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RESEARCH ON THERAPY OF PULMONARY EDEMA ASSOCIATED WITH OXIDIZERS

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FOREWORD

This research was performed at The Hine Laboratories, Inc., San Francisco, California, under Contract No. AF 33(615)-1781 in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology-Biochemistry." It was administered by the Aerospace Medical Research Laboratories, MRMPT, Wright-Patterson Air Force Base, Ohio.

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This technical report has been reviewed and is approved.

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ABSTRACT

An evaluation was made of candidate therapeutic agents for the treatment of acute pulmonary edema resulting from nitrogen dioxide exposure. Treatments consisting of hyperbaric air and oxygen; tracheal toilet; ethyl, isopropyl, and octyl alcohol vapors; hydralazine; bethanechol; physostigmine; and isoproterenol aerosols produced no change in the mortality, survival time, or lung/body weight ratios of rats suffering from NO_2-induced acute pulmonary edema. Rutin in large doses caused a decrease in mortality and an increase in survival time of exposed rats. Intravenous infusion of isoproterenol caused a decrease in mortality in rabbits exposed to NO_2. The effectiveness of hyperbaric oxygen, hydrocortisone, rutin and bethanechol against moderate exposure to NO_2 was determined by solvent uptake measurements with rats. Oxygen administered 4 hours after exposure increased solvent uptake. There were no significant effects due to the other compounds.

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SECTION I

INTRODUCTION

NO_2 liberated during rocket firing is potentially hazardous to the health of those civilians and military personnel exposed at the site. The optimum treatment of NO_2 casualties has not yet been established; therefore, an investigation for such a problem was considered advisable. This laboratory was previously engaged in a study of NO_2 toxicity in which special attention was paid to the sequence of events in the development of pulmonary pathology following exposure of various species to graded doses (ref. 1). Based on these experiments, it was concluded that the initial toxic response to NO_2 was characterized by the formation of pulmonary edema which, like that due to most other irritant gases, is morphologically and physiologically similar to that following vagal section (ref. 2). This type, in which hyperemic consolidation is the first event, is most likely due to loss of sympathetic nervous system outflow to the lung.

Pulmonary fibrosis, while persisting as long as 6 months in some animals surviving an acute NO_2 gassing, generally resolved or diminished to levels indistinguishable from the sporadic fibrosis which occurs in the older rat. Bronchiolitis fibrosis obliterans, which occasionally occurs in human beings subsequent to NO_2 intoxication, could not be produced in a variety of animals (ref. 1).

Based on these observations, we concluded that the majority of effort should be directed toward a study of possible ameliorating effects on the production of acute pulmonary edema and an evaluation of therapeutic agents which might achieve this. Candidate agents included physiologic support, anti-inflammatory substances, vasodilators, beta-adrenergic and cholinergic compounds. As a measure of potential effectiveness, we used the following criteria: decreased mortality, increased survival time, decreased lung/body weight ratios and solvent uptake.

SECTION II

EXPERIMENTAL APPROACH

GENERAL PROCEDURE

NO_2 Gas

The NO_2 used in these experiments was purchased from The Matheson Company in size 4 cylinders. The minimum purity of the gas was stated to be 99.5%.

Experimental Animals

All of the rats used in these experiments were males of the Sprague-Dawley strain. The animals used varied in weight between 150 and 300 grams. All of the rabbits used were male New Zealand Whites weighing from 2.5 to 4 kilograms. Both rats and rabbits were carefully examined before use and only healthy animals free from respiratory disease were used in the experiments.

Exposure Chambers

Two types of exposure chambers were used. These were of 217- or 7200-liter capacity. Three of the smaller chambers were used in the experiments. They were cylindrical in form and fabricated entirely of steel except for a glass observation port. A circulating fan was installed in the rear of the chamber for mixing. There were entry and sampling ports. A maximum of 8 rats were exposed at one time in these chambers. The 7500-liter exposure chamber was constructed of sheet steel, and measured 6' x 6' x 7'. A 40" circulating fan was mounted near the NO_2 inlet. The chamber accommodated 60 rats and 6 rabbits, with an animal to chamber volume ratio in excess of 1:100.

Exposure Methods

Through a series of preliminary tests we determined the approximate amount of NO_2 gas to be injected into the chamber to produce desired concentrations. The NO_2 was removed directly from the cylinder by use of a calibrated syringe and a suitable coupling device. The NO_2 cylinder was kept in a 37° C air bath to insure uniform volume/concentration relationships. This gas was then injected into the exposure chamber through an entry port. For the 217-liter chamber, 50 ml of pure NO_2 gas at atmospheric pressure produced a concentration varying between 150 and 210 ppm. For the 7500-liter chamber, 2 liters of pure NO_2 were needed to achieve the same concentrations. During exposure, the circulating fans were in operation. All exposures were performed under static conditions and the majority lasted for 15 minutes. In a few runs where there was a low 14-minute NO_2 concentration, the time was extended to 20 minutes to increase the C x T value of the exposure. Rats were placed in individual compartments in an exposure cage and placed in the chamber. The fan was turned on, the door sealed, and the NO_2 injected. Timing of the exposure was started when the injection was completed (maximum of three minutes). Under these exposure conditions of 150-210 ppm for 15 minutes, the average expected mortality in untreated rats would be approximately 66%. Rabbits were placed in wooden restraining boxes which allowed only the head to protrude. They were then placed on the chamber floor and the chamber was sealed. Two liters of pure NO_2 at atmospheric pressure and 37° C was then pumped into the chamber over a period of 3 minutes. All exposures were static, and lasted for 45 minutes from the completion of injection of NO_2.

Monitoring Chamber Concentration

The Saltzman method for determining NO_2, as modified by this laboratory (ref. 1), was used for all determinations. The color reagent N(1-naphthyl) ethylenediamine dihydrochloride (NED) was prepared by dissolving 0.1 gm in 100 ml of water to give a 0.1% solution. This was stored in a brown bottle. Glacial acetic acid (140 ml) was diluted to 980 ml with water and 5 gm of sulfanilic acid added with stirring until dissolved (Saltzman solution). Two ml of NED plus 98 ml of Saltzman solution (SS) were used as the absorbing agent. Standardization was obtained by using graded aliquots of standard sodium nitrite placed in a 25 ml volumetric flask and diluted to the mark with the absorbing agent. Color concentration was read at 15 minutes with a Coleman Junior spectrophotometer at a wave-length of 550 mμ. One ml of the standard was equivalent to 4 μl of NO_2 in 10 ml of the absorbing agent. In this range standards conformed to Beer's Law. Sampling of the NO_2 content was performed by drawing 10 ml of the absorber in a syringe and drawing in an amount of chamber air which would give a satisfactory reactant color. With a 10 ml air sample, the percent transmittance of the solution obtained from an atmosphere of 150-200 ppm of NO_2 was between 30 and 70.

EVALUATION AGAINST THE TOXIC EFFECTS OF NO_2 ON RATS

In all evaluations of candidate therapeutic agents we established a certain procedure to minimize effects other than those produced by the agent. These included simultaneous exposure of control (untreated) and test animals, inclusion of a negative control using the testing agent alone, preassignment before NO_2 gassing by random numbers of the animals to be subsequently treated, and repetition of the experiment with animals from different lots. All experimental animals were observed for a minimum of 10 hours after exposure or treatment. The time of death was recorded, an autopsy performed, and lungs removed for determination of lung/body weight ratios when feasible. These values were multiplied by 100 to give unit decimal numbers. Animals in which postmortem changes were advanced were discarded.

Physiologic Support

Hyperbaric Air and Hyperbaric Oxygen

Administration of oxygen under slightly increased pressure is standard clinical practice in the treatment of pulmonary edema. Our previous experience with NO_2 intoxication (ref. 1) indicated that hyperbaric O_2 when provided under controlled conditions might ameliorate intoxication and lessen severity of pulmonary edema. To determine whether hyperbaric air per se had any effect on pulmonary edema and to reassess the effects of oxygen, we carried out the following experiments. Groups of 8 rats were exposed once in 8 different experiments for 15-20 minutes to NO_2. After exposure 4 from each experiment were treated with air at a pressure of 10 cm of water above ambient pressure for 22-24 hours. Five groups of 8 rats each were exposed to NO_2, and 4 from each group were then placed in a chamber containing 100% O_2 at a pressure of 10 cm water above ambient for 6 hours.

Tracheal Toilet

One of the complications of pulmonary edema is a frothy exudate which fills the air passages and interferes with exchange of respiratory gases. If tracheal toilet is employed to remove this foam and maintain a patent airway, air exchange improves. An evaluation of this mechanical aid was undertaken. One experiment was carried out in which 8 rats were anesthetized with pentobarbital sodium and a tracheal tube inserted. Polypropylene tubing (PE 200 Clay-Adams) was inserted as an external airway with about 3/4" of tubing protruding. A 1-hour waiting period was allowed for recovery, during which time 2 animals died. The remaining 6 animals were divided into two groups while still under mild anesthesia. Three rats were then treated by applying negative pressure to the cannula in order to clear foam and debris. This was done every 15 minutes for 4.5 hours. The control group of animals received no treatment although their tubes were manipulated to stimulate the same degree of handling.

Anti-foam Agents

Anti-foam agents are used to increase surface tension and disrupt the air-liquid surface. Ethanol vapors have been used clinically in the treatment of pulmonary edema with questionable results. We selected three alcohols with low toxicity for evaluation: ethyl, isopropyl, and n-octyl. Half of each group of animals which was gassed with NO_2 was subsequently exposed in a dynamic system to saturated alcohol vapors immediately on completion of gassing. A negative con-

trol group was exposed to the saturated alcohol vapors only. All animals were returned to individual cages for observation.

Eight rats were treated with ethanol vapors for 4 hours. Eight rats were used as the negative controls. Thirty rats were exposed to isopropanol vapors in 6 experiments; in 4 of these, vapor treatment was for 3 hours, and in 2 experiments, for 6 hours. The negative control consisted of 12 animals. Twenty rats were treated with n-octyl alcohol in 5 separate experiments. The negative controls were exposed for 2 hours only, since longer periods were detrimental.

Anti-inflammatory Agents

Hydrocortisone.

While the mechanism of the anti-inflammatory response of the glucocorticoids has not been fully elucidated, they may prevent the formation of cellular elements and liberation of endogenous compounds which are important in the genesis of irritation and edema. Hydrocortisone has a relative potency of 1 according to Goth (ref. 3). There are reports of success following its use in treatment of kerosene pneumonia and aspiration pneumonitis (refs. 4, 5).

There were 6 experiments involving a total of 64 rats. In 5 of these the animals were gassed, and half of these were then treated. In the 6th experiment only the test drug was given. Administration was made intraperitoneally or subcutaneously as an aqueous suspension. The treated groups received the drug at the following dosage and frequency:

Group 1	1 mg/kg just prior to exposure
Group 2	1 mg/kg 24 hours prior to exposure, and 5 minutes before exposure
Groups 3 and 4	1 mg/kg 24 hours before exposure, 1 mg/kg 5 minutes before exposure, and 0.5 mg/kg at 4 and 8 hours post-exposure
Group 5	3 mg/kg 24 hours prior to exposure and 1.5 mg/kg 4 and 8 hours post-exposure

Prednisolone

Prednisolone has approximately 3 times the anti-inflammatory potency of hydrocortisone (ref. 3). Since it causes less sodium retention than other cortocoids it is a popular anti-inflammatory agent for clinical use. Sixteen rats were used in two experiments. A dose of 1 mg/kg was given intraperitoneally to half the group at 24 hours and again at 5 minutes before exposure to NO_2.

Desoxycorticosterone Acetate (DOCA)

We had previously shown (ref. 1) that DOCA may have a slight ameliorating effect on NO_2 toxicity. On theoretical grounds DOCA has little or no anti-inflammatory potency. Instead, its action is on the kidney, where it promotes potassium excretion and sodium retention. Its use is included here because of its structural relationship to the anti-inflammatory steroids. Four experiments were done using 48 animals. Treated animals were administered doses of 2.0 mg/kg subcutaneously in 50:50 corn and sesame seed oil. Negative control animals were injected with the oil mixture. Three different treatment regimens were used. The first group was injected immediately post exposure. The second group was

injected twice: once 24 hours before gassing, and again immediately before gassing. The third group received a total of 8 mg/kg in 4 doses. These were given 24 hours before gassing and immediately before gassing, and 4 and 8 hours after exposure.

Non-adrenergic Hypotensive Agents

Rutin

Rutin (3,3',4',5,7-pentahydroxyflavone-3-rutinoside) (Vitamin P) is reported to decrease capillary permeability and cause long-acting arteriole dilation. It was used in these experiments because of these properties, and because of its smooth muscle relaxing effect on bronchioles. A total of 29 experiments utilizing over 300 animals was done with this agent in order to determine the optimum dose schedule for maximum protection. In seeking this dose schedule, rutin was administered at doses of 125 mg/kg or 250 mg/kg. Higher doses were not evaluated as they produced mortality in themselves. Both single and multiple administrations were made at a number of time intervals extending from 24 hours before exposure to 8 hours afterwards. All injections were intraperitoneal. Rutin was used at low doses as a suspension in water with gum acacia, or at higher doses as a solution in 100% propylene glycol because of limited solubility.

Hydralazine

Hydralazine (1-hydrazinophthalazine) is a potent anti-hypertensive drug (ref. 6) whose principal effect is on the central nervous system. It apparently acts in the midbrain where it diminishes the outflow of sympathetic vasopressor discharges (ref. 7). In addition, it increases renal blood flow as much as 100% (ref. 8). It has been employed clinically in the treatment of essential hypertension with mixed results, and in the toxemia of pregnancy with favorable results (ref. 9). A single protection experiment using 8 animals was carried out with this agent. After exposure to NO_2, 4 of the animals were immediately administered 10 mg/kg of the drug intraperitoneally. In addition, the duration of action of hydralazine in rats was determined by measuring the period of hypotension and increased cardiac rate following preparation of animals for recording these data. After recording normal values with a Statham pressure transducer connected to a Grass polygraph, the animal was given a dose of 10 mg/kg intraperitoneally and a 1-hour record of blood pressure obtained.

Sodium Pentobarbital

Although the principal action of pentobarbital is found in the central nervous system, large doses seem to depress the autonomic ganglia, causing a vasodepression and resultant fall in blood pressure (ref. 10). There is also evidence of vagal depression and increased heart rate (ref. 11). Meyers (ref. 12) has reported that pentobarbital increases the survival time of rats subjected to bilateral vagal section. A total of 40 animals was exposed to NO_2 in 5 experiments. After each exposure, one-half of each exposed group of 8 was administered sodium pentobarbital intraperitoneally. In the first experiment the animals were given 45 mg/kg; in the second, 40 mg/kg; and in 3 other experiments the dose was 30 mg/kg.

Cholinergic Agents

Bethanechol

Bethanechol simulates the action of acetycholine on the receptor cells

of cholinergic autonomic fibres. It also stimulates the cholinergic fibres of sympathetics which are dilator to the arterioles and causes a fall in peripheral blood pressure. Harris (ref. 13) has shown that acetylcholine causes dilation of the pulmonary artery. Since bethanechol is not inactivated by cholinesterase, it has a relatively long duration of action. A total of 11 experiments utilizing 220 animals was carried out. Dose levels and number of animals used were: 0.5 mg/kg in 20 rats; 1.0 mg/kg in 60 rats; 2.0 mg/kg in 60 rats; and 3 mg/kg in 80 rats. This compound was administered intraperitoneally immediately prior to exposure in 8 experiments, and immediately after exposure in 3 experiments.

Physostigmine

Physostigmine is a powerful stimulant to organs innervated by cholinergic fibres, due to its action on cholinesterase. Physostigmine combines with cholinesterase and prevents deesterification of acetylcholine, thus allowing continued activity of acetylcholine. This reaction is reversible, and as the cholinesterase is liberated, it again hydrolyzes acetylcholine and nullifies the action of the drug. In addition to its action on the intestine, eye, and skeletal muscle, physostigmine produces peripheral vasodilation, and may depress the myocardium. A total of 7 experiments utilizing 100 animals was carried out with this drug. Dose levels used were 0.05, 0.10, and 0.15 mg/kg given intraperitoneally immediately before exposure.

Adrenergic Agents

Isoproterenol

Isoproterenol is an adrenergic agent with an isopropyl group substituted for the methyl group on the nitrogen of epinephrine. This agent is very active at the beta-adrenergic receptor sites, and causes bronchodilation, tachycardia, and peripheral vasodilation. It is used clinically as a 1:200 solution for treatment of bronchial asthma. It may be administered via the respiratory route by a special nebulizer. Fifteen experiments involving a total of 128 animals were used in gauging the efficiency of isoproterenol therapy. Following exposure, half of the exposed group was placed in a 200-liter steel chamber in which was placed a Dautreband generator filled with aqueous solutions of the drug. The concentrations used were 0.10%, 1.0% and 5.0%, with an equal percent of ascorbic acid added as an antioxidant. Treatment times varied from 30 minutes to 5 hours. An ether extract of the aqueous isoproterenol was dissolved in corn oil and given intraperitoneally to two groups of rats at doses of 10 mg/kg and 20 mg/kg of the drug, immediately following NO_2 exposures.

Nylidrin

Nylidrin is an adrenergic compound which acts chiefly on the beta receptors. It causes an increase in the cardiac output and peripheral vasodilation. It has been used clinically in the treatment of essential hypertension, Raynaud's disease, and Buerger's disease. It was used here because of its vasodepressor activity. A total of 48 animals were used with 2 treatment regimens. Sixteen of the animals received nylidrin in the amount of 0.4 mg/kg intraperitoneally, post-exposure and 8 received 0.8 mg/kg. Twenty-four animals were used as the positive control. In order to determine whether an effective dose range of this material had been reached, and to determine the approximate duration of action of this drug, an experiment was carried out in which a record of the blood

pressure of the internal carotid artery of the rat was obtained before and after administration of 0.8 mg/kg of nylidrin intraperitoneally.

EVALUATION AGAINST PULMONARY EDEMA IN RABBITS

Adrenergic Agents

Isoproterenol

Seven experiments were carried out using isoproterenol as a therapeutic agent in rabbits. Immediately after exposure, the marginal ear vein was cannulated with a 1-inch, 23-gauge needle from which the hub had been removed and to which was connected a FE 50 Clay-Adams polyethylene tubing filled with an ascorbic acid solution. The cannula was connected to a 10 ml syringe held and driven by a Palmer Model F 130 slow injection apparatus. Six rabbits were treated with 4 mcg, 8 with 2 mcg, 4 with 1 mcg, and 2 with 0.5 mcg/kg/min. Solution strength was adjusted for each of the experiments so that a flow rate of 0.05 ml/min could be maintained. In all cases, equal numbers of control animals were treated with solutions of ascorbic acid equivalent to the solutions used for the treatment. Average time from completion of the exposure to the initiation of treatment was 7 minutes. Treatment time varied between 9.5 and 10.5 hours. Observations were made of the animals' condition for the next 12 hours.

Norepinephrine

It has been suggested that there is a loss of sympathetic tone in the venous bed of the lung after inhalation of irritant gases such as phosgene and NO_2 (ref. 2). Since norepinephrine is the neuromediator of the sympathetic vasomotor nerves, this agent was given to determine whether activation of alpha-adrenergic receptors in the lung could reverse the toxic effects of NO_2 exposure. Five experiments using 10 rabbits were done in the evaluation of norepinephrine. A cut-down was performed on the central ear vein and a polyethylene catheter was inserted as far down the vessel as possible. Infusion of norepinephrine was carried out as in the previous experiment except that the infusion solution was 5% glucose. Treatment time in all cases was approximately ten hours.

EVALUATION BY MEASUREMENT OF SOLVENT UPTAKE

Rats placed in an atmosphere of diethyl ether or other solvent gas at the comparatively low concentration of 1000 ppm will remove ether from the air at a certain rate. This rate is dependent upon the diffusing capacity of the lung, respiratory rate, and the airway resistance. We have previously shown that NO_2 exposure causes a reduction of the uptake of solvent vapors (ref. 14). This is primarily due to decreased pulmonary diffusion (ref. 15) but is also caused by bronchiolar constriction and circulatory shutdown. This technique provides a relatively sensitive measure of pulmonary function. We used changes in pulmonary uptake of diethyl ether as a measure of the effectiveness of 4 of the candidate therapeutic agents discussed in the previous sections. The animals were exposed to NO_2 in the 7500-liter chamber. Exposure conditions were less severe than in the acute protection experiments. NO_2 concentration was 70 ppm and exposure time was 1/2 hour.

Determination of Solvent Uptake by the Rat

The respirometers used in these experiments were essentially those used by Gardner et al. (ref. 14). These consisted of a 1.8-liter glass jar with a metal screw top. A small 6-volt electric motor with a fan blade was bolted to the top and the holes sealed with epoxy resin. A small wire basket suspended below the motor held ascarite to absorb water and CO_2. The top of the chamber also contained an 18-gauge blunt hypodermic needle with a stopcock which allowed introduction and withdrawal of gaseous samples. Rats were conditioned by placing them in individual 1.8-liter jars for several hours prior to the experiment. When conditioned, minimal changes in activity occurred during actual testing, the usual reaction being to curl up and go to sleep. Repeated tests of solvent uptake on normal rats indicated that the expected variation would not exceed ± 10% in 95% of the cases. Previous data obtained by this laboratory (ref. 14) indicated that a 35% decrease in expected solvent uptake would occur under these conditions of NO_2 exposure.

During a solvent uptake determination, the rat was placed in the chamber, a small amount of ascarite was placed in the basket, and the lid secured. The fan was started, and enough diethyl ether vapor injected through the sample port to give a concentration of approximately 1000 ppm. After 3 minutes had elapsed, a 1 ml sample was withdrawn and injected into a Wilkens Hi-Fi Gas Liquid Chromatograph Model 600 B. The column was a 10-ft. stainless steel tube 1/8" outer diameter packed with Castorwax 40% on Chromosorb 80W. Oven temperature was 150° C, injector temperature 180° C. A flame ionization detector was used. The resultant peak defined the initial concentration. At 15 minutes after the injection of the ether, a second 1 ml sample was withdrawn and injected into the GLC. This peak represented the final concentration. The percent difference between these two readings described the percent solvent uptake of the individual rat tested. Since the uptake of solvent vapors increases with body weight (ref. 14), all uptake values were divided by the weight in grams of the individual rat tested. This figure was multiplied by 100, so that solvent uptake was expressed as percent uptake per 100 grams body weight. Prior to the NO_2 exposure, at least 2 determinations of the uptake ability were done, and if these 2 were not in agreement, sufficient determinations were made to achieve a satisfactory mean.

In evaluation of each of the 4 therapeutic regimens we used 72 rats, giving a total of 288 animals tested. We chose 3 exposure-treatment intervals in evaluating the importance of the time of administration of therapy. These were immediately after exposure to NO_2, 4 hours and 24 hours post-exposure. Both positive and negative control groups were included so that one group was exposed to NO_2 but not treated, one group was treated but not exposed to NO_2, and one group was both exposed to NO_2 and treated. Eight animals were used in each of these groups. All animals were tested for solvent uptake immediately before administration of the treatment, and again one hour after treatment. Animals gassed with NO_2, but not treated, were tested in the same time sequence. All three groups were again tested at 72 hours, after which they were sacrificed with excess pentobarbital anesthesia, and the lungs collected for histologic examination and organ/body weight measurements.

Lung sections were examined by the pathologist, Dr. Wright, without knowledge of the particular group from which they were drawn. Pathologic changes of congestion, edema, interstitial fibrosis, bronchiolitis, and interstitial irritation were graded on an arbitrary scale from 0 (normal) to 4 (maximal change).

The significance of data on solvent uptake was analyzed by both comparison of mean values between appropriate groups and by paired grouping. Analysis was done on an IBM 1402 computer programmed to determine significance by T testing. Values of p .05 were accepted as showing significant differences. Significance of data on lung/body weight ratios was determined by the rank order test.

Candidate Therapeutic Agent Evaluated

Oxygen

100% oxygen was administered to the rats at a pressure of 10 cm water above ambient pressure for two hours.

Hydrocortisone

Hydrocortisone was given subcutaneously as a suspension in water at a dose of 2 mg/kg.

Rutin

Rutin was administered as an 8% solution in 100% propylene glycol, intraperitoneally at a single dose of 125 mg/kg.

Bethanechol

Bethanechol was given intraperitoneally as a solution in water at a dose of 2 mg/kg.

SECTION III

PRESENTATION OF DATA

EFFECTIVENESS AGAINST ACUTE TOXICITY IN RATS

Hyperbaric Air and Oxygen

A total of 48 control animals was used in the 9 experiments. The overall mortality was 50%, with a median survival time of 7.1 hours. Among 36 treated animals there was a 36.1% mortality. There were no significant effects on mortality or survival time with either hyperbaric air or oxygen. Supporting data are given in Tables I and II. These data do not support the contention that hyperbaria is an effective treatment of acute pulmonary edema.

TABLE I

SUMMARY OF RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH HYPERBARIC AIR

NO_2 Concentration (ppm)		Exposure Time (Minutes)	Mortality		Survival Time (Hours)	
Initial	Final		T	C	T	C
160	140	15	1/4	0/4	20	
160	120	15	1/4	1/4	24	24
184	156	15	0/8	0/8		
120	88	20	0/4	2/4		18
188	152	15	3/4	2/4	1.5 4 20	3 3.5
208	152	15	2/4	3/4	0.7 1.7	2 24 3
224	136	20	6/8	7/8	3 4 5 12 3 12	1 2 2 4 2 2 12

TABLE II

SUMMARY OF RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH HYPERBARIC OXYGEN

NO_2-Concentration (ppm)		Exposure Time (Minutes)	Mortality		Survival Time (Hours)	
Initial	Final		T	C	T	C
224	144	15	8/8	8/8	0.5 1.5 1 1.5 2 2 2 1.5	1.5 0.2 1.5 1 8 1 2 1.5
182	128	15	1/4	2/4	10	2.5 2.7

Tracheal Toilet

Two of the 3 treated animals had a longer than expected survival time. We did not continue the experiment, however, since the excessive handling of the animals was considered as undesirable and the use of the pentobarbital anesthesia introduced a second undesirable factor. Supporting data are found in Table III.

TABLE III

SUMMARY OF RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH TRACHEAL TOILET

NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
	T	C	T	C	T	C
170	3/3	3/3	36	4	a	0.97

[a] Lungs not collected since the rats died between 11 P.M. and 8 A.M.

Anti-Foam Agents

Ethyl Alcohol

None of the untreated NO_2-gassed rats died. One treated animal died in 2.5 hours. All vapor-exposed animals survived and animals from all groups had signs of mild alcohol intoxication.

Isopropyl Alcohol

Mean mortality in untreated animals was 62% and in treated animals 45%. None of the animals exposed to vapor alone died. Corresponding lung/body weight ratios were 1.85 and 1.60, while median survival times were 2.7 and 3.6 hours. While all three criteria tended to indicate a beneficial effect from the treatment, the differences were not significant.

n-Octyl Alcohol

Mortality in untreated animals was 30% and in treated animals 35%. None of the animals exposed to alcohol vapor alone died. Corresponding lung/body weight ratios were 1.70 in both treated and untreated groups, and median survival times were 12 and 6.5 hours, respectively. No beneficial effect of treatment could be ascertained. Supporting data are presented in Table IV.

With the exception of the ethyl alcohol group, in which mortality among NO_2-exposed animals was too low to permit evaluation, none of the anti-foam agents had a significant beneficial effect.

TABLE IV

SUMMARY OF RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH THREE ANTI-FOAM AGENTS

Alcohol	Hours Treated with Saturated Vapors	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
			T	C	T	C	T	C
Ethyl	4	167	1/4	0/4	2.5	---	1.90	---
		170	0/4	0/4	---	---	---	---
Isopropyl	3	180	6/16	9/16	2.75	2.50	1.40	1.80
	6	160	5/8	6/8	4.50	3.00	1.80	1.90
Octyl	2	148	0/4	1/4				
		124	2/4	3/4				
		127	1/4	0/4	12.0[a]	6.5[a]	1.70[a]	1.70[a]
		180	3/4	1/4				
		160	1/4	1/4				

[a] Median values for the 5 experiments with octyl alcohol

TABLE V

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH HYDROCORTISONE

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Lung/Body Weight Ratio x 100	
		T	C	T	C	T	C
1 mg/kg just before exposure	150	4/4	4/4	3	4	1.82	1.54
1 mg/kg 24 hours and 5 minutes before exposure	140	6/8	4/8	5	1.5	2.09	2.26
1 mg/kg 24 hours and 5 minutes before exposure, 0.5 mg/kg 4 and 8 hours after exposure	160	2/4	4/4	4.5	2	1.84	1.97
1 mg/kg 24 hours and 5 minutes before exposure, and 4 and 8 hours after exposure	160	5/8	6/8	4	2	1.89	1.49
3 mg/kg immediately after exposure	164	3/4	3/4	2.6	9	1.74	1.49

Hydrocortisone

At the 1 mg/kg dose the cumulative mortality of the positive control (NO_2-gassed) group was 75%, of the negative controls (treated only) 0%, and of the gassed and treated group, 70%. Higher or more frequently administered doses had no significant effect. Supporting data are presented in Table V.

Prednisolone

Mortality in both control and treated groups was 87%. The lung/body weight ratios for controls was 1.91 and for treated 2.4. Median survival times were 5 and 2 hours respectively. Supporting data appear in Table VI. No significant differences could be seen due to treatment.

TABLE VI

SUMMARY OF RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH PREDNISOLONE

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
		T	C	T	C	T	C
1 mg/kg 24 hours and 5 minutes before exposure	150	7/8	7/8	2	5	2.4	1.91

Desoxycorticosterone Acetate (DOCA).

The mortality following the 3 treatment schedules was 62.5, 75, and 50%, while the mean of the 3 positive control groups was 50%. No deaths occurred with the negative controls. Corresponding median survival times were 12, 3 and 4 hours for the treated and 6 hours for the controls. Supporting data are given in Table VII. No significant differences could be ascribed to treatment.

TABLE VII

SUMMARY OF RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH DESOXYCORTICOSTERONE ACETATE (DOCA)

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
		T	C	T	C	T	C
2 mg/kg immediately before exposure	150	5/8	4/8	12	8	2.15	---
2 mg/kg at 24 hours and immediately before exposure	160	6/8	5/8	3	3.3	---	---
2 mg/kg at 24 hours and immediately before exposure and at 4 and 8 hours after exposure	150	2/4	1/4	4	6.8	---	---

Non-adrenergic Hypotensive Agents

Rutin

Exclusive of the last treatment schedule, overall mortality in the control groups averaged 58%, with a range of 37 to 100%, and in the treated groups 56%, with an identical range. The median survival time was 9.1 for control animals and 12.1 hours for treated. When we used a dose schedule of 250 mg/kg 16 hours before exposure, 125 mg/kg immediately after exposure, and 125 mg/kg 8 hours after exposure, the mortality for control animals was 71%, and for treated 40%. Median survival times were 3 hours and 12 hours respectively for control and treated rats. In both instances these differences were significant. Doses higher or more frequent than the above caused fatalities in unexposed animals. Supporting data will be found in Table VIII.

Hydralazine

. Mortality was 100% for both treated and control rats. Median survival time for the controls was 12 hours, while that of the treated rats was 2 hours. The blood pressure record of the 2 rats tested showed a transient fall in systolic blood pressure which lasted 15 minutes. In view of the decreased survival time of rats treated with hydralazine and the short duration of action of this compound, we discontinued its use as a possible single dose therapy for pulmonary edema.

Sodium Pentobarbital

At 45 mg/kg: the mortality of the control rats was 75% and the treated 100%. Average lung/body weight ratios were 2.01 for the control and 1.6 for the treated. Median survival times were 4 and 2 hours for control and treated rats respectively.

At 40 mg/kg: the mortality in both groups was 75%. Median survival time was 2 hours for both groups. However, the mean lung/body weight ratio for control animals was 2.29, while that of the treated rats was 1.37.

At 30 mg/kg: mortality of the control group was 66%, and the treated 50%. Median survival time was 2 hours for the treated group and 2.5 hours for the control. The average lung/body weight ratio for controls was 2.24, with a range of 1.79 to 2.66. The mode was 2-2.5, and the median was 2.22. The average lung/body weight ratio of the treated rats was 1.43, with a range of 1.11 to 1.97. The mode was 1.6-1.9, and the median was 1.63. There were no significant effects on mortality or median survival time; the lung/body weight ratios were lowered by 36.2%. Supporting data will be found in Table IX.

Cholinergic Agents

Bethanechol

At 0.5 mg/kg: the mortality of both the control and treated groups was 40%. Median survival time was 43 hours in both groups. Mean lung/body weight ratio of the treated group was 1.31, and that of the control was 2.28.

At 1.0 mg/kg: the mortality for the control group was 45%, and for

TABLE VIII

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH RUTIN

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Survival Time (Hours)			
				T		C	
		T	C	Mean	Median	Mean	Median
125 mg/kg 6 hrs. before exposure	130	6/8	6/8	14	12	12	12
125 mg/kg 24 hrs. before exposure	140	12/12	12/12	3	1	10	1.75
125 mg/kg 24 hrs. and immediately before	175	6/16	11/16	9.4	12	8.6	5.5
125 mg/kg immediately after exposure	173	12/16	10/16	7.6	4.5	7.1	4
250 mg/kg 6 hrs. before exposure	100	1/4	1/4	36	24	36	24
250 mg/kg 24 hrs. before exposure	140	6/12	9/12	1.5	0.75	2.8	2.5
250 mg/kg immediately after exposure	162	2/4	2/4	3.1	3.75	3.6	3.1
250 mg/kg 24 hrs. before and 125 mg/kg immediately before exposure	125	4/8	4/8	66	72	42	48
250 mg/kg 5 hrs. before and 125 mg/kg immediately after exposure	150	8/15	6/15	12.9	3.25	13	3.25
250 mg/kg 16 hrs. before and 125 mg/kg immediately before exposure	120	6/8	7/8	24	12	1.4	1.75
250 mg/kg 16 hrs. before, 125 mg/kg immediately before, and 125 mg/kg 8 hrs. after exposure	182	5/16	7/16	16.4	24	13	8
250 mg/kg 16 hrs. and immediately before exposure; 125 mg/kg immediately after and 8 hrs. after exposure	188	18/45	32/45	19	12	5.1	3

TABLE IX

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH HYDRALAZINE AND PENTOBARBITAL

Agent	Specific Treatment (mg/kg I.P.)	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
			T	C	T	C	T	C
Hydralazine	10	170	4/4	4/4	12.0	9.0	a	a
Pentobarbital	45	130	4/4	3/4	2.0	4.0	1.6	2.01
Pentobarbital	40	154	3/4	3/4	2.0	2.0	1.37	2.29
Pentobarbital	30	164	6/12	8/12	2.0	2.5	1.43	2.24

[a]Data inadvertently not recorded

the treated rats 40%. Median survival time was 34 hours for control and 14 hours for treated rats. The mean lung/body weight ratio for the controls was 2.16, and that of the treated rats was 1.62.

At 2.0 mg/kg: the mortality for the control group was 76.6%, and for the treated 53.3%. The median survival time for control rats was 2.5 hours and for the treated rats 21 hours. Mean lung/body weight ratios were 2.53 for control rats and 2.02 for treated rats.

At 3.0 mg/kg: the mortality for the control group was 65%, and for the treated rats 70%. Median survival time was approximately 2 hours for both groups. The mean lung/body weight ratio was 2.09 for the control rats and 2.23 for the treated rats. In the group which received treatment after exposure at 3 mg/kg, the mortality was 85%, while that of the corresponding control was 90%. Median survival time was 2 hours for both groups. The mean lung/body weight ratio of the controls was 2.03, while that of the treated rats was 1.93. The beneficial effect seen with the dose of 2 mg/kg amounted to a 23.3% decrease in mortality and an increase of 18 hours in median survival time. In five of the six treatment groups the lung/body weight ratios were less than their controls. Mean mortality for all control groups was 69%. Supporting data are given in Table X.

Physostigmine

At 0.05 mg/kg: the mortality for the control group was 83.3%, and for the treated group 20.8%; mortality was significantly decreased by this treatment. Median survival time was 12 hours for the control animals and 2.25 hours for the treated rats, so that treated animals which died survived for a significantly shorter time. Mean lung/body weight ratio for control rats was 1.68, and 1.73 for treated animals.

At 0.10 and 0.15: no significant protective effects were seen on mortality and the treated rats died more rapidly. There were signs of physostigmine toxicity in both treated rats and negative controls at these doses. Supporting data are given in Table XI.

TABLE X

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH BETHANECHOL

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
		T	C	T	C	T	C
0.5 mg/kg before gassing	200	4/10	4/10	43	43	1.31	2.28
1.0 mg/kg before gassing	198	8/20	9/20	14	34	1.62	2.16
2 mg/kg before gassing	204	16/30	23/30	21.3	2.5	2.02	2.53
3 mg/kg before gassing	198	15/30	18/30	1.5	2.0	2.23	2.09
3 mg/kg immediately after gassing	161	17/20	19/20	1.75	2.0	1.93	2.03
1 mg/kg before gassing and 1 mg/kg immediately after gassing	215	9/10	8/10	22	2.5	1.87	3.86

TABLE XI

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH PHYSOSTIGMINE

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
		T	C	T	C	T	C
0.05 mg/kg Physostigmine I.P. before exposure	181	5/24	20/24	2.25	12	1.73	1.68
0.1 mg/kg Physostigmine I.P. before exposure	147	16/21	10/21	4	16	1.56	1.52
0.15 mg/kg Physostigmine I.P. before exposure	144	4/4	2/4	2	12	-	-

Adrenergic Agents

Isoproterenol

At 0.01% aerosol for 2 hours: the mortality of the control group was 88%, and that of the treated was 100%. Median survival time was 2 hours for

each group, and the mean lung/body weight ratio was 2.13 and 1.91 for control and treated rats respectively.

At 0.1% aerosol for 5 hours: the mortality of the control group was 85.5%, and that of the treated rats was 100%. Median survival time was 2 hours for both groups, and the mean lung/body weight ratio was 2.27 for the control rats and 2.47 for the treated animals.

At 1% aerosol for 4 hours: the mortality of the control group was 83%, and that of the treated group was 100%. Median survival time was 2.5 hours for the control group and 1.75 hours for the treated group. Mean lung/body weight ratios were 2.65 for treated and 1.8 for controls.

At 5.0% aerosol for 0.5 hour: the mortality was 100% for the treated animals and 50% for the controls. Median survival time was 24 hours for controls and 0.5 hour for the treated.

When given intraperitoneally at 10-20 mg/kg, no significant effects were apparent on mortality or survival time. Negative controls exhibited central nervous system stimulation. We concluded that none of the therapeutic regimens exerted a beneficial effect. The lowest solution strengths produced no signs of any of the actions of isoproterenol on unexposed rats. The aerosol exposure at 1 and 5% and the intraperitoneal injections at 10 and 20 mg/kg produced jitteriness and hyperreflexia in unexposed rats. Supporting data appear in Table XII.

TABLE XII

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH ISOPROTERENOL

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
		T	C	T	C	T	C
Isoproterenol 5% Aerosol 0.5 hours	135	4/4	2/4	0.5	24	2.35	-
Isoproterenol 1% Aerosol 4 hours	146	12/12	10/12	1.75	2.5	2.65	1.8
Isoproterenol 0.5% Aerosol 5 hours	138	8/8	8/8	0.75	1	2.54	2.39
Isoproterenol 0.1% Aerosol 5 hours	167	8/8	7/8	2	2	2.47	2.27
Isoproterenol 0.01% Aerosol 2 hours	158	8/8	7/8	1.5	1.6	1.91	2.13
Isoproterenol I.P. in Oil 10 mg/kg	120	3/4	2/4	12	7	-	-
Isoproterenol I.P.	160	4/4	2/4	2	4	1.9	2.45

Nylidrin

At 0.4 mg/kg: the mortality for the control group was 75%, and that for the treated was 87%. Median survival times were not significantly different.

At 0.8 mg/kg: the mortality in both groups was 87.5%. Median survival times were 4 hours for the control group and 2 hours for the treated group.

Approximately 1 minute after the injection of nylidrin, a sharp drop in the blood pressure was observed. This lasted for 12 minutes, after which time the blood pressure returned to the pre-injection level. This very short action is insufficient to protect against pulmonary edema, and repeated injections were not undertaken as the extra handling of the animals involved in such a procedure was considered detrimental. Supporting data are given in Table XIII.

TABLE XIII

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA IN RATS WITH NYLIDRIN

Specific Treatment	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)	
		T	C	T	C
Nylidrin 0.4 mg/kg after exposure	166	14/16	12/16	3	7
Nylidrin 0.8 mg/kg after exposure	143	7/8	7/8	2	4

EFFECTIVENESS AGAINST PULMONARY EDEMA IN RABBITS

Adrenergic Agents

Isoproterenol

At 0.5 mcg/kg/min: the mortality in the control group was 100%, and in the treated group 50%. Median survival time was 3.35 hours for the control and 5.45 hours for the treated. Mean lung/body weight ratios were 1.03 and 1.25 for control and treated, respectively.

At 1 mcg/kg/min: the mortality in the control group was 100%, and in the treated group 50%. Median survival time was 0.83 hours for the control group and 1.35 hours for the treated rabbits. The mean lung/body weight ratios were 1.5 for controls, and 1.2 for treated rabbits.

At 2 mcg/kg/min: the mortality in the control group was 50% and for the treated group 25%. Median survival times were 1.25 hours for the controls and 5.75 hours for the treated. Mean lung/body weight ratios for the controls were 1.18 and for the treated 0.97

At 4 mcg/kg/min: the mortality in the control group was 33%, and in the treated group 83%. Median survival time was 4 hours for the control group

and 12 hours for the treated.

In all surviving rabbits, 6 hours after the start of the infusion there was some infiltration of the tissue in the region of the base of the ear. This may have lessened the effective dose delivered. Perhaps the use of a larger vessel would have yielded better results. When the treatment range varied between 0.5 and 2.0 mcg/kg/min the total mortality was 5/14, which was significantly different from that of the control. The higher dose of 4 mcg showed a deleterious effect on mortality, but animals survived for a significantly longer period. There was a significant difference in survival time. No consistent change in lung/body weight ratios occurred.

Norepinephrine

Mortality was 60% for the control rabbits and for treated rabbits 20%. Median survival time was 1 hour for control rabbits and 10 hours for treated rabbits. Mean lung/body weight ratios for control animals was 1.50, while that of the treated rabbit was 1.12. It appears that norepinephrine may have a beneficial effect on the mortality and survival time of rabbits exposed to NO_2. This point should be confirmed by use of larger numbers of animals and by varying doses. Supporting data appear in Table XIV.

TABLE XIV

RESULTS OF TREATMENT OF NO_2-INDUCED PULMONARY EDEMA WITH INTRAVENOUS NOREPINEPHRINE AND ISOPROTERENOL IN THE RABBIT

Agent	Mcg/kg/min	NO_2 Concentration (ppm)	Mortality		Median Survival Time (Hours)		Mean Lung/Body Weight Ratio x 100	
			T	C	T	C	T	C
Norepinephrine	1.0	160	1/5	3/5	10.0	1.0	1.12	1.5
Isoproterenol	0.5	170	1/2	2/2	5.45	3.35	1.25	1.03
Isoproterenol	1.0	170	2/4	4/4	1.35	0.83	1.2	1.5
Isoproterenol	2.0	170	2/8	4/8	5.75	1.25	0.97	1.18
Isoproterenol	4.0	170	5/6	2/6	12.0	4.0	1.4	1.15

EFFECTIVENESS ON THE PULMONARY UPTAKE OF DIETHYL ETHER

GENERAL RESULTS

Over 1,200 solvent uptake determinations were made in the 288 rats which were divided among the 36 test groups. The solvent uptake of normal rats ranged from 14.79 to 22.48%, Tables XV, XVIII, XXI and XXIV. Twenty-four groups of 8 animals were gassed with a C x T product of 70 ppm x 0.5 hours = 35 ppm-hours. All gassed animals showed signs of mild NO_2 intoxication characterized by arching of the body, ruffling of fur, closed eyelids, lethargy, and changes in respiratory rate and volume. Seventeen of the groups had a significantly decreased solvent uptake immediately after gassing when significance was determined by group mean comparison and 19 when paired differences were tested. After 4 hours, impairment of solvent uptake had improved in 4 of the

6 untreated groups that had shown initial impairment, while at 24 hours 5 of the 6 groups whose solvent uptake had been impaired and had not received treatment remained impaired. At 72 hours post-exposure, 10 of the 12 groups which had been exposed to NO_2 but not treated had returned to their pre-exposure uptake levels, while 2 still showed impairment. There was an effect of treatment alone in rats not exposed to NO_2 in 3 of the 36 time-treatment sequences. Oxygen treatment increased uptake in 1 instance while both bethanechol and rutin decreased it in another. The paired value comparisons showed differences between groups slightly more frequently than did group mean comparisons.

Oxygen

The test group which was treated 1 hour after NO_2 exposure did not have the expected decrease in solvent uptake. Consequently the effect of treatment could not be assessed at this time period. However, the solvent uptake of the group treated at 4 hours after exposure was depressed 1/3 in the immediate post-exposure period. After treatment was initiated, there was a mean increase in solvent uptake of 25%. This improvement remained at 72 hours, and the uptake at that time was significantly greater than pre-gassing values. These differences were significant at the $p = 0.5$ level. When oxygen therapy was postponed until 24 hours, no beneficial effect was seen, although by 72 hours the solvent uptake had returned to normal values. Supporting data are given in Tables XV and XVI. There were no significant differences in the lung/body weight ratios of rats both treated and exposed and rats exposed only at any exposure-treatment interval. The difference between exposed rats and rats not exposed was significant at the $p = 0.01$ level. The histologic picture of all the rats was similar, and no significant variation between groups was present. Supporting data are given in Table XVII.

Hydrocortisone

There were no significant changes in solvent uptake which could be ascribed to treatment 1 hour after treatment at the immediate, 4-, or 24-hour exposure-treatment intervals. However, evaluation was complicated by the return to pre-exposure solvent uptake values in the 4-hour treatment group. Seventy-two hours after NO_2 exposure, there were no significant differences between any of the 3 groups at any of the exposure-treatment intervals, Tables XVIII and XIX. There were no significant differences in the lung/body weight ratios of treated and exposed rats when compared to the exposed control rats at any exposure-treatment interval. The difference between exposed and unexposed rats was significant at the $p = 0.01$ level. The histological picture of the lungs was similar for all 3 groups of rats at exposure-treatment times of immediate and 24-hour post-exposure. However, when treatment was given at 4 hours after exposure, there was a decrease in the occurrence and intensity of pathologic changes in the exposed and treated group. Mean values for congestion were 0.5 for gassed and treated rats, 2.2 for exposed only rats, and 1.2 for treated only rats. Mean values for edema were 0.2 for exposed and treated rats, 2.2 for exposed only rats, and 0.0 for treated only rats. There was no interstitial irritation or bronchiolitis in either the exposed and treated rats or the treated only rats, whereas the mean values for the exposed only rats was 1.2 for irritation, and 0.8 for bronchiolitis. Table XX gives supporting data.

TABLE XV

SUMMARY OF MEAN SOLVENT UPTAKE[a] FOR RATS TREATED WITH OXYGEN AFTER EXPOSURE TO NO_2

Time of Uptake Measurement	Group and Exposure-Treatment Interval in Hours: Exposed and Treated 1	4	24	Exposed Only 1	4	24	Treated Only 1	4	24
Immediate Pre-Exposure	15.70	15.79	16.95	16.38	16.53	18.24	16.40	17.03	18.40
	0.98	0.68	1.32	0.33	0.77	1.24	1.19	0.54	1.03
Immediate Post-Exposure	12.83	10.36	16.63	10.10	10.89	10.45	b	b	b
	2.71	0.87	0.95	3.20	1.68	1.28			
Immediate Pre-Treatment	12.83	12.11	11.70	b	15.80[c]	b	16.40	12.63	16.98
	2.71	0.39	1.17		0.62		1.19	0.67	1.64
1 Hour Post-Treatment	12.03	16.21	12.15	9.35	17.60	14.23	15.33	16.76	15.64
	1.43	1.36	2.66	1.15	0.93	0.63	0.51	0.82	0.60
72 Hours Post-Exposure	11.67	18.84	15.10	10.90	16.37	15.05	15.50	17.04	17.03
	1.40	0.93	0.68	2.50	1.21	1.54	1.24	0.70	0.89

[a] Expressed as % diethyl ether removed in 15 minutes per 100 grams of rat plus the standard error.

[b] Experimental design did not call for testing at this time period.

[c] Internal control to check 1-hour values.

TABLE XVI

SUMMARY OF P VALUES OBTAINED FROM SIGNIFICANCE TESTS FOR SOLVENT UPTAKE VALUES ACCORDING TO GROUP, EXPOSURE-TREATMENT INTERVAL, AND THE TIME OF TESTING OF NO_2-EXPOSED RATS TREATED WITH OXYGEN

		Exposure-Treatment Intervals in Hours					
		1		4		24	
Comparison	Group	GM[a]	PM[b]	GM[a]	PM[b]	GM[a]	PM[b]
Before Gassing vs. Immediately After	A[c]	--[f]	--	0.01	0.01	0.05	0.05
	B[d]	--	--	0.01	0.05	0.01	0.01
	C[e]						
Immediate Post-Gassing vs. Pre-Treatment	A			--	--	--	--
	B			0.05	0.05	--	--
	C						
Immediate Pre-Treatment vs. 1 Hour Post-Treatment	A	--	--	0.05	0.05	--	--
	B						
	C	--	--	0.01	0.02	--	--
Immediate Pre-Treatment vs. > 48 Hours Post-Treatment	A	--	--	0.01	0.01	--	--
	B	--	--	0.05	0.01	--	--
	C	--	--	--	0.01	--	0.05
Before Gassing vs. 72 Hours Post-Gassing	A	--	--	0.05	0.05	--	--
	B	--	--	--	--	--	--
	C	--	--	--	--	--	--

[a] Group mean

[b] Mean differences of paired values

[c] Exposed and treated rats (N=8)

[d] Exposed only rats (N=8)

[e] Treated only rats (N=8)

[f] -- Indicates no significant difference

TABLE XVII

RESULTS OF TREATMENT OF NO_2-EXPOSED RATS WITH OXYGEN ON PATHOLOGY AND LUNG/BODY WEIGHT RATIOS

Exposure Treatment Interval	Group	Mean Lung/Body x 100	Mean Numerical Score[a]			
			Congestion	Edema	Interstitial Irritation	Bronchiolitis
Immediate	Exposed and Treated	1.38				
Immediate	Exposed Only	1.26				
Immediate	Treated Only	0.60				
4 Hours	Exposed and Treated	0.85	1.0	0.2	1.2	0.2
4 Hours	Exposed Only	0.87	0.0	0.0	0.0	0.0
4 Hours	Treated Only	0.55	0.2	0.2	0.2	0.2
24 Hours	Exposed and Treated	0.98	3.5	3.6	2.0	1.0
24 Hours	Exposed Only	1.52	2.2	2.5	2.5	2.0
24 Hours	Treated Only	1.71	1.2	0.2	1.2	0.0

[a] Based on scale 0 (normal) to 4 (maximal change)

TABLE XVIII

SUMMARY OF MEAN SOLVENT UPTAKE[a] FOR RATS TREATED WITH HYDROCORTISONE AFTER EXPOSURE TO NO_2

Time of Uptake Measurement	Group and Exposure-Treatment Interval in Hours								
	Exposed and Treated			Exposed Only			Treated Only		
	1	4	24	1	4	24	1	4	24
Immediate Pre-Exposure	14.83	16.83	17.72	14.93	15.60	17.60	15.60	16.11	16.88
	0.69	1.16	0.84	0.75	1.03	0.88	0.68	0.79	2.54
Immediate Post-Exposure	10.68	11.19	14.04	10.84	9.98	11.80			
	0.45	0.68	1.54	0.98	0.85	0.48			
Immediate Pre-Treatment	10.68	14.53	13.64				15.60	16.11	19.51
	0.45	0.46	1.17				0.68	0.80	1.03
1 Hour Post-Treatment	12.83	13.71	13.81		14.77	14.71	16.24	16.14	19.34
	1.57	0.45	1.64		1.06	0.61	0.87	0.88	1.44
72 Hours Post-Exposure	15.23	15.06	15.48	16.41	16.17	15.21	15.45	15.06	17.40
	0.67	0.68	2.40	1.13	1.23	1.27	1.00	0.73	2.12

[a] Expressed as % diethyl ether removed in 15 minutes per 100 grams of rat plus the standard error.

TABLE XIX

SUMMARY OF P VALUES OBTAINED FROM SIGNIFICANCE TESTS FOR SOLVENT UPTAKE VALUES ACCORDING TO GROUP, EXPOSURE-TREATMENT INTERVAL, AND THE TIME OF TESTING OF NO_2-EXPOSED RATS TREATED WITH HYDROCORTISONE

		Exposure-Treatment in Hours					
		1		4		24	
Comparison	Group	GM[a]	PM[b]	GM[a]	PM[b]	GM[a]	PM[b]
Before Gassing vs. Immediately After	A[c]	0.01	0.01	0.01	0.01	--[f]	0.02
	B[d]	0.02	0.02	0.01	0.01	0.01	0.01
	C[e]						
Immediate Post-Gassing vs. Pre-Treatment	A			0.01	0.01	--	--
	B			0.01	0.05	0.01	--
	C						
Immediate Pre-Treatment vs. 1 Hour Post-Treatment	A	--	--	--	--	--	--
	B						
	C	--	--	--	--	--	--
Immediate Pre-Treatment vs. > 48 Hours Post-Treatment	A	0.01	0.01	--	--	--	--
	B	0.01	0.05	0.01	0.01	--	--
	C	--	--	--	--	--	--
Before Gassing vs. 72 Hours Post-Gassing	A	--	--	--	--	--	--
	B	--	--	--	--	--	--
	C	--	--	--	--	--	--

[a] Group mean

[b] Mean differences of paired values

[c] Exposed and treated rats (N=8)

[d] Exposed only rats (N=8)

[e] Treated only rats (N=8)

[f] -- Indicates no significant difference

TABLE XX

RESULTS OF TREATMENT OF NO_2-EXPOSED RATS WITH HYDROCORTISONE ON PATHOLOGY AND LUNG/BODY WEIGHT RATIOS

Exposure Treatment Interval	Group	Mean Lung/Body x 100	Mean Numerical Score[a]			
			Congestion	Edema	Interstitial Irritation	Bronchiolitis
Immediate	Exposed and Treated	0.75	1.0	0.2	0.0	1.2
Immediate	Exposed Only	0.68	1.6	0.3	0.0	0.6
Immediate	Treated Only	0.44	2.8	1.0	0.8	0.8
4 Hours	Exposed and Treated	0.80	0.5	0.2	0.0	0.0
4 Hours	Exposed Only	0.84	2.2	2.2	1.2	0.8
4 Hours	Treated Only	0.49	1.2	0.0	0.0	0.0
24 Hours	Exposed and Treated	0.80	2.0	0.8	0.0	0.0
24 Hours	Exposed Only	0.76	2.0	0.8	1.0	0.0
24 Hours	Treated Only	0.60	1.2	0.8	1.8	0.0

[a] Based on scale 0 (normal) to 4 (maximal change)

Rutin

There were no significant changes in solvent uptake 1 hour after treatment or at any of the exposure-treatment intervals. Seventy-two hours after exposure, there was a significant decrease in the solvent uptake of the exposed and treated group in comparison with its pre-exposure value. Also, when rutin was administered 24 hours after exposure and the uptake measured 48 hours later, a significant depression of solvent uptake occurred, Tables XXI and XXII. There were no significant differences in the lung/body weight ratios of treated and exposed rats and exposed only rats at any exposure-treatment interval. The difference between exposed and unexposed rats is significant at the $p = 0.01$ level. Histological examination of the lungs showed similar findings among all three groups at each exposure-treatment interval, Table XXIII.

Bethanechol

There were no significant changes in solvent uptake 1 hour after treatment at any of the exposure-treatment intervals. Unfortunately there were no significant changes in solvent uptake of the groups treated at 4- and 24-hour post gassing when compared with their pre gassing uptakes. Seventy-two hours after exposure, there were no significant differences between exposed and treated rats, exposed only rats, and treated only rats, Tables XXIV and XXV. There were no significant differences in the lung/body weight ratios of treated and exposed rats in comparison to the positive control, exposed only, rats at any exposure treatment interval. The difference between exposed and not exposed rats was significant at the $p = 0.01$ level. The histologic picture of the lungs was similar for all three groups at each exposure-treatment interval. Table XXVI gives supporting data.

TABLE XXI

SUMMARY OF MEAN SOLVENT UPTAKE[a] FOR RATS TREATED WITH RUTIN AFTER EXPOSURE TO NO_2

Time of Uptake Measurement	Group and Exposure-Treatment Interval in Hours								
	Exposed and Treated			Exposed Only			Treated Only		
	1	4	24	1	4	24	1	4	24
Immediate Pre-Exposure	16.28	22.48	16.60	17.31	18.40	18.01	17.63	15.47	16.30
	0.59	1.23	1.47	0.53	1.35	0.64	1.24	0.86	2.21
Immediate Post-Exposure	13.75	14.75	12.89	9.93	15.14	14.10			
	0.88	1.99	1.76	1.27	2.38	1.12			
Immediate Pre-Treatment	13.75	14.89	14.75					15.47	15.67
	0.88	0.74	1.00					0.86	1.06
1 Hour Post-Treatment	15.40	10.96	16.71		13.60	19.20	14.56	15.18	14.61
	2.10	1.77	1.93		1.38	2.40	1.35	1.75	1.78
72 Hours Post-Exposure	14.94	10.80	16.42	16.86	15.35	14.70	16.08	13.49	15.58
	1.23	1.62	2.40	0.64	1.43	1.02	1.28	0.92	0.83

[a] Expressed as % diethyl ether removed in 15 minutes per 100 grams of rat plus the standard error.

TABLE XXII

SUMMARY OF P VALUES OBTAINED FROM SIGNIFICANCE TESTS FOR SOLVENT UPTAKE VALUES ACCORDING TO GROUP, EXPOSURE-TREATMENT INTERVAL, AND THE TIME OF TESTING OF NO_2-EXPOSED RATS TREATED WITH RUTIN

		Exposure-Treatment in Hours					
		1		4		24	
Comparison	Group	GM[a]	PM[b]	GM[a]	PM[b]	GM[a]	PM[b]
Before Gassing vs. Immediately After	A[c]	0.05	0.05	0.01	0.01	--[f]	--
	B[d]	0.01	0.01	--	0.05	0.02	0.02
	C[e]						
Immediate Post-Gassing vs. Pre-Treatment	A			--	--	--	--
	B			--	--	--	--
	C						
Immediate Pre-Treatment vs. 1 Hour Post-Treatment	A	--	--	--	--	--	--
	B						
	C	--	--	--	--	--	--
Immediate Pre-Treatment vs. >48 Hours Post-Treatment	A	--	--	--	0.05	--	--
	B	0.01	0.01	--	--	--	--
	C	--	--	--	--	--	--
Before Gassing vs. 72 Hours Post-Gassing	A	--	--	0.01	0.01	--	--
	B	--	--	--	0.05	--	0.02
	C	--	--	--	--	--	0.01

[a] Group mean

[b] Mean differences of paired values

[c] Exposed and treated rats (N=8)

[d] Exposed only rats (N=8)

[e] Treated only rats (N=8)

[f] -- Indicates no significant difference

TABLE XXIII

RESULTS OF TREATMENT OF NO_2-EXPOSED RATS WITH RUTIN ON PATHOLOGY AND LUNG/BODY WEIGHT RATIOS

Exposure Treatment Interval	Group	Mean Lung/Body x 100	Mean Numerical Score[a]			
			Congestion	Edema	Interstitial Irritation	Bronchiolitis
Immediate	Exposed and Treated	0.83	1.2	0.7	2.0	0.5
Immediate	Exposed Only	0.82	1.5	0.5	0.7	0.5
Immediate	Treated Only	0.60	0.5	0.7	0.2	0.0
4 Hours	Exposed and Treated	0.95	1.2	0.2	0.5	0.2
4 Hours	Exposed Only	0.65	0.0	0.7	1.0	0.2
4 Hours	Treated Only	0.61	1.0	0.0	0.0	0.0
24 Hours	Exposed and Treated	0.94	1.5	0.5	0.5	0.5
24 Hours	Exposed Only	0.84	1.5	1.0	0.5	0.0
24 Hours	Treated Only	0.71	2.3	1.6	2.3	0.0

[a] Based on scale 0 (normal) to 4 (maximal change)

TABLE XXIV

SUMMARY OF MEAN SOLVENT UPTAKE[a] FOR RATS TREATED WITH BETHANECHOL AFTER EXPOSURE TO NO_2

Time of Uptake Measurement	Group and Exposure-Treatment Interval in Hours								
	Exposed and Treated			Exposed Only			Treated Only		
	1	4	24	1	4	24	1	4	24
Immediate Pre-Exposure	15.88	14.79	15.91	15.96	15.05	17.98	17.21	15.91	15.39
	0.65	0.66	1.34	0.80	0.83	1.24	0.87	0.60	1.13
Immediate Post-Exposure	10.26	14.45	11.60	12.16	12.24	9.63			
	1.03	2.14	1.34	0.42	0.63	1.08			
Immediate Pre-Treatment	10.26	14.08	15.14		14.28		17.41	14.68	14.70
	1.03	2.08	1.24		0.97		1.38	0.56	0.89
1 Hour Post-Treatment	13.44	12.31	16.18			15.71	14.84	14.93	12.76
	2.24	1.59	1.77			2.54	0.68	0.47	1.64
72 Hours Post-Exposure	15.50	17.95	15.09	14.53	14.63	16.78	14.60	15.70	15.20
	1.87	1.91	1.14	0.72	1.46	0.84	0.74	0.47	1.58

[a] Expressed as % diethyl ether removed in 15 minutes per 100 grams of rat plus the standard error.

TABLE XXV

SUMMARY OF P VALUES OBTAINED FROM SIGNIFICANCE TESTS FOR SOLVENT UPTAKE VALUES ACCORDING TO GROUP, EXPOSURE-TREATMENT INTERVAL, AND THE TIME OF TESTING OF NO_2-EXPOSED RATS TREATED WITH BETHANECHOL

Comparison	Group	Exposure-Treatment in Hours: 1		4		24	
		GM[a]	PM[b]	GM[a]	PM[b]	GM[a]	PM[b]
Before Gassing vs. Immediately After	A[c]	0.01	0.01	--[f]	--	--	--
	B[d]	0.01	0.01	0.05	0.02	0.01	0.01
	C[e]						
Immediate Post-Gassing vs. Pre-Treatment	A			--	--	--	0.05
	B			--	--	--	0.01
	C						
Immediate Pre-Treatment vs. 1 Hour Post-Treatment	A	--	--	--	--	--	--
	B						
	C	--	--	--	--	--	--
Immediate Pre-Treatment vs. >48 Hours Post-Treatment	A	0.05	0.05	--	--	--	--
	B	0.05	0.05	--	--	--	0.01
	C	--	0.02	--	--	--	--
Before Gassing vs. 72 Hours Post-Gassing	A	--	--	--	--	--	--
	B	--	--	--	--	--	--
	C	--	0.02	--	--	--	--

[a] Group mean

[b] Mean difference of paired values

[c] Exposed and treated rats (N=8)

[d] Exposed only rats (N=8)

[e] Treated only rats (N=8)

[f] -- Indicates no significant difference

TABLE XXVI

RESULTS OF TREATMENT OF NO_2-EXPOSED RATS WITH BETHANECHOL ON PATHOLOGY AND LUNG/BODY WEIGHT RATIOS

Exposure Treatment Interval	Group	Mean Lung/Body x 100	Mean Numerical Score[a]			
			Congestion	Edema	Interstitial Irritation	Bronchiolitis
Immediate	Exposed and Treated	0.92	1.0	0.0	0.0	0.0
Immediate	Exposed Only	0.96	1.2	0.2	0.7	0.5
Immediate	Treated Only	0.52	2.0	1.0	0.5	0.7
4 Hours	Exposed and Treated	1.12	1.6	1.3	0.6	0.6
4 Hours	Exposed Only	0.91	0.3	0.0	0.0	0.6
4 Hours	Treated Only	0.48	0.7	0.5	1.5	1.2
24 Hours	Exposed and Treated	0.93	0.2	0.5	1.0	1.0
24 Hours	Exposed Only	0.72	2.0	1.2	0.2	0.0
24 Hours	Treated Only	0.65	3.0	0.7	0.7	0.0

[a] Based on scale 0 (normal) to 4 (maximal change)

SUMMARY

1. Exposure of rats to NO_2 at nominal concentrations of 200 ppm for 15 minutes resulted in a mean mortality of 65%.

2. Treatment of these rats with hyperbaric air and oxygen, tracheal toilet, ethyl, octyl and isopropyl alcohol vapors, hydrocortisone, prednisolone, desoxycorticosterone acetate, hydralazine, pentobarbital, bethanechol, physostigmine, and isoproterenol after exposure produced no beneficial effects as indicated by mortality, survival time, and lung/body weight ratios.

3. Treatment of these rats with rutin at a dose schedule of 250 mg/kg 16 hours pre-exposure, 125 mg/kg immediately after exposure, and 125 mg/kg 8 hours post-exposure, lowered mortality and increased survival time.

4. Exposure of rabbits to NO_2 at 170 ppm for 45 minutes produced a mean mortality of 60%.

5. Treatment of these rabbits by intravenous administration of isoproterenol in doses of 0.5 to 2 mcg/kg/min significantly lowered the mortality and increased mean survival time.

6. Treatment of these rabbits by intravenous administration of norepinephrine at a dose of 1 mcg/kg/min caused a decrease in mortality and an increase in survival time.

7. Exposure of rats to NO_2 at 70 ppm for 30 minutes produced a 14.8 - 22.5% decrease in solvent uptake, an increase in lung/body weight ratios, and diffuse pathologic changes in the lung.

8. Treatment of these animals immediately after and at 4 and 24 hours after exposure with hyperbaric oxygen, hydrocortisone, rutin and bethanechol did not beneficially affect any of the undesirable changes, with the exception of oxygen administered at 4 hours, which improved solvent uptake, and hydrocortisone at 4 hours, which decreased the extent of the pathologic lesions.

SECTION IV

CONCLUSIONS

The experimental design was sufficiently critical to permit an adequate appraisal of the candidate therapeutic agents. Conditions of exposure resulted in mean mortalities of untreated animals of greater than 50% in both rats and rabbits and a decrease in the uptake of solvent vapors by nearly all rats tested. Microscopic evidence of local cellular changes in pulmonary tissue, including edema, congestion, interstitial irritation and bronchiolitis, was present to some degree in all gassed rats from which specimens were taken. An increase in the lung/body weight ratios clearly distinguished NO_2-exposed rats from their negative controls. Survival times could be simply measured and generally were inversely proportioned to the severity of gassing. Using these criteria of quantitative evaluation it was not possible to demonstrate a positive beneficial effect for the majority of agents tested. This was true for those agents and procedures which are now employed clinically as well as the drugs appraised on purely theoretical grounds. Specifically, anti-foam agents, hyperbaric air and oxygen, and anti-inflammatory corticoids did not prove beneficial, with a few exceptions.

There are several mechanisms by which NO_2 produces injury to the respiratory passages and lung tissue. Laryngeal spasm is a relatively rare event, and was not recognized as the cause of death in any of our test animals. An effect on respiration was observed in the majority of animals within 2 to 10 minutes of commencing the exposure. This was manifest as an increase in rate and decrease in depth. Only pentobarbital administered before exposure affected this to any extent; while rats treated with this agent had slower rates and presumably did not have as great an intake of the gas, no statistically significant improvement occurred. Pulmonary edema usually occurred early, frequently before removal of the animals from the chamber. Manifest as a frothy exudate from the nares, there was frequently accompanying mild bleeding.

In our concept of this injury from NO_2, agents which cause relaxation of bronchial musculature, decrease capillary permeability, lower arteriolar pressure, or cause vasodilatation should be effective therapeutic agents. The only drugs which did so were rutin, evaluated against rats, and isoproterenol, against rabbits. Both of these were effective over a relatively short range of doses and produced a deleterious effect of themselves when given in larger quantities.

No satisfactory preparation of rutin is now available for clinical trial, in the quantities necessary for beneficial effects. Also, unfortunately, treatment prior to gassing was required for significant reduction in mortality. The success with isoproterenol was encouraging, and a further study with this and related agents should be undertaken.

Secondary infection with diffuse bronchial pneumonia is an important complication of NO_2 gassing in man. We did not evaluate chemotherapeutic agents in our tests, however, as the majority of animals which survived the critical period of pulmonary edema recovered.

It is apparent that the optimum therapeutic regimen has not as yet been obtained. The seriousness of the problem warrants further study. Based on our experience, infusion of the rabbit seems to be the best method of administering the test agents. The solvent uptake measurement of pulmonary dysfunction, while highly sensitive, is too laborious a method for use with the large numbers of animals required for statistical evaluation. Further, as changes in function occur at levels of impairment below those which are of concern in the clinically ill, gassed person, were favorable effects obtained, the findings would not directly relate to the more serious case.

We recommend a further study of both non-adrenergic hypotensive and adrenergic agents in the search for a more satisfactory agent in the treatment of NO_2-induced pulmonary edema. The present findings do not substantiate a specific treatment regimen. Based on this work, isoproterenol in doses of 0.5 to 2.0 mcg/kg/minute might be used to augment standard treatment. A more critical evaluation by slow infusion of isoproterenol and other related agents is recommended.

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13. ABSTRACT

An evaluaton was made of candidate therapeutic agents for the treatment of acute pulmonary edema resulting from nitrogen dioxide exposure. Treatments consisting of hyperbaric air and oxygen; tracheal toilet; ethyl, isopropyl, and octyl alcolhol vapors; hydralazine; bethanechol; physostigmine; and isoproterenol aerosols produced no change in the mortality, survival time, or lung/body weight ratios of rats suffering from NO2 --induced acute pulmonary edema. Rutin in large doses caused a decrease in mortality and an increase in survival time of exposed rats. Intravenous infusion of isoproterenol caused a decrease in mortality in rabbits exposed to NO2. The effectiveness of hyperbaric oxygen, hydrocortisone rutin and bethanechol against moderate exposure to NO2 was determined by solvent uptake measurements with rats. Oxygen administered 4 hours after exposure increased solvent uptake. There were no significant effects due to the other compounds.

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