Pulmonary embolism causes hypoxemia by redistributing regional blood flow without changing ventilation

WILLIAM A. ALTEMIEIER,1 H. THOMAS ROBERTSON,1 STEVE MCKINNEY,2 AND ROBB W. GLENNY1,2

Departments of 1Medicine and of 2Physiology and Biophysics, University of Washington, Seattle, Washington 98195-6522

Pulmonary embolism occurs in an estimated 500,000 people per year in the US (24), and many of these patients develop clinically significant hypoxemia. The exact mechanism by which this hypoxemia occurs remains in dispute. Potential mechanisms of hypoxemia in pulmonary embolism include regions of low ventilation-to-perfusion ratio (VA/Q) (2, 20), right-to-left shunting of deoxygenated blood (1, 23), diffusion limitation at the alveolar-capillary interface due to regional decreases in capillary transit time (25), and decreased mixed venous oxygen tension secondary to decreased cardiac output (14, 15).

Although studies of pulmonary embolism in humans with use of the multiple inert-gas-elimination technique (MIGET) have demonstrated the dominant role of VA/Q heterogeneity in the genesis of arterial hypoxemia (10, 20), the mechanisms by which this VA/Q heterogeneity and, in particular, low-VA/Q regions develop remain unknown. Regions of low VA/Q may develop from redistribution of perfusion to areas of low ventilation or from decreased regional ventilation in areas of preserved perfusion. Redistribution of ventilation after vascular embolization has been variably reported as nonexistent to significant by using a variety of methods and models (2, 13, 17, 20).

Robertson and co-workers (18) recently introduced a method for determining regional ventilation by using aerosolized 1-µm fluorescent microspheres. By combining this technique with simultaneously injected 15-µm fluorescent microspheres (6), measurements of regional VA/Q matching can be obtained. To better understand the determinants of hypoxemia after acute pulmonary vascular embolization, we simultaneously administered aerosolized and intravenous microspheres before and after vascular embolization with 780-µm polystyrene beads.

METHODS

Animal preparation. The experiments were approved by the Animal Care Committee at the University of Washington. Five juvenile pigs, of either gender and weighing 12–18 kg, were studied. Anesthesia was induced with ketamine (20 mg/kg) and xylazine (2 mg) injected intramuscularly. In the first animal, anesthesia was maintained with a continuous infusion of a 2:1 mixture of ketamine and valium. In all subsequent animals, anesthesia was maintained with a continuous infusion of thiopental sodium at 160–200 mg/h as needed to inhibit inspiratory efforts. The animals were mechanically ventilated via tracheostomy with a tidal volume of 10–15 ml/kg and a respiratory rate adequate to maintain an arterial carbon dioxide tension (PaCO2) of 30–35 Torr. Tidal volumes and respiratory rates, once set, were kept constant throughout the study. One carotid and one femoral artery and one external jugular and both femoral veins were cannulated. A pulmonary artery catheter was inserted through the remaining jugular vein, and the animal was placed in the prone posture. A 30-ml/kg bolus of 0.9% saline was given at the beginning of the study to maintain hemodynamic stability after vascular embolization. After a stable minute ventilation was achieved, an intravenous infusion of a standard solution of six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) was allowed to equilibrate for a minimum of 30 min.

Study protocol. Figure 1 is the timeline for the experimental protocol. Before data collections or interventions, each animal was hyperinflated to twice the tidal volume to minimize atelectasis. Baseline values for temperature, mean arterial pressure, pulmonary artery pressure, and peak airway pressure were recorded, along with an average of three thermodilution cardiac output measurements. Tidal volume was measured with a Wright spirometer and averaged over 10 breaths. The respiratory exchange ratio was calculated with the inspired and expired fractions of O2 and CO2.
measured by mass spectrometry (MGA1100, Perkin-Elmer, Norwalk, CT). Arterial and mixed venous blood and mixed expired gas were collected for MIGET analysis and blood-gas determination. Aerosolized 1-µm microspheres were delivered over a 10-min period as described by Robertson et al. (18). Simultaneously, 15-µm microspheres were intravenously injected in multiple, small, evenly spaced increments over the duration of aerosol delivery. Full data collection, including administration of a second pair of aerosolized and injected microspheres, was repeated after 30 min to assess temporal variation in ventilation and perfusion distributions.

After the second microsphere administration, each animal received a 600- to 800-mg-bolus injection of polystyrene beads (mean diameter 780 µm, range 710–850 µm; Bangs Laboratories, Fisher, IN) suspended in 0.9% saline with 0.05% Tween-80. Arterial and pulmonary artery pressures were monitored continuously. Ten minutes after embolization, arterial blood gases were determined to ensure sufficiency of gas exchange abnormalities, defined as an increase in alveolar-arterial oxygen difference (A-aDO2) three times above baseline and a decrease in arterial oxygen tension (PaO2) of 30 Torr, were present. In two of the five animals, a second injection of 200–300 mg of the 780-µm beads was required to achieve the desired gas exchange abnormalities. Each animal was observed for an additional 10 min to ensure stability of the gas exchange abnormalities. Repeat physiological data, arterial and mixed venous blood samples, and mixed expired gas samples were collected, and a final set of aerosolized and intravenous microspheres was given. After the last measurement, 10,000 U of heparin and 1.5 ml of papaverine were intravenously administered, and the animals were euthanized by exsanguination under deep anesthesia.

Lung preparation and data collection. A sternotomy was performed, the main pulmonary artery and left atrium were cannulated, and the aorta was ligated. The pulmonary vasculature was flushed with a dextran solution by gravity feed, cannulated, and the aorta was ligated. The pulmonary vascularity was assessed to determine whether systemic shunting of fluorescent microspheres occurred. Inert gas concentrations in arterial and mixed venous blood and exhaled gas were measured on a gas chromatograph (model 3300, Varian, Palo Alto, CA) by using previously described techniques (8).

Data analysis and statistics. All values are presented as means ± SD. Paired t-tests are used for statistical comparisons among hemodynamic and gas exchange, with P < 0.05 considered a significant difference.

Between 41 and 161 pieces per animal were excluded from analysis because of airway content ≥25%. Background fluorescence for the six colors was subtracted from each piece, and remaining fluorescent signals were converted to millimeters per minute by using measured values for cardiac output and minute ventilation. Up to eight pieces per animal were excluded from each data set because of an isolated ventilation signal greater than three times any other ventilation or blood flow signal. These rare, unexplained signals were confined almost exclusively to the orange color and mirror the findings of Robertson et al. (18).

Temporal variability was evaluated by calculating the Pearson correlation coefficient (r) between time 1 and time 2 (preembolism) for regional perfusion (r[Q1:Q2]), and for ventilation (r[V1:V2]). The effect of vascular embolization on regional perfusion and ventilation distribution was evaluated by calculating r between pre- and postembolization times for perfusion (r[Q2:Q3]) and for ventilation (r[V2:V3]).

Confidence intervals were calculated for each correlation by using the bootstrap technique (3). Briefly, a data set for one animal consists of n number of pieces with measurements of perfusion and ventilation at times 1, 2, and 3. Because regional perfusion is spatially correlated and therefore dependent on neighboring regional perfusion (5), each piece was grouped with the 30 closest pieces, yielding n clusters. A new data set was then generated by sampling the clustered set n/30 times, with replacement allowing some clusters to be sampled more than once and some not to be sampled at all. A new set of correlations for perfusion and ventilation between times 1 and 2 and between times 2 and 3 were calculated. This was repeated 2,000 times, generating distributions for r[Q1:Q2], r[Q2:Q3], r[V1:V2], and r[V2:V3] from which 95% confidence intervals were calculated. If the 95% confidence intervals for r[Q1:Q2] and r[Q2:Q3] (or for r[V1:V2] and r[V2:V3]) did not overlap, a significant effect of embolization was identified.
The effect of embolization on regional perfusion and ventilation heterogeneity was quantified by ANOVA. The total variability of regional perfusion and ventilation within each animal across the entire experiment may be partitioned into components that are determined by structure, effect of vascular embolization, and temporal and error variation. By structure, we mean an average flow of inhaled gas or blood for a piece (designated by $i$ and ranging from 1 to the number of pieces in a given animal, $n$) across all experimental conditions and replications. Thus structure defines those influences that remain constant over time and experimental condition. In the case of blood flow, this is thought to be due to flow resistance in the branching vascular structure (7). For ventilation, this would include influences from airflow and local parenchymal compliance differences. The embolization and time/error effects may be thought of as adding or subtracting from the structural component. The embolization effect is designated by $j$, with values of 1 or 2 for pre- or postembolization condition. The time/error effect is estimated with repeated measurements designated by $k$ within an experimental condition.

The variance component due to changes in measured ventilation from time and method error ($\sigma^2_{\text{time/error}}$) is estimated by

$$\sigma^2_{\text{time/error}} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{2} \sum_{k=1}^{n} (\bar{V}_{i,j,k} - \bar{V}_{i,j})^2}{n}$$

where $\bar{V}_{i,j,k}$ is the ventilation to piece $i$, with condition $j$, during replication $k$; and $\bar{V}_{i,j}$ is the mean ventilation to piece $i$ across replications within condition $j$. Because repeat measurements were not made after embolization, only data from condition $j = 1$ are used in estimating $\sigma^2_{\text{time/error}}$. The variance component due to changes in ventilation with embolization ($\sigma^2_{\text{emboli}}$) is estimated by

$$\sigma^2_{\text{emboli}} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{2} W_i (\bar{V}_{i,j} - \bar{V}_i)^2}{n}$$

where $\bar{V}_i$ is the mean ventilation to piece $i$ across all replications and experimental conditions. Because there are two measurements preembolization and only one after embolization, the numerator is weighted by $W_i$, which has a value of two for the preembolization condition ($j = 1$) and a value of one for the postembolization condition ($j = 2$). The variance component due to changes in ventilation across pulmonary structure ($\sigma^2_{\text{structure}}$) is estimated by

$$\sigma^2_{\text{structure}} = \frac{\sum_{i=1}^{n} (\bar{V}_i - \bar{V})^2}{n - 1}$$

where $\bar{V}$ is the mean ventilation to all pieces across all replications and experimental conditions.

The contribution of each component to the total variance in ventilation can be calculated as a percentage to aid in comparison across animals. The same analysis is repeated for perfusion in each animal.

To evaluate whether regional $\text{Va}/\text{Q}$ is determined by changes in regional perfusion or ventilation after vascular embolization, each piece was plotted with the log ratio of perfusion or ventilation postembolization to preembolization on the abscissa and log $\text{Va}/\text{Q}$ postembolization on the ordinate.

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### RESULTS

In four of five animals, measurements were obtained twice before embolization and once after embolization. The first animal had only one successful preembolization measurement due to a malfunction of the aerosol generator that prevented adequate microsphere delivery.

No significant differences were seen in the measured physiological parameters between the two preembolization periods. After embolization, $P_{aO_2}$ decreased by an average of $44.1 \pm 3.6$ Torr, and $P_{aCO_2}$ increased by $9.8 \pm 0.3$ Torr. $A-aDO_2$ increased by $32.4 \pm 3.7$ Torr, and mean pulmonary pressure increased by $17.1 \pm 6.2$ (SE) mmHg. Cardiac output and mean arterial pressure were not significantly changed by embolization.

No regions of intrapulmonary shunt ($\text{Va}/\text{Q} = 0$) with perfusion >0 were detected by the microsphere method. Extrapulmonary shunt, estimated from the presence of fluorescent microspheres in the kidneys, was not detected in the three animals examined. The average shunt measured by MIGET was 1.5% of the cardiac output and did not change significantly after embolization.

$\text{Va}/\text{Q}$ heterogeneity increased and mean perfusion-weighted $\text{Va}/\text{Q}$ decreased after embolization as measured by both MIGET and microsphere data. After embolization, the coefficient of variation for regional

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### Table 1. Summary of coefficient of variations and correlations for regional perfusion and ventilation heterogeneity before and after embolization

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>CV of Regional Perfusion</th>
<th>CV of Regional Ventilation</th>
<th>$\rho$ of Ventilation and Perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preemboli</td>
<td>Postemboli</td>
<td>Preemboli</td>
</tr>
<tr>
<td>1</td>
<td>0.56</td>
<td>0.84</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>0.55</td>
<td>0.82</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>0.58</td>
<td>0.79</td>
<td>0.62</td>
</tr>
<tr>
<td>4</td>
<td>0.68</td>
<td>1.01</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>0.75</td>
<td>0.75</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.57 ± 0.07</td>
<td>0.84 ± 0.10</td>
<td>0.55 ± 0.08</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; $\rho$, Pearson correlation coefficient.
perfusion increased from a mean of 0.57 to 0.84. In contrast, the coefficient of variation for regional ventilation was not significantly different. Correlation of regional ventilation and perfusion decreased from a mean of 0.89 preembolization to 0.67 after embolization (Table 1).

Correlations for regional ventilation and perfusion (Fig. 2) are shown in Table 2. Both $r_{Q1:Q2}$ and $r_{V1:V2}$ were due to temporal variability and method error. After embolization, regional perfusion was redistributed as shown by nonoverlapping confidence intervals between $r_{Q1:Q2}$ and $r_{Q2:Q3}$ for all animals. A consistently smaller difference existed between $r_{V1:V2}$ and $r_{V2:V3}$ with 95% confidence intervals overlapping in three of four animals (Fig. 3). This suggests possible redistribution of regional ventilation after embolization, albeit to a much lesser degree than regional perfusion redistribution.

The percent variances from pulmonary structure, embolization, and error/time for regional perfusion and ventilation in each animal are shown in Table 3. Redistribution after embolization was responsible for an average of 30.6% of the total variance in perfusion over the entire experiment. In contrast, the average percent variance in ventilation from embolization was 11.0% of the total variance. The percent variances of both regional perfusion and ventilation measurements due to temporal variability and method error were

Table 2. Correlations of regional perfusion or ventilation pre- and postembolization with 95% confidence intervals

<table>
<thead>
<tr>
<th>Animal No</th>
<th>$r_{Q1:Q2}$</th>
<th>$r_{Q2:Q3}$</th>
<th>$r_{V1:V2}$</th>
<th>$r_{V2:V3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.96 (0.94, 0.98)</td>
<td>0.78 (0.74, 0.82)</td>
<td>0.91 (0.89, 0.94)</td>
<td>0.95 (0.93, 0.97)</td>
</tr>
<tr>
<td>2</td>
<td>0.97 (0.95, 0.98)</td>
<td>0.79 (0.72, 0.83)</td>
<td>0.91 (0.89, 0.94)</td>
<td>0.87 (0.83, 0.90)</td>
</tr>
<tr>
<td>3</td>
<td>0.95 (0.94, 0.97)</td>
<td>0.81 (0.77, 0.85)</td>
<td>0.99 (0.97, 0.99)</td>
<td>0.90 (0.87, 0.94)</td>
</tr>
<tr>
<td>4</td>
<td>0.95 (0.94, 0.97)</td>
<td>0.75 (0.73, 0.79)</td>
<td>0.94 (0.92, 0.96)</td>
<td>0.92 (0.85, 0.96)</td>
</tr>
<tr>
<td>5</td>
<td>0.92 (0.91, 0.94)</td>
<td>0.75 (0.70, 0.80)</td>
<td>0.95 (0.93, 0.96)</td>
<td>0.94 (0.93, 0.96)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.95 ± 0.02</td>
<td>0.78 ± 0.03</td>
<td>0.95 ± 0.03</td>
<td>0.92 ± 0.03</td>
</tr>
</tbody>
</table>

Confidence intervals are in parentheses. $Q_1$, $Q_2$, $Q_3$: perfusion at times 1 and 2 (preembolization) and time 3 (postembolization), respectively; $V_1$, $V_2$, $V_3$, ventilation at times 1, 2, and 3.
similar at 5.0 and 4.9%, respectively. ANOVA could not be calculated in the first animal because only one preembolization measurement of regional ventilation and perfusion was obtained. However, the correlation between pre- and postembolization regional ventilation for all pieces was 0.95 compared with 0.78 for regional perfusion. This supports ANOVA results from the other four animals showing a much greater redistribution of regional perfusion than regional ventilation after embolization.

To evaluate effects of perfusion redistribution on gas exchange abnormalities, the postembolization log \( V_{A}/Q_{A} \) was plotted against the ratio of perfusion postembolization to preembolization (Fig. 4). The average coefficient of determination \( (\rho^2) \) for the five experiments was 0.77. The same analysis using ventilation post- and preembolization gave an average \( \rho^2 \) of 0.07 (Table 4).

**DISCUSSION**

The important finding from this study is that hypoxemia after acute pulmonary embolism can be explained by new, low-\( V_{A}/Q_{A} \) regions resulting from redistribution of regional perfusion without adequate compensatory changes in regional ventilation. Hypoxemia from pulmonary embolization must be due to \( V_{A}/Q_{A} \) heterogeneity, shunt, or diffusion limitation. Decreased mixed venous \( O_2 \) content due to reduced cardiac output may also contribute to hypoxemia. In this study, inert bead microembolization in anesthetized, mechanically ventilated pigs caused significant gas-exchange abnormalities due to increased \( V_{A}/Q_{A} \) mismatch. Shunt did not increase after embolization; however, these animals were mechanically ventilated with fixed tidal volumes and hyperinflated before any measurements to minimize atelectasis. In a spontaneously breathing animal, shunt may occur after embolization if pulmonary perfusion redistributed to regions of atelectasis.

\( V_{A}/Q_{A} \) heterogeneity after pulmonary embolization may result from redistribution of regional perfusion alone or from poorly matched regional ventilation and perfusion redistribution. Our high-spatial-resolution measurements show significant redistribution of regional perfusion after embolization that correlates poorly with baseline regional perfusion. In contrast, regional ventilation postembolization remains highly correlated with baseline values, indicating minimal redistribution. ANOVA analysis quantifies the contribution of regional perfusion redistribution to overall postembolization perfusion heterogeneity to be \( \sim 31\% \). In contrast, ANOVA analysis indicates that regional ventilation remains much more dependent on innate structural determinants after embolization.

Wilson and Beck (26) described \( V_{A}/Q_{A} \) heterogeneity as a sum of the individual heterogeneities of perfusion and ventilation minus a component due to their correlation. Our study demonstrates that, in normal lungs, \( V_{A}/Q_{A} \) matching is preserved despite significant heterogeneity in both regional perfusion and ventilation by

![Fig. 4. Log ventilation-to-perfusion ratio (\( V_{A}/Q_{A} \)) as determined by change in regional perfusion or ventilation in a typical animal. A: log \( V_{A}/Q_{A} \) is significantly determined by change in regional perfusion measured by ratio of postembolization blood flow (\( Q_{3} \)) to preembolization blood flow (\( Q_{2} \)). B: log \( V_{A}/Q_{A} \) is minimally determined by changes in regional ventilation after embolization. n, No. of pieces.](http://jap.physiology.org/)
the close correlation between ventilation and perfusion. After vascular embolization, increased V˙A/Q heterogeneity was associated with both an increase in regional perfusion heterogeneity and a fall in correlation between ventilation and perfusion.

We estimate that regional perfusion and ventilation redistribution accounts for 77 and 7% of V˙A/Q heterogeneity postembolization, respectively. This is derived from logarithmic plots of V˙A/Q as a function of perfusion or ventilation. This analysis can potentially force a correlation between logV˙A/Q and logQ˙3/Q˙2 because the independent and dependent variables are coupled by the common presence of Q˙3 (12, 16). The same should be true of logV˙A/Q and logV˙3/V˙2, yet little correlation is present. Another way to evaluate the dependence of postembolization V˙A/Q heterogeneity is to examine a plot of ventilation vs. perfusion (Fig. 5), wherein a line drawn through any given point and the origin has a slope equal to the V˙A/Q at that point. Each point can by classified by its relative change in ventilation or perfusion, and the relationship between this change and the postembolization V˙A/Q can be evaluated. Figure 5A demonstrates that pieces with ≥20% increase in perfusion after embolization have a low V˙A/Q, pieces with ≥20% decrease in perfusion have a high V˙A/Q, and pieces with <20% change in perfusion have a V˙A/Q near one. In contrast, Figure 5B shows the majority of pieces to have <20% change in ventilation after embolization, and those pieces that changed by ≥20% appear not to predict V˙A/Q.

Changes in regional ventilation after clinical pulmonary embolism may exceed those seen in our experimental model. Redistribution of ventilation after pulmonary artery occlusion has been attributed to alveolar hypocapnia (11, 21, 22). In these studies, occlusion of a main pulmonary artery and, in one study, flushing of the common dead space with 100% O2 resulted in significant alveolar hypocapnia. In our study, high dead space from the ventilator circuit and small size of the regions embolized may have prevented adequate alveolar hypocapnia from developing because of reinspiration of dead space gas. In a spontaneously breathing person, atelectasis due to splinting on the affected side or alteration in surfactant production (4) would cause low-V˙A/Q areas and/or shunt if any perfusion persisted. Biologically active mediators released by a thrombus

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>r(log V˙A/Q:log Q˙post/Q˙pre)</th>
<th>r(log V˙A/Q:log V˙post/V˙pre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.83</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.68</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td>0.79</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.79</td>
<td>0.12</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.77 ± 0.06</td>
<td>0.07 ± 0.05</td>
</tr>
</tbody>
</table>

r², Coefficient of determination; V˙A/Q, ventilation-to-perfusion ratio; Q˙pre, Q˙post, pre- and postembolic perfusion, respectively; V˙pre, V˙post, pre- and postembolic ventilation, respectively.

**Fig. 5.** Scattergrams of regional ventilation and perfusion after embolization classified by change in perfusion (A) or ventilation (B) in a typical animal. A: Δ, pieces in which perfusion increased ≥20% after embolization; □, pieces in which perfusion decreased ≥20% after embolization; ●, pieces in which perfusion changed <20%. Note that pieces with increased perfusion have a low V˙A/Q and pieces with decreased perfusion have a high V˙A/Q. B: Δ, pieces in which ventilation increased ≥20% after embolization; □, pieces in which ventilation decreased ≥20%; ●, pieces in which ventilation changed <20%. Note that increased or decreased ventilation does not correspond to high or low V˙A/Q.
may cause bronchoconstriction and a greater degree of regional ventilation redistribution than seen with inert emboli. In a spontaneously breathing human, the normal response to pulmonary embolism is hyperventilation, demonstrated by decreased arterial CO₂ tension. This causes a rightward shift in the V˙A/Q˙ distribution minimizing low-V˙A/Q˙ areas and hypoxemia. However, V˙A/Q˙ heterogeneity, demonstrated by an increased A-aDO₂, is still present, as a manifestation of mechanico-redistribution of regional perfusion.

The results of this study emphasize the importance of the determinants of regional ventilation and perfusion as well as the degree of correlation between the two in determining gas exchange. These findings also suggest that inadequate compensatory changes in regional ventilation after perfusion redistribution may play an important role in hypoxemia seen with clinical diseases such as pulmonary embolism. Determinants of basal regional ventilation may play a more significant role in determining gas exchange than local ventilatory regulation. Given the degree of regional ventilation heterogeneity observed recently (9, 18, 19), further research into the determinants of ventilation distribution along with mechanisms that match regional ventilation and perfusion is warranted.

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