Theoretic Limitations of the Nitrous Oxide Method for the Determination of Regional Blood Flow

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The nitrous oxide method for regional blood flow determination is based on the assumption that concentration equilibrium for nitrous oxide between the organ and its venous blood is reached. This assumption is examined, and it is shown mathematically that faulty equilibrium is not revealed by presently available criteria. It is shown that quantitatively and qualitatively erroneous results may be obtained by the use of this method for the estimation of regional blood flow.

In a previous communication from this laboratory, Sapirstein and Mellette investigated the possibility of using antipyrine as a substitute for nitrous oxide in the measurement of regional blood flow by the Kety-Schmidt technic. It was found that though apparent constancy of calculated flow for the area drained by the jugular vein was attained within twenty minutes, constancy was not reached in the area drained by the femoral vein, even after forty minutes. It was further noted that a persistent small arteriovenous concentration difference existed in both areas, suggesting that some portion of the tissues remained unequilibrated with antipyrine. Since the validity of the Kety-Schmidt method depends critically upon the establishment of concentration equilibrium between the organ whose blood flow is to be measured and the venous blood which drains that organ, we undertook an analysis of the significance of small arteriovenous concentration differences in relation to organ concentration equilibrium. It is the purpose of this communication to show that even the most minor arteriovenous concentration differences, which may be of the same order of magnitude as analytical errors, may represent major defects in organ equilibrium. It will also be shown that the criteria presently employed for the demonstration of organ concentration equilibrium are inadequate to do so.

The argument is presented first as a formal mathematical one. The significance of the results is considered in the discussion.

MATHEMATICAL BASIS

In figure 1, an organ is represented as consisting of a number of masses \( M_1, M_2, \ldots M_n \) which are perfused with blood at rates \( Q_1, Q_2, \ldots Q_n \). The masses are arranged in order of decreasing perfusion rates, i.e.,

\[
\frac{Q_1}{M_1} > \frac{Q_2}{M_2} > \cdots > \frac{Q_n}{M_n}
\]

The effluent volumes of blood \( E_1, E_2, \ldots E_n \) are considered to have gas concentrations \( V_1, V_2, \ldots V_n \) and \( V_{comb} \). The composition of the mixed venous blood is then given by equation 1:

\[
V_{comb} = \frac{Q_1V_1 + Q_2V_2 + \cdots Q_nV_n}{Q_1 + Q_2 + \cdots Q_n}
\]

To show the relationship between \( V_{comb} \) and time, the behavior of the individual \( V \)'s should be considered. It is assumed for the sake of simplicity that the arterioles are constant and that the venous blood for each tissue segment is in concentration equilibrium with the tissue mass which it drains. (Neither assumption is vital to the argument.) The change in the amount of nitrous oxide \( (MdV) \) within any one of the tissue masses \( M \) will vary with time \( (dt) \) as the product of blood flow \( Q \) through that mass and arteriovenous concentration difference through that mass \( (A - V) \). This is expressed by equation 2:

\[
M \frac{dV}{dt} = QA - V
\]
REGIONAL BLOOD FLOW WITH NITROUS OXIDE

**Fig. 1.** $M_1, M_2, \ldots, M_n$ are tissue masses. $Q_1, Q_2, \ldots, Q_n$ are the corresponding minute blood flows. $V_1, V_2, \ldots, V_n$ are the venous concentrations of the measuring agent through each tissue mass. $V_{comb}$ is the concentration of the measuring agent in the mixed venous blood ($E_{comb}$). The masses are arranged in order of diminishing perfusion rate ($Q/M$).

This may be solved to yield:

$$V = A[1 - e^{(-Q/M)t}]$$

Equation 3 describes the nitrous oxide concentration in the venous blood leaving any one of the tissue masses as a function of time. The composition of the mixed venous blood is determined by making the appropriate substitutions in equation 1. This leads, after rearrangement to:

$$V_{comb} = A\left[1 - \frac{Q_1 e^{(-Q_1/M_1)t} + \ldots + Q_n e^{(-Q_n/M_n)t}}{Q_1 + Q_2 + \ldots + Q_n}\right]$$

The value for $V_{comb}$ may now be substituted in the familiar Kety-Schmidt equation 2 to yield equation 5:

$$\frac{Q}{M} = \int_0^t \frac{V_{comb} S}{(A - V_{comb})} dt$$

In this equation, $S$ is the partition coefficient.

Upon integration and rearrangement, this yields equation 6. The partition coefficient which is close to 1.00 can be omitted.

$$\frac{Q}{M} = \frac{Q_1 + Q_2 + \ldots + Q_n - Q_1 e^{(-Q_1/M_1)t} - \ldots - Q_1 e^{(-Q_1/M_2)t} - \ldots - Q_1 e^{(-Q_1/M_n)t}}{M_1 + M_2 + \ldots + M_n - M_1 e^{(-Q_1/M_1)t} - \ldots - M_1 e^{(-Q_1/M_2)t} - \ldots - M_1 e^{(-Q_1/M_n)t}}$$

Equation 6 describes the manner in which the blood flow per unit of tissue mass seems to vary with time as equilibrium is approached. Note that at infinite time (complete concentration equilibration) all terms containing exponential functions vanish, and an identity results.

The mathematical model used in the derivation of these equations incorporates two simplifying assumptions in addition to those already noted. The first of these is that the individual tissue masses do not communicate with each other through extravascular channels. The second is that there are no losses of the measuring substance from the surface of the entire organ. It will be appreciated that the effect of the first assumption is to cause the mathematical model to be less homogeneous than the organ it describes. The second assumption is, in a sense, a corollary of the first, for if concentration equilibrium is rapid throughout the tissue, it must be rapid from the tissue outward. The effect of making the second assumption, however, is to make the mathematical model more homogeneous than the tissue which it describes. Since the geometry of the individual tissue masses is unknown (and may change from moment to moment) it is impossible to assess the relative importance of these two factors. The quantitative aspects of the subsequent arguments must therefore be taken only as illustrative examples.

Diffusion from the surface of the measured organ has the further effect of confounding the anatomical boundary of the organ with the virtual diffusion boundary. This source of error may be considerable in the case of an organ separated from other structures by fluids in which the measuring substance is soluble. This type of error is not, however, considered in the analysis developed here.

**Discussion**

Equation 4 shows that the venous blood concentration approaches equilibrium by multiple exponential processes. It will, however, be noted that equilibrium deficiencies are related to blood flows ($Q$) and perfusion ratios ($Q/M$) and never to tissue masses as such. That is to say that concentration equilibrium with respect to the blood may be almost complete, whereas poorly perfused masses of tissue of almost any size may remain unequilibrated.

Equation 6 shows that the estimated blood
flow per unit of mass changes with time. At the time when the last exponential approach to equilibrium dominates the process, the deficiency in the denominator of the ratio will be greater than the deficiency in its numerator by the ratio $M_n/Q_n$. (The tissue masses, it will be recalled, are arranged in order of diminishing perfusion ratios, $Q/M$.) Thus, if there is a very large tissue mass ($M_n$) having a very small perfusion ($Q_n$), the value for estimated flow per unit of mass will be erroneously high at all times short of complete equilibration. The venous blood, however, may not reveal the existence of a defect in equilibration, as indicated in the preceding paragraph.

These points may be illustrated by an analysis of the results of a hypothetical flow measurement made with nitrous oxide.

Figure 2 shows a plot of samples of arterial and venous blood perfusing a hypothetical tissue when venous blood approaches concentration equilibrium at first rapidly and then more slowly. The venous concentration at ten minutes is represented as 95 per cent of the arterial. The secondary approach to equilibrium is indicated as a very slow one.

The calculated flow at 10 minutes is given by the value for $V_{10}$ divided by the area $O, O, A_{10}, V_{10}$. At 15 minutes the increase in area $A_{10}, V_{15}, V_{25}, A_{15}$ is quite small. Accordingly, the flow is essentially the same whether it is calculated at 10 or 15 minutes. Note, however, that the concentration deficit, though small, is persistent. On a longer time base it enters materially into the calculation. In the illustrated case at 120 minutes, the arteriovenous integral, $O, O, A_{120}, V_{120}$ is twice as great as that at 10 minutes. The calculated blood flow at 120 minutes is only half as great as that at 10 minutes. This indicates that despite apparent stability of flow at successive time intervals close together and near concentration equilibrium between arterial and venous blood, a prolongation of the time base may serve to reveal massive equilibration defects.

The same principles will now be demonstrated numerically for a hypothetical organ consisting of three types of tissue with perfusion rates of 1.0, 0.1 and 0.01 ml./Gm./min., distributed through masses of 100, 100 and 300 Gm. respectively. These figures might represent a tissue such as a cold active forearm. The muscles might correspond to the most actively perfused tissue, the bone to the moderately perfused tissues, and the connective tissue and skin to the least perfused tissues. It will be appreciated that we have deliberately made the least perfused portion of the tissue the largest in mass. Of course, it is precisely in this type of situation (e.g. in atherosclerosis, thrombosis and infarction) that measurements of regional blood flow are presumably of the greatest clinical significance.

The time course of the mixed venous blood concentration of nitrous oxide (calculated from equation 4) is given in the second column of table 1.

Note that within 6 minutes the mixed venous blood is at 93 per cent of its equilibrium concentration, and that by 10 minutes it has reached 95 per cent of the equilibrium value. On cursory inspection it would appear quite legitimate to
assume that equilibration was complete at ten minutes.

But it must be recalled that the concentration deficit though small, is persistent and represents a large mass of tissue which is hardly penetrated at 10 minutes. In this case, the mass is almost half that of the total organ. This is shown in the third column of the Table which presents the blood flow per unit of equilibrated tissue mass as measured in the Kety-Schmidt equation as a function of time from 0 to 20 minutes and at equilibrium. The values are obtained from equation 6. The flow values decline rapidly at first, signifying rapid equilibration of the well vascularized tissues; then very slowly, indicating the slow penetration of the poorly vascularized portion of the organ. At 10 minutes, when the venous concentration is 95 per cent of its equilibrium value, the organ is less than half equilibrated.

The apparent flow decreases continually in the period from 10 to 20 minutes in the numerical example given here. However, the decrease is so gradual that in any real experiment, where biological and analytic inaccuracies make the measurements uncertain, it might reasonably be interpreted to indicate that calculated flow constancy (i.e., equilibrium) had been achieved. Not only is there a quantitative defect in the estimate, but even directional errors in the evaluation of changing blood flow can be made.

Consider, for example, the consequences of reducing blood flow in the moderately perfused portion of the organ considered above to the level prevailing in the poorly perfused area.

The fourth column of table 1 shows how the mixed venous blood from such an ischemic organ would change with time. The values are again calculated from equation 4. During ischemia the venous blood of this organ evidently comes into near equilibrium with the arterial blood more rapidly than when it is normally perfused. On the other hand, the calculated flow values, given in the fifth column, indicate clearly that the organ equilibrium is extremely defective.

The deficiency of the equilibrium at 10 minutes is more evident in the ischemic organ than in the normally perfused one. Yet, it is likely that an experimenter might consider that equilibrium had been attained in ten minutes, since the apparent flow changes after this time are relatively slight. He might attribute this to experimental errors or contamination of the organ blood by venous blood from some other source. The flow through the ischemic organ would then be estimated at 10 minutes as 0.73 ml./Gm./min., compared to the value in the normal tissue of 0.52 ml./Gm./min. Hence, the blood flow through the ischemic organ would appear to have increased. Evidently, at true equilibration, this error would not be made, for it is due solely to the relatively great defect in equilibration of the ischemic tissue at the time chosen for the measurement. Nevertheless, in the absence of an adequate criterion for the recognition of the equilibrium defect, it is impossible to see how the error could be avoided.

Similarly, it can be shown that an increase in blood flow through the moderately perfused area would appear as a decrease in the total organ flow when measured by the nitrous oxide method.

The examples chosen for illustration do not demonstrate maximal errors. The error becomes greater as the proportion of poorly perfused tissue to well perfused tissue increases. If, for example, an arteriovenous shunt makes up 1 per cent of the mass of an otherwise poorly perfused organ, the venous concentration curve

\[
\text{TABLE 1.—Approach of Mixed Venous Blood to Concentration Equilibrium and Calculated Blood Flows in a Non-Homogeneous Organ}
\]

<table>
<thead>
<tr>
<th>Time (Minutes)</th>
<th>Normal</th>
<th>Ischemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appear. Flow ml./Gm./min.</td>
<td>Appear. Flow ml./Gm./min.</td>
<td></td>
</tr>
<tr>
<td>(V_{\text{normal/4 per cent}})</td>
<td>(V_{\text{normal/4 per cent}})</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>.83</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
<td>.71</td>
</tr>
<tr>
<td>6</td>
<td>93</td>
<td>.64</td>
</tr>
<tr>
<td>8</td>
<td>94</td>
<td>.59</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>.55</td>
</tr>
<tr>
<td>12</td>
<td>96</td>
<td>.52</td>
</tr>
<tr>
<td>15</td>
<td>96</td>
<td>.49</td>
</tr>
<tr>
<td>20</td>
<td>97</td>
<td>.45</td>
</tr>
<tr>
<td>Infinite</td>
<td>100</td>
<td>.23</td>
</tr>
</tbody>
</table>

* Calculated from equation 4.
† Calculated from equation 6.
will be so completely dominated by blood coming from the shunt that the poorly perfused portion of the organ (99 per cent of the mass) will scarcely manifest itself in the concentration curve. If the poorly perfused portion of the organ had a perfusion rate per gram 1/1000 as great as that of the shunt, the apparent flow value per gram of tissue obtained by the nitrous oxide method would represent only the flow per gram of the shunt portion. To consider that this represents the whole organ would result in a flow estimation 100 times too great. Yet there is no obvious procedure for either the detection or correction of this error.

The "tissue" perfused by an arteriovenous shunt is, of course, the blood content of the shunt itself. It is often forgotten that the blood contained in an organ enters as materially into the Kety-Schmidt calculation as does the organ itself. Arteriovenous shunts are of special interest in this connection because there the equilibration of their "tissue" is hardly likely to influence the equilibrium of the remainder of the organ and yet, in a poorly perfused tissue they may dominate the venous concentration curve and the flow calculation completely.

One point deserves further emphasis. It was recognized originally by Kety and Schmidt* that the approach of the venous blood to concentration equilibrium may be a multiexponential process. It is not commonly appreciated, however, that analytical inaccuracy and phenomenological inconstancy which exist in the systems used by experimental biologists prevent the accurate distinction between multiexponential and single exponential processes. "Homogeneity" of an organ with respect to perfusion cannot be established by the existence of a "satisfactory" fit of data to a single exponential function.

Concentration equilibrium for nitrous oxide has been demonstrated to occur within ten minutes in several healthy dog brains. It is not certain whether this occurs in the same time interval in either normal or diseased human brains or other regions of man. Until convincing evidence is put forward in man that equilibration occurs regularly in the time of the estimate and in the condition investigated, the significance of nitrous oxide measurements of regional blood flow remains in doubt.

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