DMO Method for Intracellular pH

Neely, Whitmer, and Ravetto in your December issue (Circ. Res. 37: 733-741, 1975) claim to have measured intracellular pH in ischaemia by use of 4-C-5,5-dimethyl-2,4-oxazolidinedione (the DMO method); others have also used this technique. Though a knowledge of intracellular pH is of great importance to the physiology and biochemistry of ischaemia I wish to suggest that the DMO method should not be used in ischaemia and that the results may be inaccurate.

The method depends on the assumption that DMO, a weak acid, distributes itself across the cell membrane in a manner related to the intra- and extracellular pH. The weak acid is ionised on each side of the membrane which is assumed to be very much more permeable to the unionised than the ionised form of the weak acid. If a steady state is reached it is possible to calculate a value for the “overall” or “average” intracellular pH (Waddell and Butler, J. Clin. Invest. 38: 720-729, 1959).

The method is inapplicable to ischaemia for the following reasons.

1. No new steady-state is usually achieved because the tissue is in the process of dying. In the paper of Neely et al. systolic pressure, glucose utilisation and CO₂ production were measured and were changing.

2. Ischaemia may alter the permeability of the membrane to the ionised form of the weak acid and the extent to which this may occur is unknown and may alter with time. Thus, the essential assumption of the method may become invalid.

3. The value used for extracellular pH and extracellular DMO concentration in the calculation of intracellular pH cannot be determined since by definition the tissue has a limited perfusion. The pH or the DMO concentration of the venous effluent may be different in an unknown manner to that of the extracellular fluid in the ischaemic area.

4. There is doubt whether the use of isotopic markers to determine the extracellular space is still valid in severe ischaemia.

5. Analysis of the efflux of 4-C-DMO from the rabbit myocardium (Poole-Wilson, Clin. Sci. Mol. Med. 50: 9P, 1976) indicates that there is a slowly exchanging compartment in the myocardium which would probably not have been detected by the methods used by Neely et al. The short equilibration time may lead to spurious results and may itself be altered in ischaemia.

It is no defence of a theoretically unsatisfactory method to claim that it gives similar results, to better or equally inaccurate alternative methods. Unfortunately most of the problems mentioned above will contribute to an apparent intracellular acidosis and thus provide the authors with the result they were probably anticipating. For example if the cell membrane became totally permeable to both the ionised and un-ionised forms of DMO then the value of intracellular pH would approach that of the extracellular pH. That is exactly what is reported in Table 2 of the above paper.

I would suggest that unless the DMO method can be validated in ischaemia, it should not be used under such circumstances. Results such as those of Neely et al. may be misleading and should be regarded with scepticism.

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REPLY TO THE ABOVE LETTER

In response to Dr. Poole’s “Letter to the Editor” concerning the use of DMO to estimate intracellular pH in ischemic hearts, I am in complete agreement on some points, but disagree on others.

I agree that the data derived from this procedure should be interpreted with caution, especially when obtained from ischemic myocardium. This argument also applies to all procedures available for estimation of intracellular pH. Nonetheless, the technique should not be ruled out as useless as Dr. Poole implies until either (a) a better technique is developed, or (b) the possible problems that Dr. Poole outlines are shown to be reality and to negate the use of this procedure. Dr. Poole makes several points that may or may not apply to the work with ischemic hearts.

1. True, the DMO procedure only measures an average intracellular pH. To my knowledge, however, there exists no procedure which will allow the pH of individual cellular compartments to be determined. An average value can be useful and perhaps is better than none.

2. True, ischemic tissue is a dying tissue and is not in a "steady-state." Mechanically, and metabolically the tissue is deteriorating, but this also applies to every in vitro preparation used. However, the rate of deterioration is slow in comparison to the time required for DMO equilibration. Although the value for intracellular pH obtained at any given time after inducing ischemia may not be the absolute value at that time, it probably represents an average change that has occurred up to that time minus the time required for DMO equilibration. Since it takes 2 minutes for DMO equilibration to occur, pH values obtained at 20 minutes should reflect the pH that existed at about 18 minutes.

3. True, the permeability of the membrane may be altered and based on the release of enzymes from ischemic tissue, most likely is altered in some way. However, there is no evidence that the membrane becomes more permeable to weak acids either ionized or un-ionized. In fact, since lactate accumulates to very high levels in the tissue, the contrary may be true. The cells do not become freely permeable to other substances such as glucose. In the absence of insulin, free intracellular glucose cannot be detected.
4. True, one must know extracellular pH to calculate intracellular pH by this procedure. It was pointed out by us that it is impossible to measure the true interstitial pH in the ischemic heart since a gradient of H⁺ must exist between interstitial and vascular fluids. Use of the coronary effluent pH must overestimate the interstitial pH. This would, therefore, overestimate the intracellular pH calculated from DMO distribution. However, this argument should not apply to the value used for extracellular DMO. In this case, the tissue was fully equilibrated with DMO before inducing ischemia and the perfusate concentration of DMO was maintained constant throughout the period of ischemia. There was no difference between arterial and venous concentration of DMO as was true with pH. Therefore, there is no reason to believe that the interstitial concentration of DMO was significantly different than the perfusate concentration.

5. Dr. Poole states that "there is doubt whether the use of isotopic markers to determine extracellular space is still valid in severe ischemia." The basis for this doubt, however, is not disclosed. If there is doubt, one would expect that it is because the cell membrane might become leaky to the extracellular marker and, therefore, would overestimate the extracellular space and underestimate the intracellular space. There is no evidence that sorbitol, the extracellular marker used, actually penetrated the cell membrane.

6. Dr. Poole states that in his studies on efflux of 14C-DMO a slowly exchanging compartment was indicated. Analysis of the efflux of several compounds from the myocardium shows a slowly exchanging compartment. Sorbitol, for example, has a slowly exchanging compartment (Morgan et al., J. Biol. Chem. 236: 253, 1961) which may represent extracellular fluid located in the transverse tubules of the sarcoplasmic reticulum. However, this compartment is small in comparison to total extracellular space and, in addition, it should be fully equilibrated with DMO under the conditions used in the studies referred to by Dr. Poole.

7. Dr. Poole states if the cell membrane became totally permeable to both the ionized and un-ionized forms of DMO, the value of intracellular pH would approach that of extracellular pH. This change in pH was observed in the experiments referred to. However, if it were simply a problem of the membrane becoming freely permeable to DMO during ischemia, the intracellular pH which is normally about 7.0 would be expected to increase to the pH of the arterial perfusate (7.4). Just the opposite occurred. The pH in both compartments decreased to values which were dependent on the degree of restriction in coronary flow; the lowest values obtained were about 6.8. In the studies referred to, the coronary effluent pH was measured with a pH electrode. There is no reason to doubt the validity of this procedure. The coronary effluent pH dropped from 7.25 in control hearts to 6.8 in severely ischemic hearts. It is difficult to understand how the perfusate pH could decrease from 7.4 (arterial) to 6.8 on one passage through the heart unless the cells become acidic and exported H⁺. As mentioned above and as pointed out in the paper, the coronary effluent pH must reflect the amount of H⁺ picked up from the interstitial space and indicates that the pH of this space cannot be higher; most likely it is lower than the coronary effluent pH. If the membrane does become freely permeable to weak acids it most likely also becomes freely permeable to H⁺ so that the pH's of the two compartments would, in fact, equilibrate.

The problems encountered in estimating intracellular pH in a globally ischemic preparation in which some minimal rate of blood flow is maintained should be less severe than in a model of regional ischemia. With regional ischemia, areas of tissue with essentially zero blood flow are present, and it may not be possible to achieve DMO distribution prior to induction of ischemia.

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Circ Res. 1976;39:141-142
doi: 10.1161/01.RES.39.1.141

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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/39/1/141.citation

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