The Transport of Radioactive Inorganic Phosphate by the Pulmonary Circulation

By Hampden C. Lawson, Ph.D., M.D., Henry C. Mellette, Ph.D., and Everett S. Coleman, A.B.

Radio-phosphorus in the form $\text{H}_3\text{PO}_4$, injected in aqueous solution, is found to give flow-dilution curves and values for cardiac output indistinguishable from those obtained with T-1824 in normal anesthetized dogs. The fairly rapid disappearance of the $\text{P}^{32}$ from systemic blood is a technical advantage if the measurement of output is to be repeated at short intervals.

In the measurement of cardiac output by the flow-dilution procedure,\(^1\) the injected substance must pass through the pulmonary circulation without loss from blood. The ideal substance for this purpose would, on the other hand, completely disappear in one passage through the systemic circulation, and thus obviate the possibility of errors due to recirculation. The use of such substances has been discouraged by reports that diffusible dyes such as phenoltetraiodophthalein\(^2\) and salts such as sodium thiocyanate\(^3\) undergo considerable loss during primary passage through the lungs.

There is an additional need for rapid clearance of the injected material from the systemic circulation if radioactive substances are used. If the determination of output is repeated when the mixed blood activity levels are still high from a previous injection, an increased dosage is required to keep the statistical reliability of the flow-dilution curves constant.\(^4\) The progressively increasing dosage or decreasing reliability sets a practical limit to the number of determinations which can be made at reasonably short intervals.

The present study was stimulated by reports, incompletely documented, that highly diffusible ions such as $\text{K}^{42}$ and $\text{Na}^{24}$ pass through the pulmonary circulation with negligible loss, although they rapidly disappear from the systemic circulation.\(^5\) Radioactive phosphate was chosen for the study because of its availability and its many technical advantages as a tracer. It is known to disappear from blood rapidly, only about ten per cent of the injected $\text{P}^{32}$ activity remaining in the plasma at 10 minutes.\(^6\), \(^7\)

**Methods**

The study was done by injecting into the right ventricle of barbitalized dogs (250 mg. per kilogram) a solution containing the dye T-1824 and tracer concentrations of $\text{P}^{32}$ as $\text{H}_3\text{PO}_4$. Flow-dilution curves were constructed for both the dye and the $\text{P}^{32}$ from dye-density and radioactivity measurements made on serial samples taken from a carotid artery. The injected solution was made up in 0.9 per cent sodium chloride, to contain approximately 0.5 mg. per cubic centimeter of dye, and 10 $\mu$g. per cubic centimeter of $\text{P}^{32}$. The phosphate content of the solution, calculated from the specific activity of the isotope materials, was of the order of 0.75 $\mu$g. $\text{PO}_4$ per cubic centimeter.

Injections were made with calibrated syringes and catheters, the net injection volume being 3 to 4 cc. The injections were made as rapidly as possible, usually being completed within about 0.5 second. Carotid sampling was started at the time of injection, and samples were collected on a rotating drum in heparinized vials at the rate of one in about 0.4 second. Sample volumes were approximately 1 cc. each. The rapid sampling rate permitted alternate samples to be used for the measurement of dye and of $\text{P}^{32}$. This was found to be technically advantageous, since resuspension of cells to obtain a valid whole-blood aliquot for the isotope determination sometimes produced sufficient hemolysis to interfere with dye determination in a plasma aliquot.

Dye densities were read in a Coleman spectrophotometer, model 6A, using semimicrocuvettes. For the carotid samples, undiluted plasma was read against predrawn undyed plasma. A comparable density determination of the injected solution was obtained on a diluted aliquot made up in 0.9 per cent sodium chloride, to contain 1 cc. of the injected...
solution, and 2 cc. of undyed plasma in a total volume of 50 cc. This was read against a 2:50 dilution of undyed plasma in saline. Hematocrits were obtained on two or more carotid samples collected between the time of injection and initial dye appearance, by a standardized centrifuging procedure.\(^4\) Hematocrit values were corrected to 0.95 of the observed values, for occluded plasma.

Samples to be used for determination of P\(^{32}\) were hemolyzed by the addition of a few granules (about 5 mg.) of dry sodium lauryl sulfate and agitation. They were then transferred to the multieled sample containers described elsewhere,\(^8\) and one-minute beta counts obtained on each sample, using a thin glass-walled G.M. tube. The isotope flow-dilution curves obtained with these procedures in medium-sized dogs (12 to 20 Kg.) contained a total 5,000 to 10,000 counts in excess of background or mixed blood levels. Peak sample activity was usually between 1,200 and 5,000 counts per minute. Comparable activity determinations on the injected solution were obtained from a diluted aliquot made by adding 1 cc. of the solution to 25 cc. of inactive whole-blood and hemolyzing with sodium lauryl sulfate.

When more than one injection was made, the samples collected between injection and appearance were used to obtain, as an average value, the mixed blood levels of dye and of isotope. These were subtracted individually from the readings on the curves. Independent values for cardiac output were calculated from the dye and the P\(^{32}\) curves, using the general formula for output per minute

\[
F = \frac{Q \times 60}{A_o + A_e}
\]

where \(Q\) is a measure of the total quantity of injected substance, \(A_o\) is the area of its flow-dilution curve plotted in seconds to the instant of recognizable recirculation, and \(A_e\) the additional area obtained by exponential extrapolation to complete disappearance.\(^4\) The flows calculated from the isotope data are volumes of whole-blood. Those obtained from the dye data are volumes of plasma, converted to whole-blood by the multiplier 100/\(P\), where \(P\) is the volume percentage of plasma read from the hematocrit. A similar conversion, plus numerical equalization of dosage, is required for the plotting of equivalent dye and isotope curves. This was achieved by multiplying all dye-density readings by \(Q_i/Q_o \times P/100\), where \(Q_i\) and \(Q_o\) are the numerical measures of total injected isotope and dye, respectively.

**Observations and Comments**

Figure 1 shows simultaneous flow-dilution curves for dye and phosphate in a typical experiment. The smoothed curve drawn through the dye data appears to fit the isotope data nearly as well as any which could be drawn. The areas of the two curves, computed from the raw data (not smoothed), are in good agreement. Particularly notable is the lack of a time-lag in the P\(^{32}\) curve such as would be expected if diffusion gradients produced a reversible exchange between blood and extravascular fluid as the material moves through the pulmonary circuit.

Additional data are required on the levels at which the dye and the isotope return to the heart and begin to recirculate. If, as is generally assumed, the instant of this occurrence is signaled on the flow-dilution curve by an upward deflection from the exponential downslope, the data of figure 1 mean that the dye and the isotope not only pass through the pulmonary circulation, but also through some systemic circuit, with equal loss from blood. Examination of all our curves which contain such evidence of recirculation fails to show any consistent difference in the initial recirculation levels of the two substances.

![Fig. 1. Simultaneous flow-dilution curves for dye and P\(^{32}\). Time from injection in seconds on abscissa. Ordinate values are isotope activity in counts per minute, and dye density \(\times 2,247\) (see text for adjustment of dye density to equivalent dosage and whole-blood distribution). The smoothed curve is drawn through the dye data. The numbers at upper right corner give cardiac output, in cubic centimeters per minute.](http://circres.ahajournals.org/content/34/1/249)
TABLE 1.—Cardiac Output as Whole Blood Flow Dilution Volume of P³² and of T-18²⁴

<table>
<thead>
<tr>
<th>Dog</th>
<th>Trial</th>
<th>P³² cc/min.</th>
<th>T-18²⁴ cc/min.</th>
<th>P³² X 10⁶</th>
<th>T-18²⁴ X 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>4135</td>
<td>4125</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3960</td>
<td>3890</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3850</td>
<td>3878</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3713</td>
<td>3620</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3640</td>
<td>3580</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1890</td>
<td>1925</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1103</td>
<td>1140</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>2151</td>
<td>2205</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2105</td>
<td>2060</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1783</td>
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<tr>
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<td>4</td>
<td>1558</td>
<td>1538</td>
<td>95</td>
<td></td>
</tr>
</tbody>
</table>

Mean . . . . . . . . . . 11 100.7
S.D. . . . . . . . . . . ±2.93

These data offer no support to the hope entertained at the outset that P³², injected in this form, would be so rapidly removed from the systemic circulation as to minimize recirculation errors. The relative levels of dye and of isotope in blood after a lapse of approximately fifteen minutes, however, have been found in this study to be markedly different, as would be expected from previous studies. In our series, the average dye density in whole blood at this interval was approximately 1.4 X 10⁻⁴ X the density of the injected solution, while the P³² activity was about 1.5 X 10⁻¹ X the activity of the injected solution. Dye retention within the circulation over this period of time thus appears to be nearly ten times the retention of the isotope. After as many as six isotope injections at such intervals, cumulative blood levels in our animals have been in the neighborhood of 75 cpm., with negligible effects on the reliability of the curves.

Table 1 summarizes all the data in terms of the values calculated for cardiac output from the isotope and from the dye curves. It is apparent that a much larger series would be required to demonstrate a difference, if one exists. At a probability of 0.05, the two substances give results agreeing within ±6 per cent, with no apparent difference in the means. This agreement is all the more remarkable in view of the inclusion of an admittedly approxi-

mate hematocrit value in the dye calculation, and its absence from the isotope calculation.

Further study is required to explain the similarity in behavior of a relatively non-diffusible dye and a presumably highly diffusible ion in the pulmonary vascular bed.

SUMMARY

In intact, barbitalized dogs, a solution containing T-18²⁴ and P³² as H₃PO₄ was injected into the right ventricle. Flow-dilution curves for the two substances were constructed from data obtained on arterial samples. When adjusted to equivalent dosage, the curves are superimposable, and yield values for cardiac output which agree within ±6 per cent, at 0.05 probability. Clearance of P³² from the circulation is sufficiently rapid to permit repeated injection at intervals of a few minutes without producing high activity levels in mixed blood.

REFERENCES

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