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Exposure of Mice to Nitrogen Dioxide—a Constant Pressure System

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Introduction

INVESTIGATION of the respiratory effects of NO_2 requires a method for delivering a constant known concentration of NO_2 into an animal chamber. Continuous delivery of low concentrations of NO_2 cannot be accomplished with commonly available gas flow meters. Ripperton and Johnston[1] obtained dilute concentrations of NO_2 by displacing with air an intermediate concentration of NO_2 from a series of bottles into a main flow of air entering an animal exposure chamber. The concentration of NO_2 in the chamber varied from 0.7 ppm immediately after charging the bottles with NO_2 to 0.15 ppm after 23 hours. The present paper presents a modified system for maintaining a more nearly constant concentration of NO_2.

Materials and Methods

The system utilizes a plastic bag made of 6 mil polyethylene which is available from most large building supply companies. It is 23 by 24 inches and made by folding a sheet of polyethylene and sealing the edges under waxed paper with a household iron. We have demonstrated that no measureable amount of 100% NO_2 will diffuse through a 12 square inch piece of this polyethylene material during a 24 hour period. Condensation of water within the plastic bag when not in use is controlled by adding a small amount of indicating silica gel (baker analized reagent). Before the bag is used, the silica gel is shaken out and contained air is removed by evacuation with a water aspirator.

Bottles A and B are 5 gal distilled water bottles which have been calibrated in liters.

The mouse exposure chamber is a one liter low form culture flask with the neck cut off to fit a large rubber stopper. This glass chamber allows continuous observation of the mice and eliminates excessive dead space.

Sampling and injection sites consist of a glass tube with a short side arm capped with a sleeve-type serum bottle stopper.

Indicating silica gel (Baker analized reagent) is used to dry the air before it is mixed with NO_2. The NO_2 was obtained in lecture bottles from Matheson, Coleman and Bell.

Operation of Exposure System

Figure 1 is a diagram of the system designed for exposure of mice to various concentrations of NO_2 at a flow rate of approximately 500 ml of gas per minute for a total of 30 minutes. To charge the system bottle A is filled with the diluent gas, in this case with room air or oxygen, and allowed to come to atmospheric pressure. Valve V_1 is positioned to allow tap water to enter bottle A. Valve V_2 is positioned to allow gas forced from bottle A to flow through the desiccator trap. Valve V_3 is positioned to allow dry air to enter the previously evacuated polyethylene bag which is suspended freely by the top edge and may be supported partially from below. To avoid significant positive pressure in the bag the total charge of gas never should exceed about 30% of the volume of the bag.

To obtain a calculated volume of NO_2 at atmospheric pressure a three foot section of plastic tubing is attached to a lecture bottle containing pure NO_2. The valve on the bottle is opened to allow the gas to flow through the tube slowly against atmospheric pressure. After the tube has been flushed with gas which is released into a well ventilated hood, a 20 gage needle on an oiled syringe is passed through the wall of the plastic tubing and the required amount of NO_2 is withdrawn. To allow for equilibration of pressure the syringe is left in place for one minute. The NO_2 is injected into the flow of dry air as it enters the plastic bag. The syringe is flushed several times with dry air before it is removed from the line. After a measured quantity of gas

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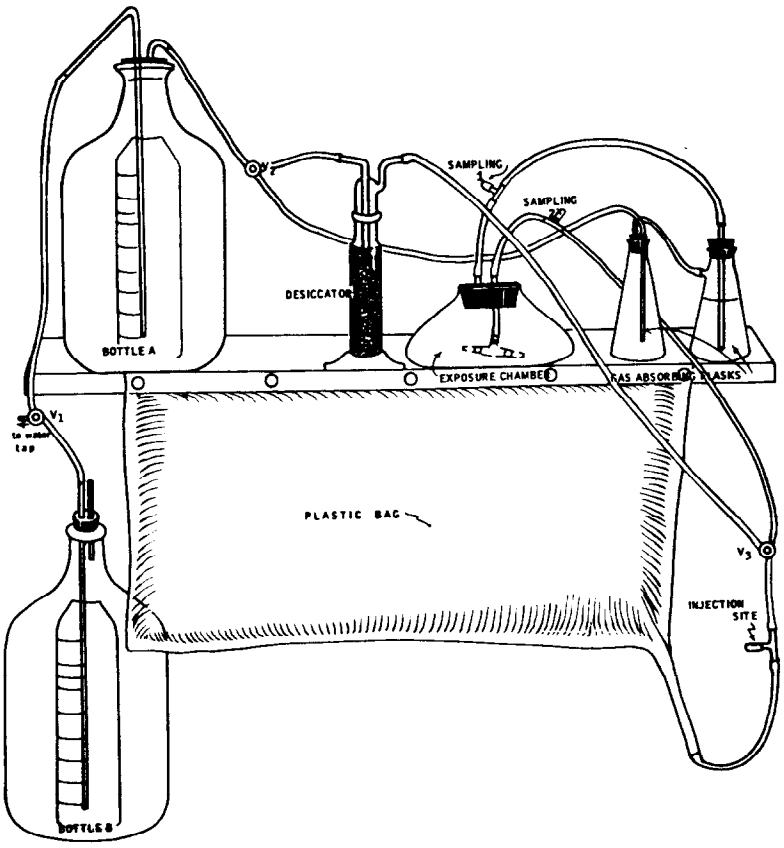

FIGURE 1. Diagram of an apparatus for exposure of mice to low concentrations of NO_2. Valve V_1 is connected to a water supply and a measured volume of air is displaced from bottle A through the desiccator into the plastic bag. While the bag is filling a known amount of NO_2 is added at the injection site. Valves V_2 and V_3 are then changed to allow diluted NO_2 to flow through the animal exposure chamber, NO_2 absorbing flask, and back into bottle A as water is siphoned into bottle B.

has been displaced from bottle A all valves are closed and the polyethylene bag is kneaded gently to assure thorough mixing of the gases. Mice are placed in the exposure chamber and the top is secured. Valves V_2 and V_3 are positioned to allow gas to flow into the exposure chamber and then through a gas washing flask for absorption of excess NO_2 and back into bottle A. To start an exposure valve V_1 is opened allowing water to siphon into bottle B and drawing an equal volume of gas through the exposure chamber. The concentration of NO_2 in the gas entering and leaving the exposure chamber may be determined after withdrawal of a measured volume of gas from the indicated sampling sites. The sample is injected into a serum bottle containing Saltzman's absorbing fluid. The syringe is rinsed several times with absorbing fluid before withdrawal from the bottle. The concentration of NO_2 is determined by the colorimetric method of Saltzman.[2]

Discussion

Proportions of NO_2 in air are determined by the volume of gases allowed to enter the plastic bag. Except for dilution of the NO_2 by air initially present in the chamber the concentration of NO_2 during the exposure is constant and within 10% of the calculated value. Because of the changing head of pressure in the siphon, the gas flow must be adjusted intermittently with valve V_1 to maintain the desired flow rate.

A nearly constant concentration of NO_2 could be maintained over this short exposure period by the method used by Ripperton and Johnston. Several bottles of the gas mixture would be re-

quired to reduce the chance of mixing the prepared NO_2 with air drawn into the bottle most removed from the chamber. The present system appears to be more convenient and it eliminates all chance of a changing concentration of NO_2 during the exposure period.

We are now experimenting with the use of a miniature positive displacement pump of the perastaltic type to deliver an intermediate concentration of NO_2 into a constant flow of air entering the animal exposure chamber. With this system the concentration of NO_2 should be independent of operating time and suitable for long term exposures.

Summary

A compliant plastic bag has been incorporated into a closed system designed for exposure of mice to nitrogen dioxide. The plastic bag allows the delivery of a constant concentration of gas at a controlled rate into an animal exposure chamber at nearly constant pressure.

References

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2. Saltsman, B. E.: Colorimetric Microdetermination of Nitrogen Dioxide in the Atmosphere. *Anal. Chem. 26:* 1949 (1954).