Assay of inert gas contamination in studies of hydrostatic pressure effects

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Li M, Hong SK, Paganelli CV, Hogan PM. Assay of inert gas contamination in studies of hydrostatic pressure effects. Undersea & Hyperbaric Med 1993; 20(2):163–169.—In hyperbaric tissue studies it is common to use gas pressure as a means for increasing the hydrostatic pressure of the preparation. When this is done, one must take the necessary precautions to avoid gas contamination of the tissue. In the present study, we demonstrate a) methods for calculating the gas contamination using particular materials in the perfusion system, and b) a technique for isolating the fluid environment from contamination by the compressing gas in a flow-through tissue chamber. The present work is exemplified with a specific apparatus, but the general principle is applicable to other studies.

hydrostatic pressure, inert gas, inert gas contamination, gas diffusion

In hydrostatic pressure experiments on tissues and cells, an inert gas such as He or N₂ is commonly used to pressurize the system. Hydrostatic compression of the contents of the tissue bath is typically achieved through the interface between the perfusate flowing from the enclosed bath and the chamber gas. Since the outflow orifice is away from the bath well, chamber gas is prevented from diffusing into the aqueous phase of the bath (1). However, where it is necessary to move the tissue chamber in and out of the hyperbaric vessel, a flexible plastic tube is often used to connect the tissue chamber to the fluid inflow line inside the pressure vessel. Gas can reach the tissue bath by diffusion across this tubing. In such cases, the contaminating gas may influence the system under investigation, and such experiments in the strictest sense may not represent the effects of hydrostatic pressure alone. It therefore becomes imperative to avoid or be aware of possible gas contamination of the tissue perfusate. In the present study, we used N₂ to demonstrate how such gas contamination through the plumbing system can be evaluated, and in addition, we show a simple way to assay the amount of contaminating gas in the tissue bath under pressure.

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MATERIALS AND METHODS

The methods and materials used in the present study were identical to those used in previous experiments performed to study effects of high hydrostatic pressure on ion transport across epithelial tissue (2). In brief, a tissue chamber was placed in a hyperbaric pressure vessel. A continuous flow of solution (5 ml · min⁻¹) was delivered to the tissue chamber by a plumbing system that included a solution reservoir, a pump, and a delivery tube. The reservoir was open to the atmosphere and connected to the pump. The pump provided a constant flow rate that was unaffected by hydrostatic back-pressure up to 400 atm. The delivery tube had two parts: approximately 122 cm of stainless-steel tubing which spanned the distance from the pump to the hyperbaric pressure vessel; and inside the vessel, the stainless-steel tubing was connected to 61 cm of polyethylene tubing (Clay Adams, Intramedic, 0.057 cm inner radius, 0.26 cm outer radius, 0.62 cm³ vol for fluid delivery). The distal end of the polyethylene tubing was inserted into the delivery port of the tissue chamber. A drainage tube from the tissue chamber drained into an open reservoir inside the pressure vessel. The potential pathway for N₂ leakage into the tissue bath is the 61 cm of polyethylene tubing. The flow stream exiting the tissue chamber prevents the entry of gas through the outflow tube.

Pressure inside the hyperbaric vessel was raised with N₂ to 100 atm. The net increase in P₉ was about 99 atm. As mentioned earlier, the perfusion drainage tubes from the tissue chamber emptied into an open reservoir inside the pressure vessel, so that pressure was transmitted hydrostatically to the tissue.

RESULTS

Theoretical estimation of N₂ leakage through perfusate delivery tube

The resultant rise in partial pressure of N₂ (ΔPN₂) in the perfusate due to N₂ diffusion across the fluid delivery tubes can be calculated using known values for the diffusion coefficient of N₂ across the fluid delivery tubing, the geometry of the tubing, and the fluid flow rate.

Dimensions of the tubing used in our experiment are shown in Fig. 1. Based on experimental conditions, the net increase of N₂ partial pressure outside the fluid delivery tube is constant at 99 atm. Because the diffusion of N₂ across the tube wall is slower than the diffusion of N₂ into the perfusate, we assume that the partial pressure of N₂ within the tube is uniform along radial lines [PN₂(r,χ) = PN₂(χ)]. We also assume that the tube carries a fluid having a flat velocity profile [V(r) = V]. The problem to be solved in N₂ mass transfer (JN₂) into a flowing fluid through the wall of a fluid delivery tube. The model for the calculation is shown in Fig. 1.

The continuity Eq. 3 for this problem is

\[
\frac{dn}{dt} = F(\chi - \Delta\chi/2)\pi r_s^2 - F(\chi + \Delta\chi/2)\pi r_s^2 + J(\chi)2\pi r_s\Delta\chi
\]  

(1)
where:

\[ n = \text{number of moles in the differential volume}, \Delta \chi \pi r_2^2 \]

\[ F(\chi) = \text{convective flux across the flat face at } \chi = PN_2(\chi)V \alpha \]

\[ V = \text{flow velocity of fluid} \]

\[ r_2 = \text{the inner radius of the tubing} \]

\[ J = \text{diffusive flux across the tubing wall} \]

\[ \alpha = \text{solubility of N}_2 \text{ in water} \]

\[ J(\chi) = \frac{D[\Delta PN_{2\infty} - \Delta PN_2(\chi)]}{r_2 \ln(r_1/r_2)} \tag{2} \]

where:

\[ D = \text{diffusion coefficient of N}_2 \text{ across the fluid delivery tubing} \]

\[ \Delta PN_{2\infty} = \text{rise in N}_2 \text{ partial pressure outside of the tube} \]

\[ \Delta PN_2(\chi) = \text{rise in N}_2 \text{ partial pressure at } \chi \]

\[ r_1 = \text{the outer radius of tubing} \]

then:

\[ \frac{\Delta n}{\Delta t} = \pi r_2^2 V \left( -\frac{2\Delta PN_2 \alpha}{2 \chi} \right) \Delta \chi + \left( \frac{2\pi D \alpha [\Delta PN_{2\infty} - \Delta PN_2(\chi)]}{\ln(r_1/r_2)} \right) \Delta \chi \tag{3} \]

In the steady state, \( \frac{dn}{dt} = 0 \), therefore

\[ \frac{d\Delta PN_2}{d\chi} = \frac{2D}{r_2^2 V \ln(r_1/r_2)} [\Delta PN_{2\infty} - \Delta PN_2(\chi)] \tag{4} \]

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**FIG. 1**—Model of calculation and geometry of the tubing. \( L \), total length of the tube (61 cm); \( r_1 \), outer radius of the tube (0.26 cm); \( r_2 \), inner radius of the tube (0.057 cm); \( V \), velocity of fluid; \( J \), \( N_2 \) flux across the tubing wall. \( PN_2(\chi) \): \( PN_2 \) at distance \( \chi \) along the tube.
If $\Delta P_{N_{20}} = \Delta P_{N_{2x=0}}$ then

$$\frac{\Delta P_{N_{2}(x)}}{\Delta P_{N_{2}}(x)} = 1 - \left(1 - \frac{\Delta P_{N_{20}}}{\Delta P_{N_{2}}(x)}\right)e^{-\lambda x}$$  \hspace{1cm} (5)$$

where:

$$\lambda = \frac{r_s^2 v \ln(r_1/r_2)}{2D}$$  \hspace{1cm} (6)$$

For our experiment, $\Delta P_{N_{2m}}$ was 99 atm; $\Delta P_{N_{20}}$ was 0 atm, $r_1$ was 0.26 cm, and $r_2$ was 0.057 cm. With the flow rate of 5 ml·min$^{-1}$ and this geometry flow velocity, $V$, was 8.13 cm·s$^{-1}$. With a known permeability of polyethylene to N$_2$ (KN$_2$) equal to $2 \times 10^{-3}$ cm$^3$ N$_2$ STP/(min·m$^{-2}$·atm$^{-1}$·cm$^{-1}$) (4), D is equal to KN$_2$·RT = 3.64 $\times$ 10$^{-9}$ cm$^2$·s$^{-1}$ (where R = 2.785 cm$^3$·torr$^{\circ}$K·cm$^{-3}$ STP; T = 298$^{\circ}$K).

$\Delta P_{N_2}$ (61 cm) ($\Delta P_{N_2}$ of the fluid at the end of the polyethylene tubing where it is connected to the tissue chamber) is calculated as follows:

$$\lambda = \frac{(0.057 \text{ cm})^2 \cdot 8.16 \text{ cm} \cdot \text{s}^{-1} \cdot \ln(0.26/0.057)}{2 \cdot 3.64 \times 10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}} = 5.5 \times 10^6 \text{ cm}$$  \hspace{1cm} (7)$$

$$\Delta P_{N_2} (61 \text{ cm}) = 99 \text{ atm} \cdot (1 - e^{-64\text{cm}/5.5 \times 10^6\text{cm}}) = 0.0011 \text{ atm}$$  \hspace{1cm} (8)$$

Thus, a calculation based on our plumbing system suggests that the inert gas contamination is insignificant.

Figure 2 shows the gain in P$_{N_2}$ of the perfusate at various perfusion rates. The five curves represent five fluid delivery tubes which have the same length but different

FIG. 2—Gain in P$_{N_2}$ of the perfusate as a function of perfusion rate. Curves were calculated with a unit length of tube of 61 cm.
GAS CONTAMINATION DURING HYDROSTATIC PRESSURE

radii (Table 1). As can be seen, perfusion rate is an important determinant of the gain in \( PN_2 \). Tubes A, B, and C (Table 1) have the same wall thickness but an increasing inner radius; therefore at the same perfusion rate there is a decreasing fluid velocity from tubes A to C and a greater gain in \( PN_2 \) in the perfusate. Thus, the rate-limiting factor of gas contamination is the linear fluid velocity inside the tube. Tube D has the same inner radius as tube A, but D has a thinner wall and therefore a higher gain in \( PN_2 \) in the perfusate at the same perfusion rate. This is expected because gas diffuses more easily across the thinner wall. Tube E has the same inner radius as tube C, but a very thin wall. Because tube E has both a slow linear fluid velocity and a thin diffusion barrier, it has the highest contamination rate.

Experimental assessments of \( \Delta PN_2 \) in the tissue bath

Because there may be some unknown pathways other than the fluid delivery tubes by which \( N_2 \) may reach the tissue bath, \( \Delta PN_2 \) of the tissue bath can also be easily assessed by the technique described below.

The experimental design for \( PN_2 \) assay is illustrated in Fig. 3. A 20-ml glass syringe located outside the pressure vessel was connected to the tissue bath through a liquid-filled, stainless-steel sampling tube (0.25 ml vol) that penetrated the pressure vessel wall. A sample of the tissue bath was drained by the sampling tube through a microvalve into the syringe. The valve separated the sampling tube into two segments: an inner segment (0.15 ml) which was in contact with the tissue bath, and an outer segment (0.1 ml) which was not. The valve was closed during compression.

Experimental procedures were as follows: Throughout the experiment, a continuous flow of solution (5 ml \( \cdot \) min \(^{-1} \)) was delivered to the tissue chamber. Before compression, the sampling tube was filled with the same perfusing solution. The sampling valve was then closed and compression began. When the tissue had been compressed to 100 atm for 2 h, the valve was opened, allowing pressure to force the bath fluid through the tube and into the syringe. Fine control of the fluid flow into the syringe was obtained by adjusting the valve. In general, the speed of drawing a sample solution should not exceed the perfusion rate. We first withdrew and discarded the dead space volume from the sampling tube. In another 5 min, 18 ml of sample solution from the tissue bath had been drawn into the syringe. At least 1 h was allowed for the dissolved \( N_2 \) to evolve. The volume of gas in the syringe was then measured, and \( \Delta PN_2 \) of the tissue bath calculated by dividing the volume of \( N_2 \) dissolved in each milliliter of sample solution with the known solubility of \( N_2 \) in

<table>
<thead>
<tr>
<th>Tube</th>
<th>( r_1 ), cm</th>
<th>( r_2 ), cm</th>
<th>Wall Thickness, cm</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>0.20357</td>
<td>0.00057</td>
<td>0.203</td>
</tr>
<tr>
<td>B</td>
<td>0.26</td>
<td>0.057</td>
<td>0.203</td>
</tr>
<tr>
<td>C</td>
<td>10.203</td>
<td>10.0</td>
<td>0.203</td>
</tr>
<tr>
<td>D</td>
<td>0.00067</td>
<td>0.00057</td>
<td>0.0001</td>
</tr>
<tr>
<td>E</td>
<td>10.0001</td>
<td>10.0000</td>
<td>0.0001</td>
</tr>
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water \((0.0155 \text{ ml N}_2 \cdot \text{ml H}_2\text{O}^{-1} \cdot \text{atm}^{-1})\). Results from our particular experiment showed that there was no visible volume of gas coming out of the sampled solution.

Perfusion drainage tubes from the tissue chamber emptied into an open reservoir inside the pressure vessel (see Materials and Methods). Drainage fluid in the reservoir was exposed to the inert gas used to pressurize the chamber and should have a high \(\Delta \text{PN}_2\). By drawing sample solutions from this reservoir the minimal amount of gas visible to the naked eye was determined. After the commencement of compression to 100 atm, we began to draw samples from the reservoir. The experimental procedures were the same as above. We first drew off the tubing dead space and then drew 1 ml of the solution directly from the reservoir under pressure. No visible bubbles were detected by eye until the chamber pressure reached 50 atm. About 0.002 ml gas/ml solution was visible immediately after collecting the solution. The corresponding \(\text{PN}_2\) of this volume of gas was 0.13 atm. We drew a sample when pressure in the chamber reached 100 atm, and three more samples at 30 min, 1 h, and 2 h later. Results from these experiments showed that there were 0.08, 0.28, 0.34, and 0.44 ml of gas/ml solution at 0 min, 30 min, 1 h, and 2 h after the \(\text{PN}_2\) in the pressure vessel reached 100 atm. The corresponding \(\Delta \text{PN}_2\) of the drainage fluid in the open reservoir was 5.26, 18, 21.9, and 28.4 atm.

By this method, the volume of gas bubbles just visible to the naked eye is about \(1-2 \times 10^{-3}\) ml. Because no bubble was observed in the 18-ml sample solution drawn directly from the tissue bath, it may be assumed that the volume of \(\text{N}_2 \cdot \text{ml}^{-1}\) of sample solution is smaller than \(0.55-1.1 \times 10^{-3}\) ml, from which one can deduce a \(\Delta \text{PN}_2\) in the bath solution of less than 0.0035 atm.
DISCUSSION

The calculations performed with the equation for N₂ diffusion across the wall of a cylindrical tube into a flowing column of fluid show that the expected amount of inert gas contamination in the tissue bath is 0.0011 atm. This low value is reasonable because the fluid delivery tube in our system has a small volume relative to the flow rate and thus a high fluid velocity which minimizes the time for gas uptake. Moreover, this calculated value is consistent with the value estimated from the observed absence of visible gas bubbles, indicating that the P N₂ of the tissue bath was below 0.0035 atm.

It is known that certain cellular functions can be affected by inert gases. For instance, at 25°C, N₂ in the range of 5–15 atm inhibited rat intestinal Na-K-Mg ATPase activity and stimulated this enzyme when at higher than 15 atm (5). At 37°C, a progressive increase in the degree of inhibition of Na-K-Mg ATPase is noted in the P N₂ range of 8–19 atm. N₂ pressures greater than 19 atm tended to activate the ATPase. Helium at 37°C stimulated the Na-K-Mg ATPase in the range of 1–6 atm. An increasing inhibition of the same enzyme was noted in the range of 7–17 atm He. Another study showed that He, at pressures above 137 atm, affected the excitability of excised rat preganglionic sympathetic nerves (6). In another study, it was noted that certain effects of hydrostatic pressure on mammalian cardiac tissue were counteracted by dissolved inert gases in the perfusate (7). Compression of mouse sinus nodes from 1 to 150 atm of hydrostatic pressure reduced the beating frequency significantly (7). When at a hydrostatic pressure of 150 atm the perfusate was saturated with N₂ at 70 or 140 atm or with He at 140 atm, the beating frequency was increased significantly.

Thus, if in pure hydrostatic pressure studies the calculated or the measured inert gas partial pressure in the tissue perfusate is high, the effects noted may be due to pressure per se, or to the contaminating inert gas, or to a combination thereof. Therefore, one must take precautions to avoid or minimize gas contamination. We have shown that significant gas contamination can be effectively avoided by increasing the fluid velocity inside the delivery tube, which is achieved by either increasing the perfusion rate or by decreasing the inner diameter of the tube. Moreover, increasing the wall thickness or decreasing the surface area of the perfusate delivery tube will help to prevent significant gas contamination.

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References