A simplified noninvasive method to measure airway blood flow in humans

Adam Wanner, Eliana S. Mendes, and Neal D. Atkins

Division of Pulmonary and Critical Care Medicine, Department of Medicine,
University of Miami Miller School of Medicine, Miami, Florida

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Wanner, Adam, Eliana S. Mendes, and Neal D. Atkins. A simplified noninvasive method to measure airway blood flow in humans. *J Appl Physiol* 100: 1674–1678, 2006; doi:10.1152/japplphysiol.01349.2005.—Our laboratory has previously developed and validated a noninvasive soluble gas uptake method to measure airway blood flow (Qaw) in humans (Onorato DJ, Demirozu MC, Breitenbüberer A, Atkins ND, Chediak AD, and Wanner A. Am J Respir Crit Care Med 149: 1132–1137, 1994; Sciri M, McCaskill V, Chediak AD, Abraham WM, and Wanner A. *J Appl Physiol* 79: 1386–1390, 1995). The method has the disadvantage of requiring eight breath-hold maneuvers for a single Qaw measurement, a complicated data analysis, and the inhalation of a potentially explosive gas mixture containing dimethylether (DME) and O2. Because of these shortcomings, the method thus far has not been used in other laboratories. We now simplified the method by having the subjects inhale 500 ml of a 10% DME-90% N2 gas mixture to fill the anatomical dead space, followed by a 5- or 15-s breath hold, and measuring the instantaneous DME and N2 concentrations and volume at the airway opening during the subsequent exhalation. From the difference in DME concentration in phase 1 of the expired N2 wash-in curve multiplied by the phase 1 dead space volume and divided by the mean DME concentration and the solubility coefficient for DME in tissue, Qaw can be calculated by using Fick’s equation. We compared the new method to the validated old method in 10 healthy subjects and found mean ± SE Qaw values of 34.6 ± 2.3 and 34.6 ± 2.8 ml·min⁻¹·ml⁻¹, respectively (r = 0.93; upper and lower 95% confidence limit +2.48 and −2.47). Using the new method, the mean coefficient of variation for two consecutive measurements was 4.4% (range 0–10.4%); inhalation of 1.2 mg albuterol caused a 53 ± 14% increase in Qaw (P = 0.02) and inhalation of 2.4 mg methoxamine caused a 32 ± 7% decrease in Qaw (P = 0.07). We conclude that the new method provides reliable values of and detects the expected changes in Qaw with vasoactive drugs. The simplicity and improved safety of the method should improve its acceptability for the noninvasive assessment of Qaw in clinical research.

bronchial blood flow; soluble gases; adrenergic agents

O U R L A B O R A T O R Y H A S P R E V I O U S L Y D E V E L O P E D A N O N I N V A S I V E S O L U B E G AS U T A P E M E T H O D F O R T H E M E A S U R E M E N T O F B L O O D F L O W I N T H E A I R W A Y M U C O S A [a i r w a y b l o o d f l o w (Qaw)] of humans (9) and validated its accuracy with microspheres in sheep (13). The method is based on quantifying the disappearance of the soluble gas dimethylether (DME) from the anatomical dead space. The method involves inhalation of a gas mixture containing DME, helium, and O2, followed by multiple breath-hold times and subsequent exhalation into a spirometer while the instantaneous concentrations of DME (FiDME), helium, and nitrogen (N2) are recorded along with the expired volume. From these parameters, Qaw can be calculated according to Fick’s principle. Qaw values are expressed as microliters per minute per milliliter, where “milliliter” reflects the anatomical dead space volume (VD) (minus the most proximal 20–50 ml to exclude upper airway uptake). One Qaw determination takes ~7 min. This technique has been used in our laboratory in several studies involving healthy subjects and patients with airway disease (1, 2, 5, 6–8, 11).

Although this is the only currently available reliable noninvasive method to assess Qaw, it has the disadvantage of requiring eight breath-hold maneuvers for a single Qaw measurement, a complicated data analysis, and the inhalation of a gas mixture containing both DME and O2. As a result, an elaborate test apparatus is needed to prevent the possibility of accidentally igniting the gas mixture with an electrical component. Because of these shortcomings, the method thus far has not been used in other laboratories.

Recently, we introduced a new way to analyze the gas concentration and volume tracing. In the old analysis, the helium dilution-corrected mean fractional DME concentration (FDMEx) in the anatomical dead space volume after four different breath-hold times (5, 10, 15, and 20 s) was used to calculate DME uptake from the anatomical dead space volume and Qaw by Fick’s equation. Because each of the four breath holds was repeated, eight breath-hold maneuvers were required per Qaw measurement. In the new analysis, the decline in FDMEx is determined at or toward the end of phase 1 of the expired N2 curve after a short (e.g., 5-s) and long (e.g., 15-s) breath-hold period. With this approach, there is no need for helium and O2 in the gas mixture, only two breath holds are needed, the gas signals do not have to be integrated, and a safe 10% DME-90% N2 mixture can be used. Because the inspired N2 concentration is higher than the alveolar N2 concentration, the end of phase 1 can be identified on the expired N2 curve (single-breath N2 wash-in).

In this paper we describe the new, simplified DME uptake method for the measurement of Qaw and report the results of its validation by comparing Qaw values obtained with the old and new methods and by assessing the reproducibility of Qaw and determining Qaw responses to vasoactive agents using the new method. With this approach, we expected to show that the new method is feasible, reproducible, and sensitive to intervention.

MATERIALS AND METHODS

Theoretical Considerations

DME is soluble in tissue and blood (12). If a volume of DME is inhaled that is large enough to fill V0, the disappearance of DME from the dead space during a subsequent breath hold is due to DME equilibration with tissue lining the airway, blood flow through that
tissue (Qaw), and axial DME diffusion into the alveolar space where FDME is low because of DME dilution and uptake by pulmonary blood flow. Assuming that DME equilibration with airway tissue is complete within seconds and that axial DME diffusion does not occur in the VD corresponding to phase 1 of the single-breath N2 washout or wash-in curve (V(D1)), the uptake of DME from VD1 between a 5-s and 15-s breath hold must be proportional to Qaw in that segment of the conducting airways (Fig. 1). With this approach, the transient, tissue equilibration-dependent DME uptake and alveolar gas contamination can be ignored.

Using Fick’s equation for blood flow calculation, \( Q_{aw} = \frac{V_{DME}}{(F_{DME} \cdot \alpha)} \), where \( V_{DME} \) is DME uptake from VD1 during the 10 s between the 5-s and 15-s breath-hold period, \( F_{DME} \) is the mean fractional DME concentration in VD1 between the two breath holds, and \( \alpha \) is the Bunsen solubility coefficient for DME in tissue and blood at 37°C (12). The DME uptake from VD1 is obtained from the difference in \( F_{DME} \) in VD1 minus the first 20 ml of the exhaled volume to exclude oropharyngeal (defined as the combined epipharyngeal, mesopharyngeal, hypopharyngeal, and oral cavity) DME uptake between the two breath-hold periods, multiplied by VD1 (minus 20 ml) and divided by 10 s. Finally, Qaw is divided by VD1 (minus 20 ml) to normalize for the airway volume in which Qaw is determined; Qaw is expressed as microliters per minute per milliliter.

**New Method to Measure Qaw**

**Apparatus.** The equipment used to measure Qaw consisted of a pneumatic multiport valve system whose ports were connected to a mouthpiece, to a Teflon bag to hold the test gas mixture (10% DME-90% N2, purchased premixed from Praxair, Danbury, CT), and to atmosphere (Fig. 2). Between the valve system and the mouthpiece, a pneumotachograph (connected to a differential pressure gauge and demodulator; Validyne, Northridge, CA) was inserted, and the instantaneous concentrations of DME and N2 were measured in the mouthpiece by use of a mass spectrometer (Perkin-Elmer, Pomona, CA). This reduced the relevant external dead space to 6 ml. The pneumotachograph was calibrated with the test gas. A computer provided with analog-to-digital converters was used to control the filling of the Teflon bag and the sequence of the solenoids regulating the pneumatic valve system, to integrate the flow signal, and to calculate Qaw from the expired volume and integrated gas concentration signals as described above. The inspired DME concentration was monitored to ensure reproducible values for the 5-s and 15-s breath-hold pairs. The mass spectrometer inlet was not heated, and no corrections were made for water pressure. The resulting overestimation of \( F_{DME} \) was considered to be negligible (~0.003).

**Procedure.** Subjects (wearing a nose clip) inhaled room air through the mouthpiece to total lung capacity position, exhaled 500 ml and then inhaled 500 ml of the test gas mixture back to total lung capacity position in rapid sequence. The airway was occluded at this point. Then they held their breath for either 5-s or 15-s in random order. After each of the breath-hold periods, the subjects exhaled through the port with the critical flow orifice (to standardize expiratory flow rate at 0.25 l/s), and air flow and gas concentrations were measured during this maneuver. The elapsed time between the two breath holds was less than 1 min.

**Data analysis and calculation of Qaw.** As shown in Fig. 1, Qaw is determined from the exhaled volume (V), DME, and N2 signals. Expiration through a critical flow orifice after a 5-s and 15-s breath hold starts at \( t_0 \). The end of phase 1 on the N2 washout curve is reflected by \( t_2 \). At this time point, the phase 1 dead space (V(D1)) has been expired. \( t_1 \) is the time point at which the oropharyngeal volume has been expired (20 ml). The dead space interest is the volume expired between \( t_1 \) and \( t_2 \) (V(D1) - 20 ml). The mean DME concentration between \( t_1 \) and \( t_2 \) is lower after the 15-s than 5-s breath hold owing to DME uptake during the 10 s that have elapsed between them.

\[
F_{DME,5} = \frac{F_{DME,1} + F_{DME,15}}{2}
\]

\[
F_{DME,15} = \frac{F_{DME,1} + F_{DME,15}}{2}
\]

\[
\Delta F_{DME} = F_{DME,5} - F_{DME,15}
\]

\[
F_{DME} = \frac{F_{DME,5} + F_{DME,15}}{2}
\]

\[
V_{DME} = (V(D1) - 20 ml) \cdot \Delta F_{DME}
\]

\[
Q_{aw} = \frac{V_{DME}}{(F_{DME} \cdot \alpha \cdot 10 s)}
\]

where \( \alpha \) is the DME solubility coefficient (9 ml/ml) and Qaw is expressed in milliliters per second. Normalizing Qaw for the dead space volume from which DME uptake is measured (V(D1) - 20 ml), the equation reduces to

\[
Q_{aw} = \frac{\Delta F_{DME}}{(F_{DME} \cdot \alpha \cdot 10 s)}
\]

To express Qaw as microliters per minute per milliliter dead space, the obtained value is multiplied by 6 and 1,000.

**Protocol and Subjects**

Healthy lifetime nonsmokers who did not have a history of cardiovascular or lung disease and were not taking vasoactive or airway...
Q˙aw determination. Although the inhalation of 500 ml of an O2-free gas mixture is not expected to cause transient hypoxemia (a portion of the 500 ml ends up in Vd, where there is no appreciable gas exchange, and the remainder is added to a large alveolar volume, thereby only minimally lowering alveolar O2 concentration), pulse oximetry was carried out before and for 2 min after the 15-s breath hold (first Qaw determination in the reproducibility test).

VISIT 2. On this visit, the subjects inhaled the alternate adrenergic agonist, and Qaw, blood pressure, and pulse rate were measured before and after drug inhalation as on visit 1.

Systemic blood pressure and pulse rate were measured before each Qaw determination. Although the inhalation of 500 ml of an O2-free gas mixture is not expected to cause transient hypoxemia (a portion of the 500 ml ends up in Vd, where there is no appreciable gas exchange, and the remainder is added to a large alveolar volume, thereby only minimally lowering alveolar O2 concentration), pulse oximetry was carried out before and for 2 min after the 15-s breath hold (first Qaw determination in the reproducibility test).

There were two visits to the laboratory.

VISIT 1. Qaw was measured twice, less than 5 min apart (reproducibility assessment). Thereafter, the subjects inhaled either 1.2 mg albuterol or 2.4 mg methoxamine (delivered doses), chosen randomly. Qaw was remeasured 15 min later (we have previously shown that the vasoconstrictor and vasodilator effects of inhaled methoxamine and albuterol at these doses peak between 5 and 15 min after inhalation and have passed by 30 min; Refs. 1, 4). The aerosols of albuterol and methoxamine were generated with a DeVilbiss nebulizer connected to a dosimeter. Solutions of the two drugs in phosphate-buffered saline were freshly prepared on each experiment day, and the generated dose was calculated from the number of breaths and the drug concentration in the nebulized solution (4). The subjects inhaled the aerosols tidally from functional residual capacity. The study was single blinded, i.e., the subjects did not know which adrenergic agonist they inhaled on a given day. The effects of the buffered saline vehicle were not assessed because saline inhalation has previously been shown not to alter Qaw (10).

Mean Qaw was 34.6 ± 2.3 μl·min⁻¹·ml⁻¹ with the new calculation method and 34.61 ± 2.8 μl·min⁻¹·ml⁻¹ with the original calculation method. In 9 of the 10 subjects, the values fell within ±15% of the line of identity, and the correlation coefficient was 0.93 (Fig. 3). The 95% confidence limits were +2.48 and −2.47 μl·min⁻¹·ml⁻¹ (Bland-Altman comparison plot).

Mean Vd1 was 93 ± 6 ml and 96 ± 10 ml when obtained from the 5-s and 15-s breath-hold tracings, respectively [P = not significant (NS)]. In contrast, VD as determined with the Fowler technique (3) was higher when determined with the 5-s than with the 15-s breath-hold maneuver (299 ± 16 ml vs. 270 ± 11 ml; P < 0.05). This indicates that VD did not change during the 10-s time period used to calculate VDME, whereas the measured VD decreased with the longer breath-hold time, presumably because of a change in the slope of phase 3 on the N2 washout or wash-in curve as a result of gas mixing between alveoli and airways in the lung periphery.

Study 2

Reproducibility. Mean Qaw measured consecutively with the new method was 33.2 ± 3.0 and 32.0 ± 3.2 μl·min⁻¹·ml⁻¹, respectively (Table 1). The mean coefficient of variation was 4.4% (range 0–10.4%); 7 of 10 fell within ±5%, 1 of 10 within ±10%, and 2 of 10 within ±15% of the line of identity. Mean O2 saturation was 98.8 ± 0.2% before and 98.6 ± 0.3% 2 min after termination of the 15-s breath hold (P = NS); the O2 saturation did not fall by more than 1% in any of the subjects during the 2-min observation period.

Adrenergic responsiveness of Qaw. Mean Qaw increased by 53 ± 14% after albuterol (P = 0.02) and decreased by 32 ± 7% after methoxamine (P = 0.07) (Table 2). The corresponding mean absolute changes in Qaw were +18.9 ± 4.4 and −13.3 ± 3.1 μl·min⁻¹·ml⁻¹. Mean systolic/diastolic blood pressure was 16/7 ± 2/1 mm Hg.

Mean Qaw increased by 10.2 ± 3.5 ml on April 1, 2017 http://jap.physiology.org/ Downloaded from http://jap.physiology.org/ by 10.202.35.1 on April 1, 2017

Table 1. Reproducibility of Qaw measurement with the new method

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Qaw1 μl·min⁻¹·ml⁻¹</th>
<th>Qaw2 μl·min⁻¹·ml⁻¹</th>
<th>Qawm μl·min⁻¹·ml⁻¹</th>
<th>SD</th>
<th>CV %</th>
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<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>F</td>
<td>43.2</td>
<td>43.6</td>
<td>43.4</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>F</td>
<td>28.1</td>
<td>28.1</td>
<td>28.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>F</td>
<td>33.3</td>
<td>32.5</td>
<td>32.9</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>F</td>
<td>40.0</td>
<td>37.3</td>
<td>38.7</td>
<td>1.9</td>
<td>4.9</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>F</td>
<td>50.9</td>
<td>49.0</td>
<td>50.0</td>
<td>1.3</td>
<td>2.7</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>F</td>
<td>20.4</td>
<td>17.8</td>
<td>19.1</td>
<td>1.8</td>
<td>9.6</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>F</td>
<td>36.5</td>
<td>38.3</td>
<td>37.4</td>
<td>1.3</td>
<td>3.4</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>F</td>
<td>33.9</td>
<td>33.7</td>
<td>33.8</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>F</td>
<td>25.4</td>
<td>21.9</td>
<td>23.7</td>
<td>2.5</td>
<td>10.4</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>M</td>
<td>20.3</td>
<td>17.7</td>
<td>19.0</td>
<td>1.8</td>
<td>9.7</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>41 ± 3.3</td>
<td></td>
<td>33.2 ± 3.0</td>
<td>32.0 ± 3.2</td>
<td>32.6</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

Qaw, airway blood flow; F, female; M, male; SD, standard deviation; CV, coefficient of variation. Two measurements (Qaw1 and Qaw2) were taken <5 min apart. Qawm, mean of Qaw1 and Qaw2.
Table 2. Effects of adrenergic agonists on Qaw

<table>
<thead>
<tr>
<th></th>
<th>Albuterol</th>
<th>Methoxamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Postdrug</td>
</tr>
<tr>
<td>Qaw, μl·min⁻¹·ml⁻¹</td>
<td>34.3±2.8</td>
<td>53.2±5.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 subjects. *P = 0.02; †P = 0.07.

pressure was 109 ± 4/65 ± 2 mmHg before and 108 ± 3/66 ± 2 mmHg after albuterol and 110 ± 4/66 ± 2 mmHg before and 112 ± 3/68 ± 2 mmHg after methoxamine (P = NS for all). The corresponding pulse rates were 71 ± 2 and 70 ± 2 min⁻¹ for albuterol and 72 ± 2 and 73 ± 2 min⁻¹ for methoxamine (P = NS for all).

DISCUSSION

The new noninvasive method described in this study provides a reliable, quantitative assessment of blood flow in conducting airways while being simpler to perform and inherently safer than the original method (5, 9). Comparing Qaw values obtained with the two methods in the same subjects revealed a high degree of congruence. The original method has been validated in sheep with color-coded microspheres and therefore was considered to be the standard in this study (14).

The new method requires only two gases, DME and N2 (which can be purchased premixed), a volume-measuring device, a valve system that can be automated or manually operated, and a signal recording unit. In addition, a rapidly responding gas analyzer for the instantaneous measurement of DME and N2 must be available; currently, mass spectrometers are best suited for this purpose. In contrast to the eight breath holds used in the original method, only two breath holds are best suited for this purpose. In contrast to the eight breath holds used in the original method, only two breath holds are needed and the data analysis does not require signal integration. Therefore, the results can be calculated by hand and the use of a computer is a matter of convenience, not a necessity. Indeed, only the volume signal has to be calibrated because ΔFDME and FDME appear as a ratio in the equation to calculate Qaw, and the N2 signal is used merely to detect the end of phase 1 on the N2 wash-in curve. If FDME does not slope between t₀ and t₁ (Fig. 1), there is no need to subtract an oropharyngeal volume from VD₁ and the volume signal does not have to be calibrated. In this case, VD₁ cancels out in the volume-corrected Qaw equation, and Qaw = (ΔFDME/ FDME)-667, where 667 is (60·10⁻³)/(9·10⁻³), to obtain blood flow per minute. The unit for Qaw would be min⁻¹ but is better expressed as microliters per minute per milliliter for conceptual clarity. The analysis assumes that the inspired DME concentration is the same for both breath-hold maneuvers. This is guaranteed by using a premixed gas supply. To prevent gas separation in the reservoir bag, we used a magnetic agitator connected to the bag.

Some of the theoretical and practical limitations of the original method for the measurement of FDME also apply to the new method (1). They include DME equilibration with and diffusion in airway tissue, recirculation of DME, the airway depth captured by the measurement of Qaw, the hemodynamic effects of DME, and the influence of airway caliber on Qaw as measured with this technique.

The uptake of DME from the airway lumen consists of two phases, an initial transient state (during which DME dissolves in tissue and capillary blood and FDME reaches an equilibrium between the airway lumen and tissue/blood), and a steady state that is proportional to blood flow. Although the rate at which DME permeates the airway tissue during the transient state depends on the diffusivity of DME, one can assume that the amount of DME in tissue is relatively constant from the beginning of the steady state, decreasing only slightly in relation to the decrease in luminal FDME during the breath hold. Onorato et al. (9) have shown that the steady state is reached within 2 s. Therefore, VDME should reflect blood flow and be relatively independent of diffusivity during the steady state. The good correlation between Qaw measured with DME and Qaw measured with microspheres in sheep is in keeping with this assumption (13).

Axial diffusion could have accounted for some of the DME uptake from VD₁. Onorato et al. (9) examined this in an artificial airway and found stable DME concentrations for up to 20 s. That model did not realistically mimic in vivo conditions because of the absence of cardiogenic oscillations, which have a gas-mixing effect. However, the mixing effect probably would counteract rather than potentiate any gas concentration gradient along the airway. That observation and our limiting DME uptake to VD₁ suggest that peripheral airway and pulmonary blood flow was not included in our measurements. We subtracted a proximal air volume of 20 ml to exclude oropharyngeal DME uptake. To our knowledge, upper airway volume measurements during breath hold have not been reported. Recently, McRobbie and Pritchard (6), who used magnetic resonance imaging to quantitate oropharyngeal air volume in adults breathing tidally through a mouthpiece, found a mean value of 38 ml. Therefore, our Qaw measurements could have included a small fraction of oropharyngeal blood flow.

In the original method, 35% DME was used in the test gas mixture, and there was a concern that a capillary backpressure of DME could build up during the longer breath-hold periods (9). In the new method, 10% DME is used and the longest breath hold lasts 15 s. Subtracting the expired DME volume from the inspired DME volume (50 ml), we estimated a total DME uptake of 15 ml during the 15 breath-hold maneuver. Considering a dilution of this amount of DME by the cardiac output, the resulting DME backpressure in the airway capillaries must be negligible.

Hlastala (4) showed in rats in whom a subcutaneous gas pocket of diethyl ether was created that in the absence of tissue perfusion, the partial pressure of the gas falls by <25% within a tissue depth of 200 μm. Because DME has a molecular weight and tissue solubility similar to diethyl ether, we believe that the DME uptake method measures airway blood flow to a depth of ~200 μm, i.e., in the subepithelial tissue. In sheep, 85% of total bronchial blood flow is distributed to this anatomical structure in the airway wall (10). It is likely that this applies to the human airway as well.

The pharmacological profile of DME includes vasodilation; the magnitude of this action in the airway circulation cannot be estimated in humans. However, the above-mentioned correlation between the DME and microsphere method to measure Qaw in sheep suggests that the local hemodynamic effect of DME is likely to be small.
Although Qaw is normalized for VD1, this does not correct for differences in airway caliber. For example, in the presence of airway narrowing, VD1 is distributed to a greater depth into the bronchial tree where the mucosal surface-airway lumen area and hence DME uptake is greater. The magnitude of this artifact is not known but likely to be small because Vd and VD1 have been similar in healthy and asthmatic subjects and did not change after albuterol inhalation in a previous study involving the DME uptake technique (5).

We conclude that the new method to quantify Qaw is feasible, reproducible, and sensitive to changes in Qaw. Because of its simplicity, it lends itself to the study of airway vascular responses in health and disease.

GRANTS

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REFERENCES


