot of 35 c.mm. was removed for determination of the radioactivity of the solution and the remainder was injected intraperitoneally into the rat. The total radioactivity injected amounted to 2.5 to 5 \times 10\textsuperscript{5} counts per minute per rat. The total amount of CO\textsubscript{2} injected was 0.025 to 0.05 mm.

Isotopic CH\textsubscript{3}C\textsubscript{14}OOH was prepared\textsuperscript{1} according to the method of Sakami, Evans, and Gurin (4). The acetate solutions injected intraperitoneally

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Radioactive Carbon Dioxide Excretion

The amount of C\textsuperscript{14} in each experiment consisted of the amount of C\textsuperscript{14} injected at zero time and the amount of C\textsuperscript{14} and the total CO\textsubscript{2} excreted in each sample. The amount of C\textsuperscript{14} per millimole of total CO\textsubscript{2} is the specific activity of the expired C\textsuperscript{14}O\textsubscript{2}. The specific activities, adjusted to a uniform
initial injection of 100,000 counts per minute, have been plotted logarithmically against time in Fig. 1.

The data obtained in three bicarbonate experiments are individually shown and a smooth curve drawn through the points. The curve is linear for about 1 hour with a slope indicating a 50 per cent reduction in specific activity every 15 minutes. The decrease in specific activity during this period is interpreted as primarily a dilution of the isotopic CO₂ of the body fluids with non-isotopic CO₂ of metabolic processes. CO₂ incorporation in organic reactions is doubtless also occurring during this period, but the effect of these reactions on the specific activity of the inorganic C¹⁴O₂ is small compared with the effect of dilution with non-isotopic CO₂.

After an hour, the rate of decrease in specific activity becomes progressively slower, requiring at the end of the experiment more than 100 minutes to produce a 50 per cent decrease in specific activity. This slowing is interpreted as denoting dilution of the isotopic inorganic carbon with both non-isotopic carbon and isotopic carbon returning to the inorganic carbon pool.

Extrapolation of the linear portion of the curve to zero time gives a log specific activity of 4.58 or a specific activity of 38,000 counts per minute per millimole of CO₂. Based on the injection of 100,000 counts per minute,
this would indicate that the $^{14}$O$_2$ initially injected was distributed in an inorganic carbon pool of 2.63 mM per 130 gm. rat, or 2 mM per 100 gm. rat. Since the total CO$_2$ in the extracellular and intracellular fluids of a 100 gm. rat would amount to only about 1 mM of CO$_2$, the $^{14}$O$_2$ must have rapidly entered into mobile equilibrium with 1 mM of CO$_2$ elsewhere in the body. One possibility is rapid exchange with the CO$_2$ of bone, and another is incorporation into dicarboxylic and tricarboxylic acids. The present experiments do not permit a decision as to the relative importance of these two possibilities.

The data of the acetate experiments have been plotted as the average of three experiments. The curve drawn through the points shows a maximum specific activity of $^{14}$O$_2$ between 10 and 20 minutes, emphasizing the rapidity of the processes of acetate absorption, distribution, metabolism, and excretion of the $^{14}$O$_2$ formed. This curve is determined primarily by the balance between $^{14}$O$_2$ formed by oxidation and its dilution with non-isotopic CO$_2$.

During the period of 40 to 120 minutes, the decrease in the log specific activity was linear with a slope indicating a 50 per cent decrease in specific activity in 25 minutes. After this time, a slower rate of decrease was observed, becoming approximately parallel to the curve of the bicarbonate experiments during the later stages.

The data of the succinate experiments have been plotted as the average of three experiments. In the succinate experiments, the maximum specific activity of $^{14}$O$_2$ was reached at about 30 minutes. From 50 to 200 minutes, the curve was linear with a slope corresponding to 50 per cent decrease in specific activity in 35 minutes. The maximum specific activity was reached somewhat later than in the acetate experiments, but this may have been due to the larger amount of succinate injected and slower absorption from the peritoneal cavity.

Cumulative Excretion of $^{14}$O$_2$—The cumulative excretion of $^{14}$O$_2$ has also been calculated and the average values have been plotted for each series of experiments (Fig. 2). After 4 hours, the average total excretion of injected C$^{14}$ amounted to 95 per cent after isotopic bicarbonate, 87 per cent after isotopic acetate, and 86 per cent after isotopic succinate. These final values varied in individual experiments by ±5 per cent from the mean. Because of these variations, it is not to be concluded that the residual C$^{14}$ in the body of the rat at the end of 4 hours is known with certainty in any one of the present experiments. However, the data show that 50 per cent of the C$^{14}$ is excreted in 18 minutes when administered as NaH$^{14}$O$_3$, whereas after C$^{14}$ administration as carboxyl-labeled acetate and carboxyl-labeled succinate, 42 and 55 minutes, respectively, are required for 50 per cent C$^{14}$ elimination.
Radioactivity of Liver Constituents—A few results were obtained on the distribution of isotopic carbon in glycogen, fatty acids, and cholesterol isolated from the livers of the rats at the end of the experiments. Following isotopic bicarbonate injection, the liver glycogen had appreciable amounts of C\textsuperscript{14} but no C\textsuperscript{14} was detected in the fatty acid or cholesterol fractions of the liver. On the other hand, following isotopic acetate injection, isotopic carbon was present in all three liver components. After the administration of labeled succinate, isotopic carbon was found only in glycogen and not in the fatty acids or cholesterol.

Since the amount of residual radioactive carbon in the body of the rats at the time of their sacrifice was very small, the failure to find measurable amounts of radioactivity in a tissue fraction is not to be interpreted as evidence that none was there.

![Diagram](http://www.jbc.org/)

**Fig. 2.** The calculated cumulative C\textsuperscript{14}O\textsubscript{2} excretion is plotted against time, following the administration of isotopic sodium bicarbonate, acetate, and succinate.

**DISCUSSION**

The cumulative recovery curves of C\textsuperscript{14}O\textsubscript{2} excretion after the administration of labeled acetate and succinate (Fig. 2) do not show the rates of metabolism of the labeled materials without further analysis of the data.

However, such information may be obtained by calculating the distribution of the C\textsuperscript{14} in the organic and inorganic chemical species, in the system as a whole, at any one time. By "system as a whole" is meant the rat plus the total C\textsuperscript{14}O\textsubscript{2} which has been excreted. The problem is treated as one involving two consecutive processes, (1) oxidation and (2) excretion, these being of the general form:

\[ A \xrightarrow{k_1} B \xrightarrow{k_2} C \]
where $k_1$ is the velocity constant of conversion of $^{14}$C in organic form to $^{14}$CO$_2$, and $k_2$ is the velocity constant of excretion of $^{14}$CO$_2$. 
From the experiments with labeled bicarbonate (Fig. 2), the excretion of \( ^{14} \text{C} \text{O}_2 \) appears to follow a first order reaction for about 60 minutes. This may be expressed by the equation

\[
(C^{14} \text{O}_2)_t = 100e^{-kt}
\]

where \((C^{14} \text{O}_2)_t\) = the per cent of \(^{14} \text{C} \text{O}_2 \) excreted in time, \( t \), and \( k \) has the value of 0.047.

The values of \( k \) for the conversion of \(^{14} \text{C} \) in the carboxyl groups of acetate and succinate have been estimated from the calculated amount of \(^{14} \text{C} \) present in organic form (Figs. 3 and 4). (These values were obtained from the measurements of the total \(^{14} \text{C} \text{O}_2 \) excreted and the calculated \(^{14} \text{C} \text{O}_2 \) in the animal.) In the acetate experiments, the average value of \( k_1 = 0.043 \), and in the succinate experiments, \( k_2 = 0.028 \), thus showing that the rate of conversion of acetate carboxyl carbon to \( \text{CO}_2 \) is about the same as the rate of excretion of \( \text{CO}_2 \), whereas the rate of conversion of succinate carboxyl carbon to \( \text{CO}_2 \) appears to be slower.

The quantitative relations reached from the analysis of the data presented above must be regarded as tentative and subject to revision as further experiments are completed. They are presented at this time in order to illustrate the type of information which can be obtained from respiratory \( \text{CO}_2 \) data following the administration of isotopic materials. It is believed that the method may find useful application in the study of abnormal metabolic states in the intact animal.

**SUMMARY**

1. The rates of excretion of \(^{14} \text{C} \text{O}_2 \) by normal rats have been measured following the intraperitoneal injection of isotopic sodium bicarbonate, carboxyl-labeled sodium acetate, and carboxyl-labeled sodium succinate.
2. The cumulative excretion of radioactive carbon amounted in 4 hours to 95 per cent after bicarbonate, 87 per cent after acetate, and 86 per cent after succinate.
3. The rates of change of the specific activity of the \( \text{CO}_2 \) have been interpreted in terms of the metabolic reactions of the substances injected.
4. The velocity constants for the conversion of acetate and succinate carboxyl carbons to \( \text{CO}_2 \) and for the excretion of \( \text{CO}_2 \) have been calculated.

**BIBLIOGRAPHY**

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