Indicator-Dilution Studies with "Diffusible" Indicators

By Francis P. Chinard, M.D., Theodore Enns, Ph.D., and Mary F. Nolan, B.A.

The indicator-dilution principle has been applied to studies of the circulation in order to provide estimates of flows and volumes in the vascular compartment. A sine qua non of validity of these estimates is that the indicator used remain within the confines of the vascular compartment for the entire duration of the period required to obtain appropriate samples of blood. In such a symposium as this, it may appear paradoxical to have a presentation concerned with the uses of indicators that do not conform to the requirement just slated.

In fact, much of the information available on the flow of blood through the brain, through the liver, and through the kidney has been obtained by the use of such non-conforming indicators. The principles and techniques differ to some degree from those used in the single, rapid-injection procedures. To conform to the general pattern of the symposium, no further mention will be made of these other principles and techniques. Instead, an endeavor has been made to present some of the results of an extension of the indicator-dilution principles and to indicate wherein these results may have a bearing on the results obtained by the conventional techniques in the particular setting of the circulation. Some results of studies of the in vivo metabolic activities of the kidney are also presented briefly. No attempt has been made to provide a complete review of the literature on the uses of "diffusible" indicators.

The Heart-Lung System

For present purposes, the heart can be considered to be a set of two pumps, in series but separated by a capillary network (fig. 1). This capillary network is surrounded by an extravascular compartment in the form of a sheath consisting of cells and an extracellular bog. The sheathed capillary network is itself bathed in a gas phase. The two major divisions are the liquid phase and the gas phase. The blood, extravascular and gas phases are in series (fig. 2). An additional series system exists in the gas phase: the alveolar compartment and the dead space. In the extravascular compartment, the cells and the bog are probably in series as indicated, but some series-parallel arrangements are probable.

Indicators with different volumes of distribution can be expected to provide different concentration-time relationships after a single, rapid injection in equal amounts into such a system. These relationships are shown in figure 2. In general, and provided there are no losses from the system (the system is closed), the greater the volume of distribution the later and smaller will the peak be. The area under the different curves will, however, be the same when appropriate corrections have been made for recirculation.

With an indicator that suffers losses from the system into, for example, the gas phase (the system is open), then the area under the curve will be less than for the substance assumed to remain in the vascular compartment. The position of the peak will depend...
on the degree of approach to equilibrium during the time of transit through the multiphase system. The tails of the curves of such substances will be affected not only by recirculation but also by recycling occurring in the gas phase.

With appropriate indicators, injections can be made into the vascular system or into the gas phase. Samples can be obtained from the vascular system or from the gas phase. Some experiments to illustrate these possibilities follow.

**EXPERIMENTAL PROCEDURE**

All experiments were carried out on anesthetized mongrel dogs. Injections of indicators in aqueous solution were made either into a jugular vein or by way of a balloon-guided catheter into the right ventricle or pulmonary artery. Gaseous indicators were injected into a tracheal cannula. Sequential blood samples were obtained from a catheter in a carotid artery by means of an automatic sample collector. In experiments with dissolved gases and with metabolizable substances, the sequential blood samples were collected anaerobically by means of the collector shown in figure 3. This consists of a thick methyl methacrylate plate, with wells drilled into it, rotating over the end of the sampling catheter. The container, a spittoon, is filled with mercury; syringes, with their dead space filled with heparin solution, are fitted to the tops of the wells. The blood, pumped through the catheter, rises through the mercury to the top of the wells and can be collected in the syringes without exposure to air. Collections of total expired gas were made, when indicated, through connections to a tracheal cannula. Continuous analysis of expired gases was made by means of a mass spectrometer equipped with a doubleslit system. The inlet leak to the mass spectrometer was placed in the tracheal cannula. In experiments with metabolizable substances, a refrigerant was circulated through the outer container.

**ANALYTICAL PROCEDURES**

Analyses for all indicators including T-1824 were carried out on whole-blood samples. Standards were prepared by serial dilutions in whole blood of an aliquot of the solution used for injection. Accordingly, no corrections for hematocrit values were necessary. For specific methods see references 2 and 3.

**GRAPHIC REPRESENTATION**

The conventional plotting of the data obtained after a single, rapid injection of indicator involves linear coordinates relating concentration in collected samples, corrected in the usual manner for recirculation, to time after injection (fig. 4). In general, an asymmetric distribution curve is obtained, skewed to the right. Stow and Hetzel have shown that this distribution curve could be more or less well normalized by the use of a logarithmic scale for the time axis. This normalized distribution is represented by the equation

\[ C = C'_p e^{k(t - t_a)} \left( \frac{t - t_a}{t_p - t_a} \right) \]

where the C's represent the per cent recovery or the concentration, t is the time after injection, and the subscript p indicates the
value of the particular parameter at the maximum of the curve. The term \( t_a \) is an arbitrary point on the time scale for which \( C \) approaches zero in a mathematical sense. It can not be identified with an appearance time at which the first measurable value of \( C \) is obtained. The "appearance time" is in fact indeterminate since its value depends not on the actual appearance time but on the sensitivity of the analytical and sampling devices used. There is no a priori mathematical reason for not setting \( t_a \) to zero.

By the same transformation, the cumulative concentration curve, which is a skewed sigmoid with linear coordinates, becomes a more or less regular sigmoid with a logarithmic scale for the time axis. If the total cumulative recovery is assigned the value of 100 per cent at its maximum, each point on the curve represents a cumulative recovery at the given time. A further simple transformation provides a means of converting the now regular sigmoid to a more or less straight line. This is the probit transformation.5

Experimentally, we have obtained probit-log time plots which fall into several categories. Idealized examples are shown in figure 5. The first line (I) represents the pattern of what we call the reference substance; in many of our experiments this is T-1824. The probit plots for some "diffusible" substances (III) differ markedly from those of the reference substances. In general, they diverge from these. With other substances, a sigmoid pattern (COMP.) is evident, suggesting that two, or more, compartments are available for the distribution of the substance. In other words, curve "COMP." might be considered to have a contribution from curve I, representing the vascular compartment, and a contribution from curve II, representing another "parallel" compartment. As a matter of fact, curve "COMP." has been constructed by combining a 50 per cent contribution from a type I

---

**FIGURE 3**

Refrigerated anaerobic sequential sample collector. See reference 1 for details.
TABLE 1

Relative Recoveries and Mean Transit Times of T-1824, Na\(^{22}\) and Labeled Water

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Experiments</th>
<th>Mean, per cent</th>
<th>S.D.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recoveries</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water/T-1824</td>
<td>41</td>
<td>94.6</td>
<td>8.6</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Na(^{22})/T-1824</td>
<td>26</td>
<td>95.3</td>
<td>11.7</td>
<td>.02 &lt; P &lt; .05</td>
</tr>
<tr>
<td>Water/Na(^{22})</td>
<td>42</td>
<td>99.3</td>
<td>9.1</td>
<td>.4 &lt; P &lt; .05</td>
</tr>
<tr>
<td>Mean transit times</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na(^{22})/T-1824</td>
<td>26</td>
<td>99.5</td>
<td>3.2</td>
<td>.4 &lt; P &lt; .05</td>
</tr>
<tr>
<td>Water/Na(^{22})</td>
<td>42</td>
<td>120.5</td>
<td>9.9</td>
<td>&lt; &lt; .01</td>
</tr>
<tr>
<td>Water/T-1824</td>
<td>41</td>
<td>120.9</td>
<td>12.7</td>
<td>&lt; &lt; .01</td>
</tr>
</tbody>
</table>

distribution with a 50 per cent contribution from a type II distribution. Corresponding per cent recovery-time curves are shown in figure 6. The probit plots provide a simple and rapid means of assessing the relative distributions of different indicators simultaneously injected and of indicating the significance of the several possible phases of distribution.

Results With T-1824, Na\(^{22}\) and Labeled Water

A representative experiment in which T-1824, Na\(^{22}\) and DIO (deuterium hydrogen oxide) were injected is shown in figure 7. The data for all substances have been corrected for recirculation by the conventional semilog-plot extrapolation procedure. A consistent pattern was found in these experiments. The modal value for Na\(^{22}\) is slightly less than that for T-1824; in other respects, the per cent recovery-time curves for the two substances are quite similar. The curves for DIO, however, differ quite significantly from those of the other two substances: the peak is lower and is delayed and the curve shows much more dispersion. Calculations of recoveries and of the mean transit times are given in Table 1.

Attention is called to the apparent loss of 5 per cent of the labeled water and of 5 per cent of the Na\(^{22}\) relative to the T-1824. The mean transit times of Na\(^{22}\) and of T-1824 do not, however, differ significantly.

Discussion and Interpretations

Because of the orientation of this symposium, the apparent losses of Na\(^{22}\) and of labeled water will be considered first. These may be actual losses, as suggested by Bauman and colleagues and by our group. However, the recoveries of Na\(^{22}\) and of labeled water are nearly identical although their apparent volumes of distribution are vastly different. It is possible that the recoveries of T-1824 are underestimated or that the recoveries of Na\(^{22}\) and of labeled water are underestimated by systematic analytical or other errors: both situations may obtain. The calculations of recoveries depend to a certain degree on the correction made for recirculation. A procedure assumed to be correctly applicable to T-1824 is not necessarily applicable to other substances. Another possibility is that recirculation occurs much sooner than has been assumed. Recirculation would be more important for T-1824 than for the other two substances, which cross capillary boundaries elsewhere. In brief, is the conventional correction for recirculation correct?

The fact that the curves for T-1824 and Na\(^{22}\) are so similar indicates that the volumes of distribution must be quite similar. More directly, calculations of volumes as the product of flows from the T-1824 data and the appropriate mean transit times show no significant differences. In substance, then, if T-1824 does remain within the confines of the vascular system, so does the sodium ion. The pulmonary capillary endothelium would then be relatively impermeable to sodium ion. Other substances such as inulin, urea, p-aminohippurate, thiocyanate ion and...
bicarbonate ion show patterns similar to those of sodium ion. Potassium, as K\textsuperscript{42}, shows some slight displacement relative to the reference substance. This is consonant with its ability to enter cells. An alternative to the impermeability hypothesis is that there is actually very little by way of an extravascular extracellular bog for this group of substances to get into from the pulmonary capillaries.

While Na\textsuperscript{22} and T-1824 behave quite similarly on passage through the lungs, labeled water behaves quite differently. As indicated in table 1, the mean transit times are considerably greater than for the reference substance, and the per cent recovery-time curves have the expected patterns of a substance showing little loss but greater volume of distribution than the reference substance.

We have calculated the fraction of the labeled water that has appeared to go into a greater volume of distribution than the reference substance. The procedure for these calculations is given in reference 3. The results of the calculations indicate that approximately 56 per cent of the labeled water crosses the boundaries that confine the T-1824 or the Na\textsuperscript{22} (from data in reference 3). This represents a minimal value for the enormous molecular exchange taking place between the involved compartments in the liquid phases. With a cardiac output of the order of 2 liters per minute, the molecular exchange corresponds to a water-volume exchange of the order of 1 liter per minute. Roughly half the water pumped by the heart leaves the compartment defined by reference substances and returns in the time of a single passage through the lungs. These exchanges would not be detectable with the more leisurely sampling procedures used, for example in the estimation of plasma volume and in the estimation of total body water.

From these data, values can be calculated for certain apparent volumes of distribution. Such calculations presuppose uniformity of distribution of the substances in the particular compartments involved within the time of transit through the compartments. Evidence to be given later provides some support for that supposition. In order to avoid getting into the central-volume controversy, we have calculated volume differences. The blood flow is obtained from the data for the reference substance. This value multiplied by the difference of the mean transit times gives a value for the volume of distribution available to water but not available to the reference substance. The results of such calculations are shown in figure 8. The two sets of symbols indicate slightly different experimental situations. The open circles
FIGURE 6
Linear per cent recovery plots of probit plots shown in figure 5.

FIGURE 7
Per cent recovery-time curves in arterial blood following injection of indicators shown into right atrium.

represent some of our earlier experiments. In these, attempts were made to produce pulmonary edema by the administration of \( \alpha \)-naphthylthiourea. The arrows indicate the extent of the increase of the volume difference. Our data are consistent with those.
obtained in man with similar technics by Lilienfield and his colleagues.

The physiologic significance of these values is as yet uncertain. Probably this compartment represents more than just the volume of the pulmonary liquid phase involved in the gas exchanges. At least, this compartment is operationally defined. It represents a perfused capillary-cellular system. In the heart-lung system, the use of the two indicators, Na\textsuperscript{22} and labeled water, offers possibilities for the quantitative assessment of shunts. The labeled water would escape from the Na\textsuperscript{22} volume only in capillary systems; it would remain with the Na\textsuperscript{22} in the case of communications without capillaries, that is, in shunts. With the variety of injection and sampling sites now accessible, the use of this pair of indicators would seem worth exploring.

It might also be of interest to calculate the two volumes by the slope method and to compare the results of the more direct calculation with those obtained by that method.

In any event, the discrepancies between the T-1824 and the Na\textsuperscript{22} data are sufficiently small to justify the use of Na\textsuperscript{22} as a reference substance when the experimental design precludes the use of a compound with the acid-
Studies of the Carbon Dioxide System

The development of these technics provided us with the means of studying the carbon dioxide system in vivo and of determining the extent to which dissolved carbon dioxide contributes to expired carbon dioxide relative to the forms in which carbon dioxide may exist. The interrelationships of the different forms of carbon dioxide are shown in figure 9. Under physiologic conditions, reaction 7 can be ignored; there is no quantitatively significant concentration of carbonate ion in blood. Reaction 4 is ionic and may therefore be considered instantaneous. The uncatalyzed reaction 3, involving the interconversion of dissolved carbon dioxide and carbonic acid, is slow compared to the catalyzed reaction 5. The equilibrium is in the direction of the dissolved carbon dioxide. The catalyzed reaction velocities are approximately 1000 times larger than the uncatalyzed reaction velocities.

It has been suggested by Porstera as well as by others that equilibration of carbon dioxide among its several potential forms shown in figure 9 could be slow relative to the rate of contribution of dissolved carbon dioxide to the gas phase when the activity of carbonic anhydrase is reduced as by an inhibitor. A disproportionate contribution of dissolved carbon dioxide to the gas phase in the capillary-alveolar system would mean that equilibration of the carbon dioxide system would take place somewhere in the circulatory system distal to the end of the alveolar capillaries. Under such conditions, blood obtained from a peripheral artery would not then be identical in composition to end-alveolar capillary blood: the partial pressures of carbon dioxide in peripheral arterial blood and in alveolar gas would not be identical even though diffusion equilibrium with respect to carbon dioxide in the gas phase and dissolved carbon dioxide in the aqueous phase of blood might obtain. Evidence substantiating this suggestion follows.

Recoveries of Labeled Water and C¹⁴O₂ Relative to the Reference Substance: Effects of Acetazolamide

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Percentage recoveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labeled water</td>
<td>C¹⁴O₂</td>
</tr>
<tr>
<td>1 C¹⁴O₂ injected as bicarbonate—controls</td>
<td>98.8</td>
</tr>
<tr>
<td>2 C¹⁴O₂ injected as dissolved carbon dioxide—controls</td>
<td>92.9</td>
</tr>
<tr>
<td>3 C¹⁴O₂ injected as bicarbonate—after acetazolamide, 100 mg./kg.</td>
<td>97.8</td>
</tr>
<tr>
<td>4 C¹⁴O₂ injected as dissolved carbon dioxide—after acetazolamide, 100 mg./kg.</td>
<td>100.4</td>
</tr>
</tbody>
</table>
Arterial per cent recovery-time curves following injection of labeled bicarbonate and of labeled dissolved carbon dioxide under control conditions.

Probit plots of data in figure 10.
Arterial per cent recovery-time curves following injection of labeled bicarbonate and of labeled dissolved carbon dioxide after administration of acetazolamide.

Probit plots of data in figure 12.
Recovery patterns of $^{14}$O$_2$ in expired gas following injection of $^{14}$-labeled bicarbonate ion and dissolved carbon dioxide. Effects of acetazolamide.

D140. No features clearly distinguish the patterns for the two injected forms of carbon dioxide. Interconversion is rapid and most of the C$^{14}$ travels as bicarbonate ion regardless of the form in which it is injected. With prior administration of acetazolamide (Diamox®) in dosages of 100 mg./kg. of body weight, the patterns show considerable differences from those obtained under control conditions as shown in figures 12 and 13. The modal values for the recovered C$^{14}$O$_2$ are nearly the same as for the reference substance after injection of labeled bicarbonate. The probit plots for the recovered C$^{14}$O$_2$ show a marked shift from their previous position relative to the reference substance. The recovery after injection of labeled bicarbonate is nearly complete. The recovery is considerably less than complete after injection of labeled dissolved carbon dioxide. The results of these and other experiments are summarized in table 2. There is a significantly smaller recovery of C$^{14}$O$_2$ in arterial blood when C$^{14}$O$_2$ diss is injected with inhibition of the carbonic anhydrase. In brief, under these conditions a disproportionately large loss of dissolved carbon dioxide occurs. The time available for the interconversion of the different forms of carbon dioxide is the interval between the time of injection and the time of passage to the end of the alveolar capillaries. This time is too short to permit essentially complete interconversion when the activity of the carbonic anhydrase is reduced. An ancillary finding is the apparent limitation of what must be bicarbonate ion to the volume available for the distribution of the reference substance.

**RECOVERY OF C$^{14}$O$_2$ AND C$^{14}$O$_2$ INJECTED WITH ACETAZOLAMIDE**

A natural sequel to these experiments was the determination of the recovery of labeled carbon dioxide in the collected expired gases following the intravenous injection of labeled dissolved carbon dioxide and labeled bicarbonate, before and after the administration of acetazolamide. The results of a represen-
tative experiment are shown in table 3. There is essential equality of the contributions from the two species under control conditions but a fourfold increase in the contribution from C\textsuperscript{14}O\textsubscript{2}\text{diss} after the administration of acetazolamide.

In conjunction with these experiments, carbon dioxide labeled with C\textsuperscript{13} was incorporated in the injection solutions. This permitted the nearly continuous recording of the enrichment of the expired carbon dioxide in C\textsuperscript{18} in each breath under the several conditions described above. Results of such an experiment are shown in figure 14. There is some reduction in the initial contribution from HC\textsuperscript{14}O\textsubscript{3} after acetazolamide while later the contribution increases in contrast to the results of the control run. This later contribution may reflect the gradual uncatalyzed interconversion of bicarbonate ion and dissolved carbon dioxide as the blood circulates through the body. In sharp contrast, the data show a very marked increase (roughly fourfold) in the contribution from dissolved carbon dioxide after acetazolamide.

Confirmatory results with H\textsubscript{2}O\textsuperscript{18} have been reported in the original communication.\cite{8}

**DISCUSSION AND INTERPRETATIONS**

The results from these different experimental approaches are consistent and show clearly that there is a disproportionately large contribution from dissolved carbon dioxide after inhibition of carbonic anhydrase. Under these conditions, peripheral arterial blood cannot be representative in composition of end-alveolar capillary blood. Equilibrium is not established with respect to the carbon dioxide system in the time of transit defined above.\* Whether equilibrium is established under control conditions cannot be determined from these experiments. The catalyzed reactions are sufficiently rapid for this. This question remains. Is there actually equilibrium distribution of gases between liquid and gas phases in the time of transit of blood through the alveolar capillaries?\footnote{These studies have been published: Chinard, F. P., Enns, T., and Nolan, M. F.: Diffusion and solubility factors in pulmonary inert gas exchanges. J. Appl. Physiol. 16: 831, 1961.}

**Studies With "Inert" Gases†**

The evidence that equilibration of "inert" gas does occur within the time of transit of the blood through the lungs, although considerable, is indirect. It can be shown by some simple calculations that the mean distance traveled in water by a substance with a diffusion coefficient of the order of 2x10\textsuperscript{-5} cm\textsuperscript{2}/sec. is approximately 60 microns. This is far greater than the distance between

\*We had suggested that marked pH alterations of blood might occur in the alveolar capillaries because of the loss of dissolved carbon dioxide. Alterations of pH would occur with changes of the partial pressure of carbon dioxide, which is directly related to the concentration of dissolved carbon dioxide. We are grateful to Dr. Robert W. Berliner for pointing out this error to us.

\textsuperscript{†}These studies have been published: Chinard, F. P., Enns, T., and Nolan, M. F.: Diffusion and solubility factors in pulmonary inert gas exchanges. J. Appl. Physiol. 16: 831, 1961.
the center of an alveolar capillary and the gas phase. However, the diffusion coefficient of such a substance in the alveolar-capillary barrier could be considerably smaller than its diffusion coefficient in pure water. A more direct attack on this problem was warranted.

Operationally, the question is this. Is there a diffusion limitation or a solubility limitation in the exchange of "inert" gases between the blood and gas phases?

EXPERIMENTAL PROCEDURE

The following indicators were used: T₂ (tritium gas), Kr⁸⁵, Xe¹³³, and C¹⁴-labeled ethylene. These substances have different molecular weights (and hence different diffusion coefficients) and, except for the pair ethylene and xenon, substantially different solubilities.

If diffusion in the liquid phase is a limiting factor, then, after intravenous injection of a pair of gases, the recovery-time curves of the two gases in arterial blood (and the probit plots) should have different patterns, the more slowly diffusing gas appearing earlier in the arterial blood samples in larger amounts than the more rapidly diffusing gas. The probit plots of the two gases should be displaced relative to each other. The over-all recovery of the more slowly diffusing gas should be greater. If solubility in the liquid phase is a limiting factor, then the recovery-time curves of the gases should be proportional to each other, the probit plots should be identical, and the relative recoveries of the two gases should be proportional to their relative solubilities.

In these experiments, injections of aqueous solutions were made by way of a catheter into the right ventricle or into the pulmonary artery and injections of gases were made into a tracheal cannula. The dogs were anesthetized, paralyzed with succinylcholine and ventilated by means of an intermittent positive-pressure device providing a maximal positive pressure of about 12 mm. of mercury. The pressure was briefly increased to 25 mm. of mercury periodically to prevent atelectasis.

RESULTS

The data obtained in a representative experiment in which Na²², DHO and dissolved tritium and Kr⁸⁵ were injected intravenously are shown in figure 15. The probit plot of the data is shown in figure 16. While the recoveries of the two gases are quite small compared to the reference substance and to DHO and while the peaks occur at about the same time as for the reference substance, the curves show considerable skewing to the right. This skewing is consonant with losses to the alveolar gas phase and beyond with subsequent recycling in the gas phase. Similar results were obtained with the pair ethylene, C¹⁴ and Xe¹³³.

In another set of experiments, the pairs of gases were injected as gases into the tracheal cannula at the same time that Na²² was injected into the right ventricle. The data and the probit plot of a representative experiment are shown in figures 17 and 18.
from the slight precession of the gases over the reference substance, the results are quite similar to those obtained following the intravascular injection.

The recovery ratios of the gases used in these and other similar experiments are summarized in Table 4. They are to be compared with the ratios of the solubilities in water and with the square root of the reciprocals of the molecular-weight ratios of the appropriate pairs. For present purposes, these last values can be considered a sufficiently close approximation to the ratios of the diffusion coefficients. The recovery ratios found are much closer to the ratios of the solubilities than to the ratios of diffusion coefficients.

**DISCUSSION AND INTERPRETATIONS**

The direct proportionality of the recovery curves as shown by the similarity of the probit plots, and the proportionality of the recovery ratios, point to solubility as the determining factor in these experiments. There is nothing in our data to suggest a diffusion effect. Molecular movements (diffusion rates) are so large that only solubility and gas volume factors appear to play a role in determining the amount of a gas that will remain in blood. The recovery ratios are significantly higher than the solubility ratios. This we interpret to be a reflection of the greater diffusibility of the smaller gas molecules in the gas phase:

the alveoli and the dead space. The absolute values for the gas recoveries varied considerably from one experiment to another in keeping with the experience of other investigators. This finding may be related in part to variations in the blood and gas content of the lungs during the respiratory and cardiac cycles. The fact that the recoveries were within the same range regardless of the route of injection provides some evidence against significant shunts in these preparations.

Considerable emphasis has been given during the past few years to the use of Kr\(^{85}\) for the detection of shunts. Of particular importance is the recent report by Fritts and his colleagues\(^6\) in which earlier studies are reviewed. These investigators injected T-1824 and Kr\(^{85}\) into a vein and obtained T-1824 arterial-dilution-curve samples of arterial blood for Kr\(^{85}\) analysis. With normal perfusion-ventilation relationships a very large but variable fraction of the injected Kr\(^{85}\) left the circulation. This variation precluded accurate estimation of such small shunts as might occur normally. However, large pulmonary arteriovenous shunts could be detected and estimated with some accuracy. Our results
TABLE 5
Potential Production of Carbon Dioxide From Retained Glucose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial glucose concentration (Gₐ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal blood flow (RBF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose input to kidney</td>
<td>$\text{G}_{\chi} \times \text{RBF}$</td>
<td>$100 \text{ mg./100 ml. = 5.5 \mu mole/ml.}$</td>
</tr>
<tr>
<td>Glucose retained</td>
<td>$1.5 \text{ per cent of input}$</td>
<td>$670 \mu \text{mole/min.}$</td>
</tr>
<tr>
<td>Potential CO₂ production</td>
<td>$6 \times \text{glucose retained}$</td>
<td>$60 \mu \text{mole/min.}$</td>
</tr>
<tr>
<td>Actual CO₂ production</td>
<td>$([\text{CO₂}]_v - [\text{CO₂}]_a) \times \text{RBF}$</td>
<td>$10 \mu \text{mole}$</td>
</tr>
<tr>
<td>Potential CO₂ production of glucose</td>
<td></td>
<td>$60 \text{ per cent of total}$</td>
</tr>
</tbody>
</table>
have some intrinsic interest and indicate, in addition, a possible approach to the study of myocardial metabolism.

It has been suggested that the transport of glucose by the renal tubule cells might involve the breakdown and resynthesis of the 6-carbon chain. In a series of experiments with appropriately labeled glucose it was possible for us to show that randomization of carbon atoms in the 6-carbon chain did not occur. It was thus established that glucose was transported with its 6-carbon chain intact. In these experiments, a solution containing T-1824, ordinary creatinine and labeled glucose was injected into the renal artery through a bent needle inserted into the aorta and turned into the renal artery. Sequential samples of renal venous blood were obtained from a catheter in the renal vein. The recovery-time curves of a representative experiment are shown in figure 19. The displacement of the curves for glucose and creatinine relative to that for T-1824 indicates that these substances must have a much greater volume of distribution than T-1824. Approximately 70 per cent of the injected creatinine is recovered in the renal venous blood; this is consonant with the recovery of some 30 per cent in the urine. In the case of glucose, the recovery is nearly complete. (These recoveries are based on the assumption that the recovery of T-1824 is complete.) The excess recovery of glucose over creatinine occurs in the time period after most of the T-1824 has left the kidney. This excess glucose recovery in this time period represents the glucose transported by the tubules since prior administration of phlorizin results in obliteration of the differences of the creatinine and glucose curves.

In a series of 15 similar experiments, the recoveries of glucose averaged 97.6 per cent (S.D., 10.4 per cent) while the recoveries of creatinine averaged 73.7 per cent (S.D., 8.8 per cent). This fact suggested that glucose might not be metabolized by renal tissue in vivo to the extent that would be expected from the known rapid glycolysis occurring in slices of kidney cortex. To check on the extent of glucose breakdown in the time span of these experiments, renal venous-blood samples were collected anaerobically and the C14O2 production was determined. On the average less than 0.06 per cent of the C14 injected as glucose was recovered as C14O2. The nonvolatile C14 was found to be in the form of glucose: no other forms were detected by paper chromatograms and radioautographs. On this basis, the contribution of glucose to renal production of carbon dioxide would be less than 2 per cent.

It was possible, however, that the small fraction of the injected glucose remaining in the kidney after the routine 1-minute sampling period could contribute significantly to carbon dioxide production subsequently. To that end, the residual C14 in the kidney was determined at various time intervals after the injection into the renal artery. One minute after injection, approximately 3.5 per

![Diagram](https://example.com/diagram.png)
cent of the injected glucose remained in the kidney. This had decreased to about 1.5 per cent approximately 4 minutes after the injection.

As shown in table 5, this small amount retained in the kidney could account for some 60 per cent of the total carbon dioxide production by the kidney. Glucose is not excluded as a source of carbon dioxide but its rapid disappearance from the kidney without evidence of degradation does not suggest that it is a major source.

A search was then started for other possible sources and our first efforts were directed at lactate and pyruvate. As shown in figure 20, there is a rapid and substantial production of C\textsuperscript{14}O\textsubscript{2} following the injection of DL-lactate labeled in the 1 position (carboxyl). The reaction time is of the order of 4 seconds as indicated by the difference of the modal transit times of the creatinine and C\textsuperscript{14}O\textsubscript{2}. Some 20 per cent of the injected C\textsuperscript{14} is recovered as labeled carbon dioxide. Similar results were obtained with pyruvate labeled in the 1 position. However, the reaction time was shorter by about 1 second and the recovery of C\textsuperscript{14}O\textsubscript{2} about twice as great as with correspondingly labeled DL-lactate. With lactate and pyruvate labeled in the 2 or 3 positions, the recoveries of C\textsuperscript{14}O\textsubscript{2} were much smaller. The results of these studies are summarized in table 6.

It was logical to assume that the discrepancy between the results obtained with DL-lactate and those obtained with pyruvate was due to the metabolism of the L isomer of lactate with insignificant metabolism of the D isomer. Appropriate experiments with labeled D and L-lactate verified the assumption. Accordingly, we have calculated the contribution of naturally occurring L-lactate to renal carbon dioxide production. The results of these calculations are summarized in table 7. More than 50 per cent of the renal carbon dioxide production comes from the carboxyl carbons of lactate and pyruvate. This is consistent with the very high renal respiratory quotients reported recently by Cohen.\textsuperscript{11}
It will be noted that this calculation, based on tracer experiments, indicates a greater utilization of lactate than is found from the calculation of utilization based on the product of blood-flow and arteriovenous-concentration differences. It appears that there is lactate formation as well as lactate utilization in the kidney.

In brief, glucose does not appear, at the present writing, to be metabolized to a very considerable extent by the kidney. The carboxylic carbons of lactate and pyruvate are the major immediate source of the carbon dioxide production by the dog's kidneys under the conditions of these experiments. These studies may serve to raise some questions concerning the validity of extrapolations from tissue-slice experiments. They may also serve to indicate the limitations of the deductions concerning metabolic processes drawn from the conventional in vivo substrate-utilization experiments in which utilization is calculated as the product of blood-flow and arteriovenous-concentration differences.

Summary

Some of the applications and extensions of the indicator-dilution techniques have been presented. The techniques offer possibilities for the investigation of some of the parameters of the circulatory and respiratory systems. They also offer a means of studying some of the more rapid features of organ metabolism in vivo. Compared to the degree of sophistication achieved by the more conventional dye-dilution studies, these extensions with "diffusible" indicators are in an embryonic stage of development. It is believed that they have a future.

References


Circulation Research, Volume 8, March 1962
Indicator-Dilution Studies with "Diffusible" Indicators
Francis P. Chinard, Theodore Enns and Mary F. Nolan

Circ Res. 1962;10:473-490
doi: 10.1161/01.RES.10.3.473

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/10/3/473