The results obtained so far in the preservation of frozen and liquid blood at − 3° C are sufficiently encouraging to justify further studies, which are now under way.

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A "Free Manometer" Method of Using the Standard Warburg Apparatus[1]

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In the Warburg apparatus, as commonly employed, a gas reaction occurs within a closed system of constant volume. The temperature being held constant, pressure changes (read on the open manometer arm) are related by a linear function to the volume of gas evolved or taken up. This function is known as the "vessel constant" and depends upon gas-space volume, fluid volume, temperature, solubility of the reacting gas, atmospheric pressure, and manometer fluid density (*1*).

It can be shown that within the limits of accuracy generally accepted in the Warburg technique (±1%), a constant volume is not in fact required; that if both pressure and volume are permitted to vary, a linear function (vessel constant) is still obtained; and that consequently the repeated leveling of manometer fluid, whereby a constant volume is maintained, can be eliminated.

The free manometer technique presents the following unique features: automatic recording, by a time-controlled camera, becomes feasible; certain small errors inherent in the process of leveling the manometer fluid are eliminated; readings can be made more rapidly, more frequently, and with greater ease, and arithmetical steps are greatly simplified; the capacity of the standard manometer is substantially increased, while its sensitivity is correspondingly reduced. A total gas change three to four times as great as by the constant volume method can be measured without resetting the manometer. This increased capacity has been found desirable in at least two applications: in measuring substrate oxidation by cells or tissues in the face of a high endogenous rate, and in studying the protracted time course of enzyme-substrate reactions.

The fluid on the vessel side of the manometer is set initially to the manometer midpoint and subsequent readings are made on this same arm of the manometer. The fluid adjustment is not touched again after the initial setting. A reciprocal and equal change occurs in the fluid levels of the two arms as a reaction proceeds, but the fluid in the open arm is ignored. Thermobarometer corrections, provided they are not exceedingly large (< 5 mm), are made in the usual fashion, but the thermobarometer vessel must contain the same volume of fluid as the other vessels.

If the vessel constants are determined empirically no special problems arise. If they are calculated from mercury calibration of the gas space an additional factor M is required. M is the linear volume of the manometer (cu mm/mm) and is obtained automatically if one follows the calibration method suggested by Burris (*3*, p. 50), filling the manometer first to a point above, and then to a point below the midpoint mark. The full vessel constant equation is:[2]

$$k = \left[\frac{V P_o + V_f RT\alpha_x}{P_o RT}\right]\left[\frac{p_x P_o M}{V P_o + V_f RT\alpha_x} + \frac{p_z P_o M}{V P_o + V_f RT\alpha_z} + 2\right]$$

It should be noted that the only variables requiring calibration are V and M. The observed change in level (mm) on one arm of the manometer multiplied by the vessel constant gives moles of gas reacting at NTP.

The full equation must be used for CO_2, but O_2 and other gases of low solubility can be determined with the following simplified vessel constant:

$$k = \frac{2V + M\,(P_o - p_w)}{RT} + V_f\,\alpha_x\left[\frac{2V + M\,(P_o - p_w)}{V P_o}\right]$$

where p_w is the vapor pressure of water (mm manometer fluid) at temperature T.

The method described here has been in use in this laboratory for some time. Replicate determinations of CO_2 and O_2 by free manometer and constant volume techniques under diverse conditions agree satisfactorily. It has proved convenient to calculate vessel constants by both methods and to use the free manometer technique routinely, reserving the constant volume technique for those occasions when high sensitivity is desired. Full theoretical and practical details of the method will be published elsewhere (*2*).

References

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[2] V = gas space (cu mm)
M = manometer factor (cu mm/mm)
$R = 8.21 \times 10^4 \times P_o$
T = absolute temperature
p_x = initial partial pressure of reacting gas (mm manometer fluid)
p_z = initial partial pressure of inert gas (mm manometer fluid)
P_o = atmospheric pressure (mm manometer fluid)
V_f = fluid volume (cu mm)
α_x, α_z = solubility coefficients of the gases (moles/cu mm at P_o. T)