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# Modified Technique Using Perfused Isolated Guinea Pig Lung to Determine Effect of an Aerosol Constituent on Pulmonary Dynamics

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**Abstract** □ Polyethylene glycol 400, a commonly used plasticizer in many cosmetic aerosol hair sprays, was tested to determine its effect on perfusion pressure, perfusion vascular flow rate, and tidal volume in the perfused isolated guinea pig lung. Negative pressure was maintained within the chamber housing the intact lungs, and initial perfusion of the pulmonary vasculature was accomplished *via* the right side of the heart *in situ* after guinea pigs were sacrificed by decapitation. Polyethylene glycol 400 was injected into the pulmonary arterial system in doses of 0.01–30 μg/ml after pretreatment with isoproterenol (1 μg/ml). Isoproterenol was then readministered, followed by nebulized doses of the cosmetic chemical into the trachea at 0.3, 3, and 30% concentrations. Nebulized polyethylene glycol 400 was also administered in 50, 70, and 90% concentrations. Polyethylene glycol 400 significantly increased perfusion pressure and flow rate after injection into the pulmonary arterial system of the isolated lung. In addition, nebulized administration in concentrations of 0.3–30% significantly increased the perfusion flow rate; following the 50–90% nebulized doses, a significant increase in both perfusion pressure and flow rate was observed. Tidal volume decreased, regardless of the route of administration, as increasing doses of the cosmetic constituent were delivered to the isolated lung.

**Keyphrases** □ Polyethylene glycol 400—effect on pulmonary dynamics in perfused isolated guinea pig lung □ Pulmonary dynamics—effect of polyethylene glycol 400 in perfused isolated guinea pig lung □ Aerosol constituents—polyethylene glycol 400, effect on pulmonary dynamics in perfused isolated guinea pig lung

The relation between inhalation of airborne particles and pulmonary disease has been known for some time (1). Numerous cases of pulmonary thesaurosis were attributed to the inhalation of cosmetic hair sprays, although the question of whether cosmetic aerosols actually cause pulmonary disease is still controversial (2–5). Zuskin and Bouhoys (6) observed, under controlled experimental conditions, that short lasting and significant decreases in the air expiratory flow rate from the lung occurred following acute exposures to various commercial hair sprays. The purposes of this investigation were to develop a simplified, improved version of the perfused isolated guinea pig lung and to employ it as a model by which responses to the administration of a cosmetic constituent (polyethylene glycol 400) could be monitored to determine whether or not it alters pulmonary dynamics.

## EXPERIMENTAL

**Lung Chamber and Perfusion Apparatus**—A modified method of Bhattacharya and Delaunois (7) was used for perfusion of the isolated guinea pig lung. The apparatus that housed the lungs consisted of a cylindrical Plexiglas chamber, 16 cm high and 11 cm wide. Resting within the chamber on three pegs, cemented to and situated in a triangular pattern around the inner wall, was a Plexiglas funnel with its bottom located directly above a glass mason jar. The top of the jar was screwed into the bottom of the chamber with a single hollow nylon nut and bolt.

A Plexiglas elbow and connecting tube, cemented to the exterior bottom of the chamber, led from the inside to the outside through a small hole in the chamber floor. The base of a three-way tube was connected to the outside Plexiglas tube of the elbow. One side of the three-way tube led into a calibrated mercury manometer[1]; the other side was conected to a second three-way tube, which had its base ttached to the expiration outlet of a small animal respirator[2]. The other side of the second three-way tube was connected to the air intake valve of a vacuum pump[3] (Fig. 1).

The chamber's upper end was closed with a Plexiglas plate. A piece of polyethylene tubing[4] that served as the perfusion cannula was passed through a small opening in the top of the chamber. The other end of the tubing was attached to a metal needle (20 gauge), which had the male end of a three-way valve inserted into its base. This valve served as an injection port through which test materials were administered into the pulmonary arterial vasculature of the isolated guinea pig lung.

Perfusion of the pulmonary vasculature was accomplished *via* a solid-state veristaltic pump[5]. It pumped aerated Tyrode solution from

[1] Fisher Scientific Co., Springfield, N.J.
[2] Model V5KG, E&M Instrument Co., Houston, Tex.
[3] Model 0211V45F, Gast Manufacturing Corp., Benton Harbor, Mich.
[4] PE-60 (0.08 cm i.d., 0.12 cm o.d.), Clay Adams, Becton, Dickinson and Co., Parsippany, N.J.
[5] Portable combination pressure/vacuum pump (model 0211-V45F. G8CX), Manostat Corp., New York, N.Y.



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**Table I—Mean Perfusion Pressure, Flow Rate, and Tidal Volume Expressed as a Percentage of the Control [a] following Intrapulmonary Injection of Polyethylene Glycol 400**

| | Dose, μg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.01 | 0.03 | 0.1 | 0.3 | 1 | 3 | 10 | 30 |
| Lungs per experiment [b] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| Perfusion pressure | 100.4 ± 4.0 [c] | 101.0 ± 4.0 | 103.0 ± 5.0 | 111.1 ± 4.1 [d] | 114.2 ± 3.0 [e] | 117.0 ± 4.0 [e] | 119.0 ± 4.0 [e] | 124.4 ± 6.0 [e] |
| Flow rate | 102.4 ± 6.0 | 112.0 ± 8.2 | 123.0 ± 9.0 [d] | 126.0 ± 11.0 [d] | 142.0 ± 12.0 [e] | 148.4 ± 14.0 [e] | 157.0 ± 13.0 [e] | 159.4 ± 15.0 [e] |
| Tidal volume | 102.2 ± 4.3 | 102.4 ± 5.0 | 103.0 ± 5.0 | 100.0 ± 5.4 | 96.0 ± 7.0 | 91.3 ± 10.0 | 84.4 ± 10.0 | 85.2 ± 5.0 [e] |

[a] (Final value at each dose/control value) × 100. [b] Lungs pretreated with intrapulmonary injection of 1 μg of isoproterenol/ml. [c] Mean percentage value ± *SEM*. [d] $p < 0.05$. [e] $p < 0.01$.

a bottle maintained in a water bath at 37° past both a pressure transducer[6] and flowmeter[7]. The components of the Tyrode solution (8), in grams per liter, were: sodium chloride, 8.0; potassium chloride, 0.2; magnesium chloride, 0.1; calcium chloride, 0.2; monobasic sodium phosphate, 0.05; sodium carbonate, 1.0; and glucose, 1.0. With the addition of povidone, the Tyrode solution simulated the osmotic pressure of guinea pig serum, previously determined to be 291 mosmoles. Upon exiting from the flowmeter, the fluid flowed through the metal needle before entering the chamber, which was situated in another 37° water bath (Fig. 2).

A central nylon tube and polyethylene cannula protruded downward into the chamber, and the trachea of the isolated lung was sutured onto the polyethylene tube. In passing through the central opening in the top of the chamber, the tracheal cannula bisected a flattened Plexiglas doughnut-shaped structure cemented to its inside. It was continuous with the outside through a hole in the top of the chamber, and into this opening was inserted one end of a length of tubing with a three-way valve attached to its other end. A 10-ml syringe was placed into the injection port of the valve. Encircling the lower edge of the doughnut-shaped structure were 55 pinholes, each having a depth of approximately 0.15 cm, which served as a sprinkler system to moisten the lung continuously during the experiment.

A four-pronged Plexiglas fitting was connected to the upper exposed portion of the nylon tracheal cannula. The fitting was divided by a three-way nylon tube; it ran perpendicular to the chamber and had a small opening located directly above the tracheal cannula. One of the bifurcated ends of the tube was clamped closed, and the other end had an open-ended male adapter[8] attached to it. The base of the three-way tube was connected to one end of a U tube, while the other end of the tube led into a pressure transducer permanently set to the calibration position. Four milliliters of water, always in contact with the transducer, lined the U; the distal portion of tubing was connected to the Plexiglas fitting on top of the chamber. Approximately 18 cm left in the tubing served as pulmonary "dead space" for expired air leaving the lung.

The last projection of the Plexiglas piece extended straight up and was directly in line with the opening in the nylon three-way tube and the tracheal cannula. The ejection outlet of a nebulizer[9] was connected, *via* tubing, to the Plexiglas projection. When the nebulizer was not in operation, a hemostat clamped the tubing closed just above the end of the Plexiglas extension. An air compressor[10] interfaced with an automatic timer[11] enabled the cosmetic chemical to be administered in nebulized form into the respiratory tract of the isolated lung.

**Operative Procedure**—Male English short hair guinea pigs[12], 800–1200 g and approximately 10 months old, were sacrificed by decapitation and allowed to exsanguinate. A midline incision was made from the abdomen to the cervical region, and the skin was retracted. The diaphragm was then separated from the distal end of the rib cage, and the sternum was removed to expose the heart. The heart was transversely cut in half, and the perfusion cannula was inserted into the pulmonary artery through the right ventricle.

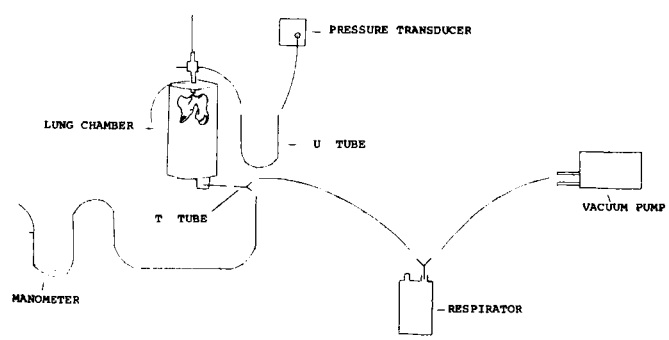


**Figure 1**—*Withdrawal of air from lung chamber.*

The cannula was then connected to an inverted bottle containing modified Tyrode solution and suspended above the guinea pig to allow for initial *in situ* perfusion of the lung. By blunt dissection, the trachea was isolated and the intact lung was gently lifted and removed from all body connections. The preparation was then placed on a dissecting tray, and the tracheal cannula was inserted into the air passage of the isolated lung. Initial *in situ* perfusion times averaged 200 sec, and the total time for complete removal of the tissue never exceeded 15 min from the time of decapitation.

The nylon end of the tracheal cannula was passed through the central opening in the chamber's top, which was then joined to the main body of the chamber with the double-cannulated lung suspended inside. The perfusion cannula and tracheal cannula were connected to the metal 20-gauge needle and four-pronged fitting, respectively, and the chamber was three-quarters submerged within the water bath.

**Treatment**—Ten lungs were initially administered 1 ml of a 1-μg/ml solution of isoproterenol[13] into the pulmonary artery, followed by intrapulmonary injections of polyethylene glycol 400[14] in doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μg/ml (1 ml injected/dose). Following the final intrapulmonary injections of polyethylene glycol 400, 1 ml of a 1-μg/ml solution of isoproterenol was reinjected into the pulmonary artery of the same lung. This injection was followed by three consecutive and separate 10-sec sprays of the cosmetic chemical, in concentrations of 0.3, 3, and 30%, into the trachea of the isolated lung. The air flow rate of the nebulizer was 2.5 liters/min, and it delivered approximately 0.006 g of test material into the lung during a single 10-sec period.

Six lungs were administered 1 ml of a 1-μg/ml solution of isoproterenol into the pulmonary artery, followed by a single 20-sec nebulized administration of polyethylene glycol 400 into the trachea of the lung in concentrations of 50, 70, and 90%.

Throughout any experiment, perfusion pressure and tidal volume were monitored on a physiograph[6]. The pulmonary vascular flow rate was recorded using the flowmeter.

**Apparatus Operation**—The veristaltic pump, which delivered the perfusion solution to the pulmonary vasculature, was operated at 20–30 mm Hg. As soon as a clear perfusate dripped from the left side of the heart onto the funnel within the chamber, the respirator was switched on. It was used at a setting of 30 ventilations/min with the inspiration to expiration ratio set at 1:1. The vacuum pump was utilized to produce negative alternating pressure within the chamber, simulating intrathoracic pressure, as recorded *via* the mercury manometer. The respirator,

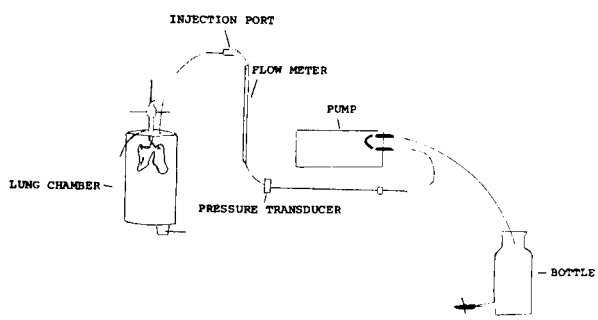


**Figure 2**—*Path of perfusing solution to lung chamber.*

[6] Narco Biosystems, Houston, Tex.
[7] Roger Gilmont Instruments, Great Neck, N.Y.
[8] Type A, Clay Adams, Becton, Dickinson and Co., Parsippany, N.J.
[9] No. 841 (particle diameter of 1–5 μm), DeVilbiss Co., Somerset, Pa.
[10] Type 501, DeVilbiss Co., Sommerset, Pa.
[11] Universal timer, Dimco-Gray Co., Dayton, Ohio.
[12] Camm Research Institute, Wayne, N.J.
[13] Lot 18123, Vitarine Co., New York, N.Y.
[14] Lot 1043JK, Ruger Chemical Co., New York, N.Y.

**Table II—Mean Perfusion Pressure, Flow Rate, and Tidal Volume Expressed as a Percentage of the Control [a] following Nebulized Administration of Polyethylene Glycol 400**

| | Dose | | |
|---|---|---|---|
| | 0.3% | 3% | 30% |
| Lungs per experiment[c] | 10 | 10 | 9 |
| Perfusion pressure | 99.4 ± 4.0[d] | 103.0 ± 6.0 | 109.0 ± 7.0 |
| Flow rate | 99.4 ± 3.0[e] | 107.0 ± 4.0[e] | 120.1 ± 6.0[f] |
| Tidal volume | 117.0 ± 11.2 | 115.0 ± 14.2 | 104.0 ± 17.4 |

[a] (Final value at each dose/control value) × 100. [b] All results represent the average of three responses. [c] Lungs previously exposed to intrapulmonary injections of polyethylene glycol 400. [d] Mean percentage value ± *SEM*. [e] $p < 0.05$. [f] $p < 0.01$.

**Table III—Mean Perfusion Pressure, Flow Rate, and Tidal Volume Expressed as a Percentage of the Control [a] following Nebulized Administration of Polyethylene Glycol 400**

| | Dose | | |
|---|---|---|---|
| | 50% | 70% | 90% |
| Lungs per experiment[b] | 6 | 6 | 6 |
| Perfusion pressure | 111.3 ± 2.4[c,d] | 120.0 ± 6.0[d] | 127.0 ± 7.1[d] |
| Flow rate | 111.0 ± 3.0[d] | 124.0 ± 5.0[d] | 131.3 ± 7.0[d] |
| Tidal volume | 92.1 ± 6.0 | 96.3 ± 6.2 | 91.1 ± 8.0 |

[a] (Final value at each dose/control value) × 100. [b] Lungs pretreated with intrapulmonary injection of 1 μg of isoproterenol/ml. [c] Mean percentage value ± *SEM*. [d] $< 0.01$.

therefore, served only to stop momentarily the continuous withdrawal of air from the chamber.

In this manner, the lung was able to expand noticeably (as negative pressure within the chamber forced atmospheric air into the lung *via* the adapter located on the four-pronged Plexiglas fitting) and collapse (as the respirator "cut" the continuous evacuation of chamber air). The lung ventilated at a rate dictated by the setting on the respirator, and expired air from the lung was diverted into the water-filled conduit of the U tube.

## RESULTS

As a result of the polyethylene glycol 400 injections into the isolated guinea pig lungs, both perfusion pressure and flow rate significantly increased (Table I). Tidal volume decreased approximately 15% in comparison with the control, although it initially increased as a result of the chemical treatment. The flow rate increase was considerably more than that of the increase observed with perfusion pressure.

Nebulized polyethylene glycol 400, delivered into the respiratory tract in a concentration of 0.3–30%, significantly increased the perfusion flow rate (Table II). Perfusion pressure and tidal volume increased and decreased, respectively, as a result of the administrations. The percent decrease in tidal volume never fell below the control value; however, the 30% dosage level response undoubtedly represents a pneumoconstricting reaction since a chemically induced increase in tidal volume (observed after the 0.3 and 3% doses) might be explained on the basis of air entering into and stretching the lung tissue during the nebulizer administration process. Thus, any decrease in tidal volume may be attributed solely to the action of nebulized polyethylene glycol 400 within the airways of the lungs.

The nebulized polyethylene glycol 400 treatment at concentrations of 50–90% magnified those results observed at the lower dosage level (Table III). Perfusion pressure and flow rate both significantly increased, while tidal volume showed a consistent drop, below control values, as a result of the administrations.

## DISCUSSION

Data presented here demonstrate that polyethylene glycol 400, when administered into either the arterial or bronchial system of the isolated guinea pig lung, is capable of altering pulmonary dynamics. Such changes may be indicative of one of three factors:

1. The chemical's viscous characteristics, which would tend to "coat" the arterial vessels of the isolated lung or to block the bronchial airways, depending on the route of administration.
2. The chemical's pharmacological activity.
3. The chemical's ability to cause the release of histamine from the connective tissue spaces of the lung or pulmonary circulation.

The third possibility may best explain the observed results. Murray (9) summarized the fate of several vasoactive substances in the pulmonary circulation of experimental animals and found that histamine was not effectively removed or inactivated by the lung. Because these findings hold true in perfusion studies as well, it is conceivable that the cosmetic constituent triggers the release of active histamine from depots in the isolated lung. Histamine and histamine-like effects may explain simultaneously the bronchial and vascular responses observed in the isolated lungs after polyethylene glycol 400 administration. Since overall, in this study, tidal volume consistently decreased, regardless of the route of administration of polyethylene glycol 400, and perfusion pressure and flow rate both increased, histamine's ability to produce constriction of the airways as well as vascular dilatation (in small vessels) and constriction (in large vessels) could be responsible.

It is important to consider, however, that decreases in tidal volume as a result of polyethylene glycol 400 injections into the pulmonary artery represent changes at the alveoli, since the perfusing solution is directed toward them and not the bronchi (10). In addition, because the nebulized particles of the polyethylene glycol 400 sprays are impacted onto the walls of the trachea and are, therefore, not deposited into the airways of the isolated lung, the nebulized effect in comparison to the injected responses is diminished.

As for the other two considerations, the glycols are generally not considered to be pharmacologically toxic (11) and the physical nature of polyethylene glycol 400 probably plays only a minor role, if any, in altering pulmonary dynamics. The findings of Zuskin and Bouhoys (6), noted previously, were postulated to be the result of histamine-induced release caused by cosmetic hair spray exposure; the present results support their supposition. In addition, this modified technique for perfusion of the isolated guinea pig lung apparently offers a simple yet sensitive method by which any agent may be tested for its effect on pulmonary dynamics.

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